Tree Physiology Advance Access published January 30, 2014

Tree Physiology 00, 1–9
doi:10.1093/treephys/tpt121

Research paper

Plant nitrogen status and co-occurrence of organic and inorganic nitrogen sources influence root uptake by Scots pine seedlings

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Received October 28, 2013; accepted December 12, 2013; handling Editor Heinz Rennenberg

Insights into how the simultaneous presence of organic and inorganic nitrogen (N) forms influences root absorption will help elucidate the relative importance of these N forms for plant nutrition in the field as well as for nursery cultivation of seedlings. Uptake of the individual N forms arginine, ammonium (NH_4^+) and nitrate (NO_3^-) was studied in Scots pine (*Pinus sylvestris* (L.)) seedlings supplied as single N sources and additionally in mixtures of NO_3^- and NH_4^+ or NO_3^- and arginine. Scots pine seedlings displayed a strong preference for NH_4^+-N and arginine-N as compared with NO_3^--N. Thus, NO_3^- uptake was generally low and decreased in the presence of NH_4^+ in the high-concentration range (500 μM N), but not in the presence of arginine. Moreover, uptake of NO_3^- and NH_4^+ was lower in seedlings displaying a high internal N status as a result of high N pretreatment, while arginine uptake was high in seedlings with a high internal N status when previously exposed to organic N. These findings may have practical implications for commercial cultivation of conifers.

Keywords: amino acids, conifer, fertilizer.

Introduction

In northern ecosystems, nitrogen (N) availability is generally limiting for plant growth (Tamm 1991, Vitousek and Howarth 1991). A wide variety of boreal forest plants, mycorrhizal as well as non-mycorrhizal, have the ability to take up organic N in the field; however, the degree to which organic N serves as an important N source for plant nutrition is still debated (Jones et al. 2005, 2013, Näsholm et al. 2009, Kuzyakov and Xu 2013). In boreal forests, organic N dominates soil N (Inselsbacher and Näsholm 2012), and may hence constitute a significant portion of the N accessible for plant uptake. Thus, in these organic N-dominated soils, mineralization rates underestimate the plant available N (Kielland 1994, Schimel and Chapin 1996, Schimel and Bennett 2004). It has been suggested that the bottleneck for plant N availability in these ecosystems is the depolymerization rates of high-molecular-weight organic compounds into low-molecular-weight organic compounds such as small peptides and amino acids (Jones and Kielland 2002, 2012, Schimel and Bennett 2004, Kielland et al. 2007, Rennenberg et al. 2009). The fast turnover of amino acids in soils implies that amino acids may serve as a significant N source even at relatively low concentrations in the soil solution (Kielland et al. 2007).

As stated above, N is present in a wide variety of chemical forms in the soil, inorganic as well as organic, and their respective concentrations may display large temporal and spatial differences in the soil. Hence, roots exploring the soil are in simultaneous contact with numerous N sources. Uptake of the inorganic N forms ammonium (NH_4^+) and nitrate (NO_3^-) and how these N forms interact at root absorption have been studied in conifers, where it has been shown that the presence of NH_4^+ inhibits the uptake of NO_3^- (Kamminga-van Wijk and Prins 1993). Uptake studies of organic N have mainly focused on the uptake capacities of single N sources, and only a few studies

have investigated the importance of interaction between organic and inorganic N sources on root uptake. Moreover, most of the studies to date have been performed on agricultural crop species such as perennial ryegrass (*Lolium perenne* L.) (Thornton and Robinson 2005) and wheat (*Triticum aestivum* L.) (Gioseffi et al. 2012) and not on commercially important forest tree species, with the exception of a study on European beech (*Fagus sylvatica* L.) (Stoelken et al. 2010). Even fewer studies have investigated whether these interactions are N concentration dependent. However, Stoelken et al. (2010) showed that the presence of glutamine or arginine in a mixed solution together with NH_4^+ and NO_3^- inhibited the uptake of NH_4^+ in the tested concentration range (55–5500 μM total N for glutamine and 75–7500 μM total N for arginine), while NO_3^- uptake was only affected by the presence of glutamine at 'excess external N concentrations' (5500 μM total N) and not by arginine.

Alongside the possible influence of co-occurrence of different N sources and effects of external N concentrations, N uptake is also affected by the internal N status of the plant. Scots pine (*Pinus sylvestris* (L.)) seedlings have been shown to decrease NH_4^+ and arginine uptake as a response to increased internal N concentrations (Öhlund and Näsholm 2004), and Persson and Näsholm (2002) showed that uptake of various inorganic and organic N sources was higher in unfertilized than in fertilized seedlings. A lot of effort has been made to elucidate the significance of organic N as an N source for conifers; however, in order to understand the complexity and relevance of organic N acquisition, studies must focus on using field-relevant concentrations as well as mixtures of different inorganic and organic N forms. A better understanding of how these organic and inorganic N forms interact may help elucidate the relative importance of these N sources for plant nutrition in the field as well as for commercial nursery cultivation of seedlings.

