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A comparative kinetic analysis of nitrate and ammonium influx in two early-successional tree species of temperate and boreal forest ecosystems

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ABSTRACT

Root NO_3^- and NH_4^+ influx systems of two early-successional species of temperate (trembling aspen: *Populus tremuloides* Michx.) and boreal (lodgepole pine: *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) forest ecosystems were characterized. NO_3^- and NH_4^+ influxes were biphasic, consisting of saturable high-affinity (HATS) and constitutive non-saturable low-affinity transport systems (LATS) that were evident at low and relatively high N concentrations, respectively. NO_3^- influx via HATS was inducible (IHATS); nitrate pre-treatment resulted in 8–10-fold increases in the V_{max} for influx in both species. By contrast, HATS for NH_4^+ were entirely constitutive. In both species, V_{max} values for NH_4^+ influx were higher than those for NO_3^- uptake; the differences were larger in pine (6-fold) than aspen (1·8-fold). In aspen, the K_m for NH_4^+ influx by HATS was approximately 3-fold higher than for IHATS NO_3^- influx, while in pine the K_m for IHATS NO_3^- influx was approximately 3-fold higher than for NH_4^+ influx. The aspen IHATS for NO_3^- influx appeared to be more efficient than that of pine (V_{max} values for aspen being approximately 10-fold higher and K_m values being approximately 13-fold lower than for pine). By contrast, only small differences in values for the NH_4^+ HATS were evident between the two species. The kinetic parameters observed here probably result from adaptations to the N availabilities in their respective natural habitats; these may contribute to the distribution and niche separation of these species.

Key-words: ammonium fluxes; kinetics of nitrate and ammonium uptake; lodgepole pine; nitrate fluxes; nitrogen nutrition; trembling aspen.

INTRODUCTION

In most late-successional communities of temperate and boreal forest ecosystems, NH_4^+ is generally the main source of soil inorganic N (e.g. Van Cleve *et al*. 1983; Chapin, Van Cleve & Tyron 1986; Lavoie, Vezina & Margolis 1992). Natural or artificial disturbances result in increased rates of nitrification, increased $[NO_3^-]$, and low $[NH_4^+]$ in the soils (e.g. Likens, Bormann & Johnson 1969; Lodhi & Killingbeck 1980; Van Cleve *et al*. 1983; Walley, Van Kessel & Pennock 1996). Plant species differ greatly in their capacities for the utilization of particular N forms and these adaptations may contribute to the unique spatial and/or temporal distributions of these species (e.g. Bledsoe & Rygiewicz 1986; Chapin, Moilanen & Kielland 1993; Kronzucker, Siddiqi & Glass 1997; Min *et al*. 1998, 1999). Thus, at relatively low external [N] ($[N]_o$), i.e. in the range of high-affinity transport systems (HATS; see Glass & Siddiqi 1995), late-successional species e.g. *Picea glauca* (white spruce) show a clear preference for the utilization of NH_4^+ over NO_3^- (Kronzucker, Glass & Siddiqi 1995a; Kronzucker, Siddiqi & Glass 1995b,c,d; 1996, 1997). By contrast, *Populus tremuloides* (trembling aspen) and *Pseudotsuga menziesii* var. *glauca* (Douglas fir), two early-successional species, have relatively high capacities for NO_3^- acquisition and can maintain apparently adequate cytoplasmic $[NO_3^-]$ ($[NO_3^-]_{cyt}$) for optimal rates of reduction even at low (0·1 mol m^{-3}) $[NO_3^-]_o$ (Min *et al*. 1999). In the same study, *Pinus contorta* var. *latifolia* (lodgepole pine), another early-successional species, showed a much lower capacity for NO_3^- uptake at 0·1 $[NO_3^-]_o$ and failed to maintain adequate $[NO_3^-]_{cyt}$ for optimal rates of reduction (Min *et al*. 1999). In pine, however, NO_3^- uptake rates and $[NO_3^-]_{cyt}$ increased greatly at the higher $[NO_3^-]_o$ (1·5 mol m^{-3}). Interestingly, lodgepole pine is generally associated with fire-disturbed sites where soil $[NO_3^-]$ may be high (Klinka *et al*. 1990; Brayshaw 1996).