The aim of the current study was to clarify how acquisition of the individual N forms NH_4^+, NO_3^- and arginine in Scots pine seedlings is affected by the internal N status of plants and by the presence of other N forms in the uptake solution. An additional aim was to investigate whether previous exposure to organic N would affect root uptake of such N forms. We hypothesized that presence of arginine would not negatively affect the uptake of NO_3^- in the same manner as has been reported for NH_4^+ in conifer seedlings. Further, we hypothesized that a high internal N status in the seedlings would result in a relatively lower subsequent N acquisition from the uptake solution and that previous exposure of roots to arginine would result in higher uptake rates of this N source.

Materials and methods

Plant material and growth conditions

Scots pine seeds originating from seed orchard Gotthardsberg (58°9′N, 55 m above sea level) were sown in 240 g of industrial quartz sand (Ø 0.55 mm) in 0.2-l pots. The sand was washed twice with the same fertilizer solution as used for the rest of the growth period (for ion exchange). Seedlings were grown in a climate chamber in a day/night 16/8 h, 20/18°C regime and with a relative humidity of 70–80%. The photon flux density was 300–350 μmol m^{-2} s^{-1}. Seedlings were rotated three times a week in order to produce seedling material of similar size. From Day 14, seedlings were fertilized twice a week with 20 ml of a fertilizer solution diluted from concentrate to 7 mM N (corresponding to a rate of 1.96 mg N per seedling per fertilization event). The fertilizer concentrate was modified from the industrial fertilizer Rika-S (Weibulls, Hammenhög, Sweden) containing (g/l): N, 78 (of which NO_3^--N, 39; NH_4^+-N, 19.5; and arginine (Arg)-N, 19.5); K, 69; P, 12; S, 9.8; Mg, 7.2; Fe, 0.84; Mn, 0.42; B, 0.18; Zn, 0.13; Mo, 0.03; Cu, 0.02. The fertilizer was kept frozen and diluted fresh for every fertilization event in order to ensure the organic N content. The pH of the fertilizer was set to 5.8.

After 8 weeks, seedlings were transferred to acclimate for 3 days in hydroponics. Sand particles were gently removed and roots were washed thoroughly in tap water. The plant material was thereafter divided into three different pre-treatments. Roots were washed in three aliquots of 0.5 mM $CaCl_2$ in order to remove any adsorbed compounds before they were transferred to the respective pre-treatment solution. For two of the pre-treatments, the N composition was identical to the fertilizer solution used for the growth period, with N distributed as 50% NO_3^--N, 25% NH_4^+-N and 25% Arg-N. This pre-treatment, which included organic N in the form of arginine, was given in two N concentrations: 20 and 1000 μM (hereafter named low ON + IN and high ON + IN, respectively), of which the lower N concentration aimed at decreasing the N status of the seedlings before the following uptake experiment. A third pre-treatment with equimolar concentrations of NO_3^--N and NH_4^+-N was given at an N concentration of 1000 μM (hereafter named high IN; Table 1). Pre-treatment solutions were mixed fresh daily. Neither growth conditions nor the subsequent uptake experiments were axenic and mycorrhizal infection was not controlled. Seedlings were placed in a black plastic container (9.5 × 9.5 × 14 cm) filled with one of the three different pre-treatments. The solutions were aerated from an aquarium pump with an outlet to each container. The seedlings were maintained afloat at the surface by the support of a piece of black foam plastic.

Uptake experiment

On the fourth day after pre-treatment in hydroponics, an uptake study was performed with isotopically labeled N sources. The organic N source was provided as U-[$^{13}C_6$], [$^{15}N_4$]-Arg, while NO_3^--N and NH_4^+-N were supplied as $^{15}KNO_3$ and $^{15}NH_4Cl$ (all compounds at a labeling rate of 96–98 atom%). The uptake study was conducted for 2 h starting at noon, thus minimizing potential fluctuations in

Table 1. Nitrogen source compositions and concentrations of pre-treatments and uptake solutions.

Pre-treatment	N source pre-treatments	Concentration (μM total N)	N source uptake solutions	Concentration (μM total N)
Single N sources				
Low ON + IN	Arg; NH_4^+; NO_3^-	20	Arg	20–1000
High ON + IN	Arg; NH_4^+; NO_3^-	1000	Arg	20–1000
High IN	NH_4^+; NO_3^-	1000	Arg	20–1000
Low ON + IN	Arg; NH_4^+; NO_3^-	20	NH_4^+	20–1000
High ON + IN	Arg; NH_4^+; NO_3^-	1000	NH_4^+	20–1000
High IN	NH_4^+; NO_3^-	1000	NH_4^+	20–1000
Low ON + IN	Arg; NH_4^+; NO_3^-	20	NO_3^-	20–1000
High ON + IN	Arg; NH_4^+; NO_3^-	1000	NO_3^-	20–1000
High IN	NH_4^+; NO_3^-	1000	NO_3^-	20–1000
Mixed N sources				
Low ON + IN	Arg; NH_4^+; NO_3^-	20	Arg (NO_3^-)	10–500 (10–500)
High ON + IN	Arg; NH_4^+; NO_3^-	1000	Arg (NO_3^-)	10–500 (10–500)
High IN	NH_4^+; NO_3^-	1000	Arg (NO_3^-)	10–500 (10–500)
Low ON + IN	Arg; NH_4^+; NO_3^-	20	NH_4^+ (NO_3^-)	10–500 (10–500)
High ON + IN	Arg; NH_4^+; NO_3^-	1000	NH_4^+ (NO_3^-)	10–500 (10–500)
High IN	NH_4^+; NO_3^-	1000	NH_4^+ (NO_3^-)	10–500 (10–500)
Low ON + IN	Arg; NH_4^+; NO_3^-	20	NO_3^- (Arg)	10–500 (10–500)
High ON + IN	Arg; NH_4^+; NO_3^-	1000	NO_3^- (Arg)	10–500 (10–500)
High IN	NH_4^+; NO_3^-	1000	NO_3^- (Arg)	10–500 (10–500)
Low ON + IN	Arg; NH_4^+; NO_3^-	20	NO_3^- (NH_4^+)	10–500 (10–500)
High ON + IN	Arg; NH_4^+; NO_3^-	1000	NO_3^- (NH_4^+)	10–500 (10–500)
High IN	NH_4^+; NO_3^-	1000	NO_3^- (NH_4^+)	10–500 (10–500)

uptake capacities caused by the transfer from pre-treatment to uptake solutions as well as fluctuations caused by diurnal variations in uptake capacities. The roots were gently blotted with tissue paper and washed in three aliquots of 0.5 mM $CaCl_2$, blotted with tissue paper again and then immediately transferred to the uptake solution. Individual seedlings were placed in 50-ml falcon tubes containing 25 ml of uptake solution with four different concentrations (20, 100, 200 and 1000 μM N) and seven different N combinations (N compositions of the uptake solutions are specified in Table 1). Micronutrients were provided at a concentration corresponding to 200 μM N in pre-treatments and uptake solutions (see the fertilizer content in the previous section). Three replicates were used enabling the use of homogeneous seedling material and allowing for precision and control during the uptake experiment. During the 2-h uptake study, the test solutions were aerated from an aquarium pump through a Ø 0.8 mm needle via a sterile filter (0.22 μm). At the end of the uptake experiment, roots were once more blotted with tissue paper, washed in three aliquots of 0.5 mM $CaCl_2$ and blotted with tissue paper again. Roots and shoots were separated by the uppermost lateral root and then transferred to oven dry at 65 °C for 72 h. It should be noted that this experimental procedure does not account for potential efflux of N from roots to the solution. Hence, data presented refer to gross uptake rates while net uptake rates may be lower due to efflux of N during the 2-h incubation period.

Stable isotope analysis

The dried root and shoot samples were weighed and thereafter milled to a fine powder in a bead mill. The ^{15}N and ^{13}C content as well as the total N concentration of the plant material was analyzed with an isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany) interfaced to an element analyzer (Flash EA 2000) modified from Ohlsson and Wallmark (1999).

Treatment of data and statistical analyses

Atom% excess of ^{15}N and ^{13}C in the seedlings was calculated by subtracting the natural abundances of the heavier isotopes from the atom% from each labeled sample and N source. Mean values of the natural abundance of non-labeled plant material ($n = 4$) were subtracted to calculate atom% excess of labeled plants. The atom% excess was thereafter converted to μmol acquired N g^{-1} root dw h^{-1}. Intact uptake of arginine was verified by comparing the relationship between excess ^{13}C and excess ^{15}N.

The effect of pre-treatment on N concentration as well as on N uptake was evaluated by one-way analysis of variance. *P*-values ≤0.05 were considered to indicate a significant difference among means. Analyses were followed by Tukey's post hoc test. Minitab 16 Statistical Software (Minitab Inc., State College, PA, USA) was used for the analyses.

The substrate concentration of each N source was used to estimate the maximum uptake rate (V_{max}) and affinity constant (K_m) by linear regression of the Hanes–Woolf plot. Standard

error (SE) for V_{max} and K_m was calculated according to Ritchie and Prvan (1996).

Results

Nitrogen concentration

The low ON + IN pre-treatment resulted in a significantly lower N concentration in the roots as well as the shoots than in the other pre-treatments, being 1.67% in roots and 1.39% in shoots ($P \leq 0.000$; Figure 1). The root N concentration of seedlings of the high ON + IN pre-treatment was highest (2.57%) and intermediate (2.33%) for the high IN pre-treatment, while N concentrations of the seedlings pre-treated with the two high N pre-treatments were similar in the shoots, being 1.87 and 1.77%, respectively ($P < 0.000$; Figure 1).

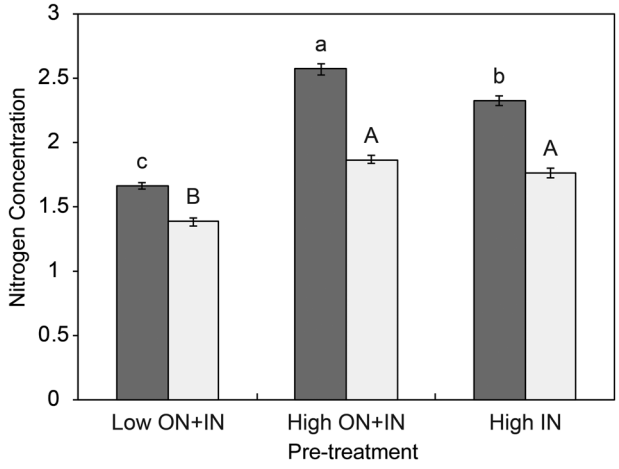

Figure 1. Nitrogen concentration (%) of roots (dark gray bars) and shoots (light gray bars) of 8-week-old Scots pine seedlings pre-treated for 3 days in low (20 µM N) or high (1000 µM N) ON + IN (50% NO_3^--N, 25% NH_4^+-N and 25% Arg-N) or high (1000 µM N) IN (50% NO_3^--N, 50% NH_4^+-N). Different lower-case and capital letters indicate significant differences in N concentration in roots and shoots, respectively at $P \leq 0.05$. Average values ± SE, $n = 84$.

Arginine-N uptake

Acquisition of Arg-N was generally much higher (10-fold) than that of NO_3^--N (Figures 2 and 4). Uptake rates of Arg-N were within the range of 5.1–28.4 µmol g^{-1} root dw h^{-1} at the tested concentration range (20–1000 µM; Figure 2). Uptake of arginine was higher in seedlings from the high ON + IN pre-treatment, which was especially pronounced at the intermediate N concentration interval between 100 and 200 µM N, where the uptake was about twice as high as for the other pre-treatments ($P = 0.001$–0.011; Figure 2a). A low internal N concentration of the seedlings (Figure 1) did not affect uptake in the low ON pre-treatment, as this was similar to that of seedlings previously exposed to a high IN pre-treatment (Figure 2a).

When supplied together with equimolar concentrations of NO_3^--N, uptake of Arg-N was similar across pre-treatments in the low micromolar range; however, at a concentration of 500 µM, uptake of seedlings pre-treated with a high ON + IN was higher than for those supplied with low or IN pre treatments ($P = 0.010$; Figure 2b).

The uptake of the N sources was further characterized by estimating kinetic parameters. The V_{max} for arginine was in the range 4.6–8.0 µmol g^{-1} root dw h^{-1} and the K_m was within the range 18.6–50.0 µM. Estimates of V_{max} as well as of K_m for arginine were higher when Arg-N was provided as a single N source than when provided in a mixture with equimolar concentrations of NO_3^--N for seedlings previously exposed to a low ON + IN or high IN pre-treatment. However, the generality of this pattern was not consistent in seedlings previously exposed to a high ON + IN, showing equally high V_{max}, while K_m was lower when arginine was provided as a single N source (Table 2).

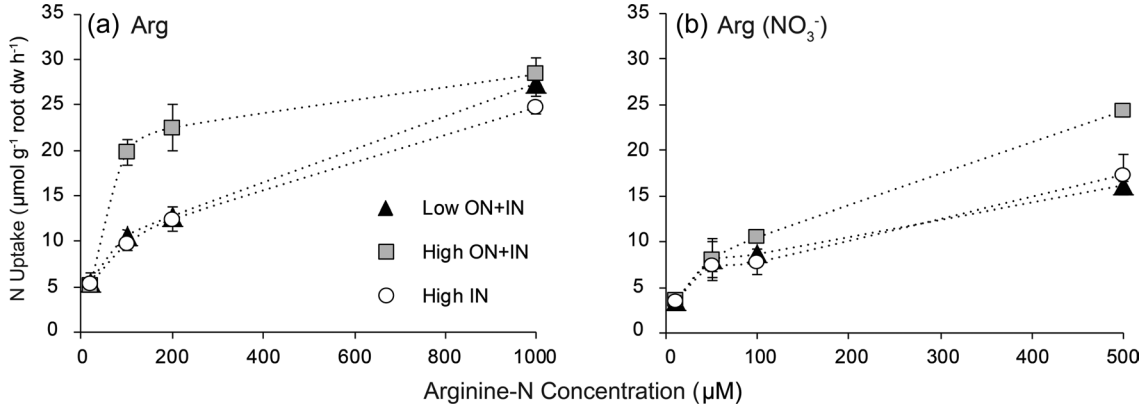

Figure 2. Uptake of arginine-N of Scots pine seedlings when supplied (a) as a single N source or (b) in a mixed solution with equimolar concentrations of NO_3^--N and acclimated in a low (20 µM N) or high (1000 µM N) ON + IN (50% NO_3^--N, 25% NH_4^+-N and 25% Arg-N) pre-treatment or high (1000 µM N) IN (50% NO_3^--N, 50% NH_4^+-N) pre-treatment. Symbols represent average values, $n = 3$. Error bars represent SE when not hidden by the symbol. Note the different scales of the x-axis.

When Arg-N was supplied together with equimolar concentrations of NO_3^--N, the estimated V_{max} was higher in seedlings pre-treated with a high ON + IN than in those pre-treated with a low ON + IN (Table 2).

NH_4^+-N uptake

Uptake of NH_4^+-N was generally high: more than 10-fold that of NO_3^--N (Figures 3 and 4). Ammonium-N uptake rates were within the range 4.7–33.6 μmol g^{-1} root dw h^{-1} at the tested concentration range (20–1000 μM; Figure 3). Hence, Arg-N and NH_4^+-N uptake rates were approximately within the same range (Figures 2 and 3). Ammonium uptake was high throughout the concentration range in seedlings previously exposed to a low ON + IN ($P = 0.008$; Figure 3a), seedlings provided with the high IN pre-treatment were intermediate, while there was a high variation in the uptake of NH_4^+-N in seedlings from the high ON + IN pre-treatment, which was especially pronounced at the concentration range 200–1000 μM. Hence, the lower uptake rates at 200 and 1000 μM compared with that at 100 μM may not reflect a true decrease in uptake rates at higher external N concentrations but may simply be due to a high variability in uptake rates.

Uptake of NH_4^+-N in the presence of NO_3^--N did not differ significantly among pre-treatments; however, there was a trend toward higher uptake throughout the concentration span in seedlings displaying low internal N concentration (Figures 1 and 3b), and at 500 μM, uptake was higher in seedlings previously exposed to a low ON + IN than in those exposed to a high IN, while those given a high ON + IN pre treatment could not be separated from either of the other pre treatments ($P = 0.056$; Figure 3b).

The estimated V_{max} for NH_4^+ was in the range 15.6–35.6 μmol g^{-1} root dw h^{-1} and the estimated K_m was within the range 59.3–119.1 μM. Estimated V_{max} and K_m for NH_4^+ when provided as a single N source could only be calculated for seedlings from the low ON + IN pre treatment, since underlying Hanes–Woolf plots were not significant in the other pre treatments. Seedlings provided with NH_4^+ as a single N source had a higher estimated V_{max} than those simultaneously provided with NO_3^-, while K_m values were similar across single and mixed N sources (Table 2).

Estimated V_{max} or K_m was similar between pre-treatments when NH_4^+ was supplied together with NO_3^- (Table 2).

NO_3^--N uptake

NO_3^--N uptake was generally low (0.1–3.7 μmol g^{-1} root dw h^{-1}; Figure 4); however, seedlings with low internal N concentration as a result of a low N pre-treatment (Figure 1) had several-fold higher NO_3^--N uptake ($P = 0.000$–0.002; Figure 4). There was a trend toward slightly lower uptake throughout the concentration range in seedlings pre-treated with high ON + IN than in those of the high IN pre-treatment.

Table 2. The V_{max} and K_m for uptake of arginine, NH_4^+ and NO_3^- in Scots pine seedlings.

Pre-treatment	Concentration (μM total N)	N source	Concentration (μM substrate)	Estimated $K_m \pm$ SE (μM)	Estimated $V_{max} \pm$ SE (μmol g^{-1} root dw h^{-1})
Low ON + IN	20	Arg	5–250	50.0 ± 10.4	8.0 ± 0.6
Low ON + IN	20	Arg (NO_3^-)	2.5–125 (10–500)	18.6 ± 3.5	4.6 ± 0.2
High ON + IN	1000	Arg	5–250	20.0 ± 6.2	7.6 ± 0.4
High ON + IN	1000	Arg (NO_3^-)	2.5–125 (10–500)	36.2 ± 7.5	7.7 ± 0.8
High IN	1000	Arg	5–250	45.7 ± 8.7	7.2 ± 0.5
High IN	1000	Arg (NO_3^-)	2.5–125 (10–500)	24.2 ± 7.9	4.9 ± 0.6
Low ON + IN	20	NH_4^+	20–1000	59.3 ± 15.4	35.6 ± 1.1
Low ON + IN	20	NH_4^+ (NO_3^-)	10–500 (10–500)	101.1 ± 26.9	29.5 ± 2.9
High ON + IN	1000	NH_4^+	20–1000	n.s.	n.s.
High ON + IN	1000	NH_4^+ (NO_3^-)	10–500 (10–500)	119.1 ± 29.0	24.9 ± 2.6
High IN	1000	NH_4^+	20–1000	n.s.	n.s.
High IN	1000	NH_4^+ (NO_3^-)	10–500 (10–500)	70.3 ± 30.3	15.6 ± 1.8
Low ON + IN	20	NO_3^-	20–1000	271.5 ± 89.5	4.5 ± 0.7
Low ON + IN	20	NO_3^- (Arg)	10–500 (2.5–125)	80.8 ± 19.6	3.5 ± 0.3
Low ON + IN	20	NO_3^- (NH_4^+)	10–500 (10–500)	n.s.	n.s.
High ON + IN	1000	NO_3^-	20–1000	399.8 ± 143.0	1.3 ± 0.3
High ON + IN	1000	NO_3^- (Arg)	10–500 (2.5–125)	443.9 ± 222.9	1.6 ± 0.7
High ON + IN	1000	NO_3^- (NH_4^+)	10–500 (10–500)	197.3 ± 50.9	1.2 ± 0.2
High IN	1000	NO_3^-	20–1000	464.9 ± 99.5	2.5 ± 0.4
High IN	1000	NO_3^- (Arg)	10–500 (2.5–125)	124.2 ± 49.9	1.3 ± 0.2
High IN	1000	NO_3^- (NH_4^+)	10–500 (10–500)	227.4 ± 79.1	1.8 ± 0.4

Kinetic parameters were estimated from substrate concentrations of each individual N source, n.s. denotes insignificant regressions in the underlying Hanes–Woolf plots.

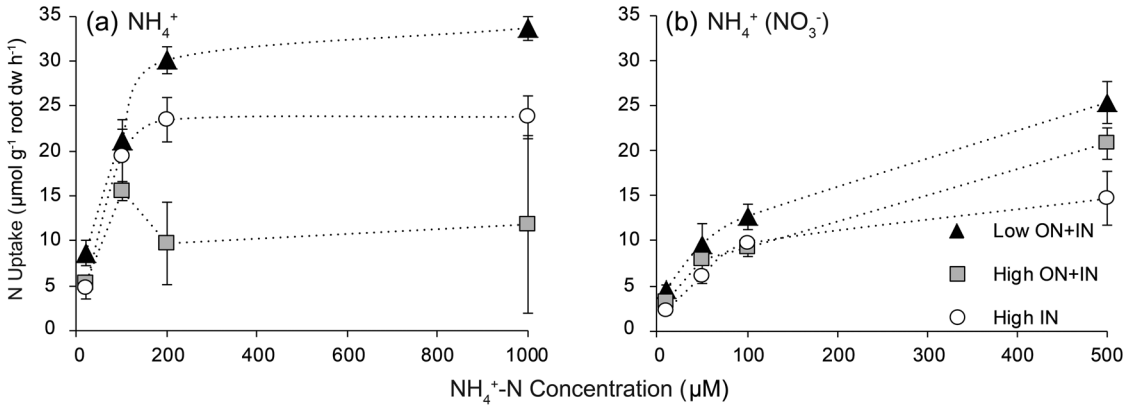

Figure 3. Uptake of NH_4^+-N of Scots pine seedlings when supplied (a) as a single N source or (b) in a mixed solution with equimolar concentrations of NO_3^--N and acclimated in a low (20 μM N) or high (1000 μM N) ON + IN (50% NO_3^--N, 25% NH_4^+-N and 25% Arg-N) pre-treatment or high (1000 μM N) IN (50% NO_3^--N, 50% NH_4^+-N) pre-treatment. Symbols represent average values, $n = 3$. Error bars represent SE when not hidden by the symbol. Note the different scales of the *x*-axis.

Uptake of NO_3^--N was highest in seedlings acclimated in a low pre-treatment, regardless if supplied together with Arg-N or NH_4^+-N (Figure 4b and c). However, uptake was lower at 500 μM in the presence of NH_4^+-N as compared with that in the presence of Arg-N ($P = 0.010$).

The maximum uptake rates of NO_3^- were generally low (1.2–4.5 μmol g^{-1} root dw h^{-1}; Table 2), with the exception of seedlings previously exposed to a low ON + IN. Estimated V_{max} and K_m were only higher for seedlings provided with NO_3^- as a single source than as a mixed source together with equimolar concentrations of Arg-N, when previously exposed to low ON + IN (Table 2).

Within the single N source tests, estimated V_{max} was highest in seedlings exposed to a low ON + IN pre-treatment and lowest in those exposed to a high ON + IN pre-treatment. Estimated V_{max} was also highest in seedlings exposed to a low ON + IN pre treatment when NO_3^- was supplied as a mixed N source together with Arg-N, while seedlings pre-treated with high IN had the lowest estimated V_{max} (Table 2). Due to an insignificant Hanes–Woolf plot in the low ON + IN pre-treatment, there were no differences in estimated V_{max} when NO_3^- was supplied as a mixed N source together with NH_4^+-N (Table 2).

Uptake of intact arginine

Intact uptake of arginine was verified by the relationship between excess ^{13}C and excess ^{15}N from U-[$^{13}C_6$], [$^{15}N_4$]-Arg, where the theoretical slope for 100% of intact uptake of arginine is 1.5. The regression (slope = 1.40; $r^2 = 0.93$) indicated that a minimum of 93% of the acquired Arg-N was derived from uptake of intact amino acid (data not shown).

Discussion

The present study shows how Scots pine seedlings regulate uptake rates depending on internal N status and how the simultaneous presence of NO_3^- and NH_4^+, as well as NO_3^- and arginine, influences root uptake. In general, uptake of NH_4^+-N and Arg-N was similar but about 10-fold higher than that of NO_3^--N. This is in line with earlier studies in suggesting that many conifer species show a strong preference for NH_4^+ as compared with NO_3^- (Marschner et al. 1991, Kamminga-van Wijk and Prins 1993, Kronzucker et al. 1996, 1997, Gessler et al. 1998, Malagoli et al. 2000, Öhlund and Näsholm 2004). Conifers have also been shown to take up comparable or even higher amounts of organic N than NH_4^+-N (Öhlund and Näsholm 2001, 2004, Persson et al. 2003, 2006, Metcalfe et al. 2011, Gruffman et al. 2013).

Seedlings were pre-treated with either low ON + IN, high ON + IN or high IN solution and in this way we obtained seedlings with different N status (Figure 1). Further, these pre-treatments enabled us to study the importance of previous exposure to organic N for uptake characteristics of such N forms. The N concentrations of seedlings with high N concentration pre-treatments (high ON + IN and high IN) were in the range considered optimal for nursery seedlings (cf. Gruffman et al. 2012), while those receiving the low N pre-treatment (low ON + IN) were suboptimal according to the same standards. Hence, the recorded uptake parameters of this study may be considered relevant for nursery-grown Scots pine seedlings. It should be stressed, however, that results from short-term uptake studies such as that reported here cannot be directly translated into uptake rates in the field or growth rates of seedlings on different N sources but are mainly indicative of physiological capacities.

Arginine uptake was not negatively affected by a high internal N status as seedlings displayed similar uptake rates when provided with the low ON + IN or high IN pre-treatment, despite significant differences in seedling N concentrations (Figure 2). Instead, the high ON + IN pre-treatment resulted in highest uptake of Arg-N, at 100 and 200 μM N, corresponding to an

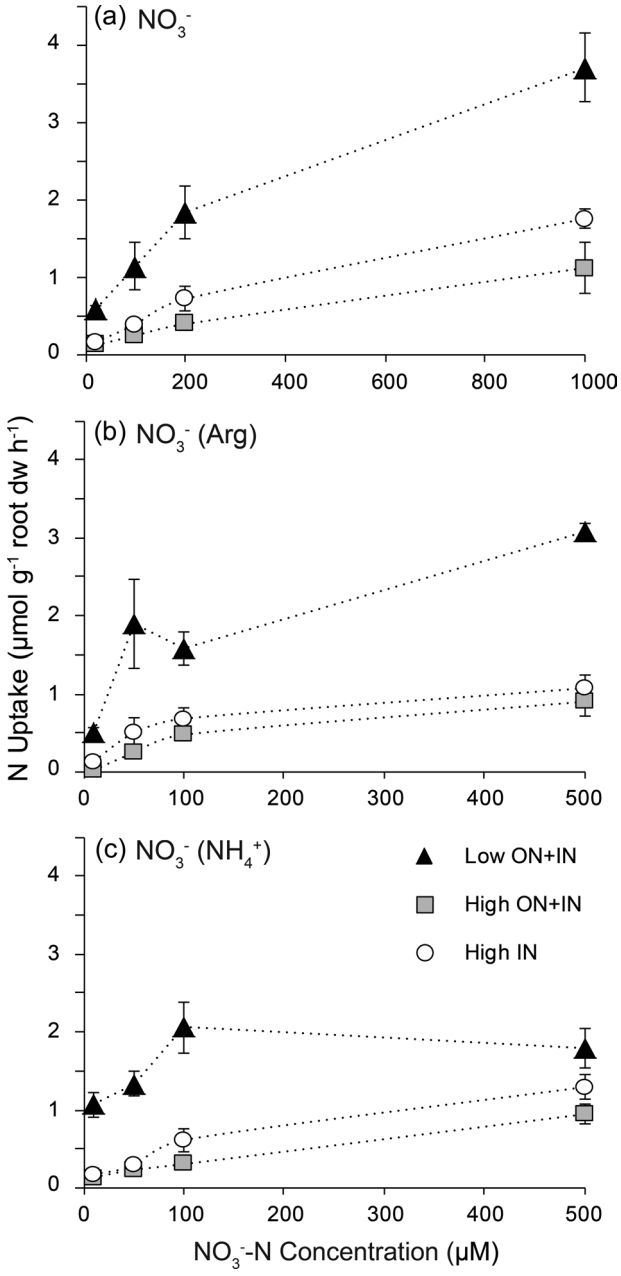

Figure 4. Uptake of NO_3^--N of Scots pine seedlings when supplied (a) as a single N source, (b) in a mixed solution with equimolar concentrations of Arg-N and (c) in a mixed solution with equimolar concentrations of NH_4^+-N and acclimated in a low (20 µM N) or high (1000 µM N) ON + IN (50% NO_3^--N, 25% NH_4^+-N and 25% Arg-N) pre-treatment or high (1000 µM N) IN (50% NO_3^--N, 50% NH_4^+-N) pre-treatment. Symbols represent average values, $n = 3$. Error bars represent SE when not hidden by the symbol. Note the different scales of the *x*-axis.

arginine concentration of 25 and 50 µM (Figure 2a). In this concentration range, seedlings had twice the N-uptake rates as in the other pre-treatments, indicating that arginine uptake was up-regulated in seedlings that had been previously exposed to higher concentrations of arginine (250 µM Arg-N). This suggests that up-regulation of arginine uptake involved a high-affinity uptake system such as the amino acid permease 5 (AAP5), previously reported to mediate uptake of arginine and lysine in *Arabidopsis thaliana* (Svennerstam et al. 2008, 2011). Induction of uptake in conifers is well known for NO_3^- (Kronzucker et al. 1995, 1997, Min et al. 1998, 2000, Tischner 2000), but only a few reports on this phenomenon regarding organic N forms are available (e.g., Persson and Näsholm 2002).

In contrast to arginine, uptake of both NH_4^+ and NO_3^- was negatively affected by a high internal N status of seedlings (Figures 3 and 4). This was apparent regardless of whether the N forms were provided as single N sources or as mixtures, indicating that seedlings down-regulated uptake of these sources as a response to high internal N status (Figures 3 and 4). This was especially evident for uptake of NO_3^--N, where uptake rates in the two high-N pre-treatments were <50% of those in the low ON + IN pre-treatment. Down-regulation of uptake has earlier been demonstrated for NO_3^- for various species, including Scots pine (Persson et al. 2006). Nitrate uptake is controlled by several internal factors such as a systemic feedback regulation as a result of high N status of the plant and increased concentrations of down-stream products of metabolized NO_3^-, i.e., amino acids (glutamine) (Vidmar et al. 2000, Miller et al. 2008, Girin et al. 2010). A complementary explanation might be that uptake of NO_3^- is associated with high energetic costs (Zerihun et al. 1998). Although it cannot be deduced from the current study, seedlings exposed to high N concentrations in the pre-treatment might be energy limited, thereby decreasing their subsequent uptake of NO_3^- (cf. Gruffman et al. 2013). The high uptake of Arg-N in seedlings previously exposed to high ON + IN would also be in agreement with this complementary explanation, provided that these seedlings are able to benefit from the carbon provided by the organic N source (Gruffman et al. 2013).

Uptake rates of NO_3^--N were generally slightly lower (although not significant) when seedlings had been exposed to a high ON + IN pre-treatment than to a high IN pre-treatment, possibly indicating that the presence of arginine in the pre-treatment solution affected the subsequent NO_3^- uptake (Figure 4). This effect may, however, be related to the somewhat higher N concentration of roots of seedlings from the high ON + IN pre-treatment (Figure 1). Pre-treatment with various amino acids, not including arginine, has earlier been shown to inhibit NO_3^- uptake in Norway spruce (*Picea abies*) and beech, when provided at high concentration (10 mM) (Gessler et al. 1998).

Nitrate-N uptake was repressed in the presence of NH_4^+-N, but not in the presence of Arg-N in the high-concentration range for seedlings pre-treated with low ON + IN (Figure 4). This result suggests that uptake mediated by low-affinity NO_3^-

transport systems may be sensitive to the simultaneous presence of NH_4^+ but not of arginine. This difference between NH_4^+ and arginine in how they affect the uptake of NO_3^- may have practical relevance; combinations of NH_4^+ and NO_3^- are commonly used as fertilizers for conifer seedlings but may display low efficiencies partly because of negative interactions between the two N sources.

The estimated V_{max} were higher for all the three N sources when provided as single N sources than when provided in mixtures (Table 2), potentially affected by the lower concentrations of N forms used in the mixture. The estimated K_m and V_{max} of arginine uptake were twice as high when arginine was provided as a single N source than when provided in a mixture with NO_3^-, the exception being seedlings previously exposed to high ON + IN, which displayed equally high estimated V_{max} of arginine regardless if provided as a single or mixed N source (Table 2). This suggests that arginine uptake was up-regulated by ON + IN pre-treatments. Estimated K_m and V_{max} of seedling uptake of the three N sources also varied depending on pre-treatment. The estimated affinities for arginine were high (18.6–50.0 μM) while they were intermediate for NH_4^+ (59.3–119.1 μM) and low for NO_3^- (80.8–464.9 μM; Table 2). High-affinity root uptake of arginine has earlier been reported for both *Arabidopsis* (Svennerstam et al. 2011) and barley (*Hordeum vulgare*) (Jämtgård et al. 2008). Maximum uptake rates were high for NH_4^+, intermediate for arginine and low for NO_3^-, again illustrating the poor efficiency of Scots pine seedlings to utilize NO_3^-. It should be noted that these values refer to uptake of the N sources, not to N uptake, implying that maximum N uptake in the form of arginine was in the same range as N uptake in the form of NH_4^+.

Large amounts of fertilizers are added during commercial cultivation of tree seedlings (Juntunen and Rikala 2001). However, the fraction of N absorbed by seedlings of the total amount of N added has been reported to be low, leading to economical and ecological sub optimization (Juntunen et al. 2002, Öhlund and Näsholm 2002). Several studies have shown that tree seedlings in general and conifer seedlings in particular display low uptake rates of NO_3^- (Kronzucker et al. 1995, Gessler et al. 1998, Öhlund and Näsholm 2001, Miller and Hawkins 2007) and that a high internal N status of seedlings leads to an even lower uptake of NO_3^- (Öhlund and Näsholm 2004). Moreover, earlier studies have shown that NO_3^- uptake is negatively affected by the presence of NH_4^+ (Kamminga-van Wijk and Prins 1993). The current study corroborates these earlier findings but contrasts these effects with results on organic N in the form of arginine. Thus, compared with the inorganic N forms, particularly NO_3^-, arginine uptake was high and positively affected by a high internal N status of seedlings. Interactions between arginine and NO_3^- were less severe than those between NH_4^+ and NO_3^-, suggesting that mixtures of arginine and NO_3^- may display higher N uptake efficiencies than mixtures of NH_4^+ and NO_3^-.

Conclusions

The present study shows that Scots pine seedlings display a strong preference for NH_4^+-N and Arg-N as compared with NO_3^--N, probably due at least partly to a strong negative effect of internal N and NH_4^+ on the uptake of NO_3^-. Generally, seedling uptake of NH_4^+-N and Arg-N was in the same order and ~10-fold that of NO_3^--N. Arginine uptake was high in seedlings previously exposed to high concentrations of Arg-N, suggesting up-regulation of transport systems in the high-affinity range regardless of a high internal N status. In contrast, uptake of NO_3^--N and NH_4^+-N was negatively affected by a high internal N status of seedlings. In the high N concentration range, seedlings with low internal N status decreased NO_3^--N uptake in the presence of NH_4^+-N, but not in the presence of Arg-N, which may have practical implications for commercial cultivation of conifers.

Acknowledgments

We wish to thank Jonas Lundholm for skillful analytical work and Anders Muszta and Kristi Kuljus for statistical advice.

Funding

This study was financed by grants from The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, FORMAS and from the SLU project Trees and Crops for the Future (TC4F).

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