The concentration-dependent differences in the capacities for N utilization among the early-successional species described above prompted us to undertake more detailed kinetic analyses of the NO_3^- and NH_4^+ influx systems of these species. In aspen and lodgepole pine, NO_3^- and NH_4^+ influxes were biphasic, as in several other species (Glass & Siddiqi 1995). A high-affinity transport system (HATS) was saturable and operated at low external N concentration, while at higher $[N]_o$ a linearly concentration-dependent,

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low-affinity transport system (LATS) was evident. There were substantial differences between the two species in the characteristics of these transport systems. Since the aim of the present study was to characterize the NO_3^- and NH_4^+ transport systems of the tree species *per se*, plants used in the present studies were grown in hydroponic systems free of mycorrhizal associations (Min *et al*. 1998, 1999; see also Kronzucker *et al*. 1997).

MATERIALS AND METHODS

Plant growth

Seeds of trembling aspen (*Populus tremuloides* Michx., seedlot 40959) and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm., seedlot 3847) were obtained from the Tree Seed Centre, Ministry of Forests, Surrey, British Columbia, Canada. Seeds were germinated in styroblocks in a 2:1 (v/v) peat/perlite mixture, containing dolomite at the rate of 4·3 kg m^{-3}. Seedlings were maintained in the peat/perlite mixture in a greenhouse for approximately 3 months. They were then transferred to hydroponic tanks (40 L capacity) containing aerated modified 0·1 strength Johnson's solution with the desired NO_3^- or NH_4^+ concentration, provided as $Ca(NO_3)_2$ or $(NH_4)_2SO_4$ (Min *et al*. 1999). The nutrient solutions were buffered at pH 6·4–6·8 by adding excess powdered $CaCO_3$. The hydroponic tanks, containing the seedlings, were maintained in a controlled environment room at 20 ± 2 °C, 70% relative humidity and 16 h light/8 h dark period. Light was provided at 250 μmol m^{-2} s^{-1} at plant level by fluorescent tubes with spectral composition similar to sunlight (Vita-Lite, Duro-Test, Fairfield, NJ, USA).

NO_3^- and NH_4^+ pre-treatments

Plants were grown in low N (a pulse of 25 mmol m^{-3}) for the first 15 d, and then transferred to solutions without N or containing 0·1 mol m^{-3} NO_3^- or NH_4^+ for 5 d prior to influx measurements. We have observed that both NO_3^- and NH_4^+ fluxes had reached steady values within 4–5 d of pre-treatment with NO_3^- and NH_4^+, respectively (Kronzucker *et al*. 1996; Min *et al*. 1998, 1999). The reason for restricting the N supply to the 5 d prior to influx determinations was that we detected appreciable formation of NO_2^- in the culture solutions when 0·1 mol m^{-3} NH_4^+ was present for 10 d or more. This probably resulted from the activity of nitrifying organisms carried over from the peat/perlite growth medium. Thus, a 5 d pre-treatment with 0·1 mol m^{-3} NH_4^+ provided a means of avoiding NO_2^- accumulation without sacrificing apparent steady-state conditions (Min *et al*. 1999).

Production and purification of $^{13}NO_3^-$ and $^{13}NH_4^+$

The radiotracer ^{13}N (half-life = 9·96 min) was produced as $^{13}NO_3^-$ by proton irradiation of H_2O at the TRIUMF Cyclotron on the campus of the University of British Columbia, Vancouver, Canada. In the case of $^{13}NO_3^-$ experiments, radio-contaminants were removed as described by Kronzucker *et al*. (1995a), except that 5 cm^3 of 0·1 mol m^{-3} (instead of 2·5 mmol m^{-3}) $Ca(NO_3)_2$ were used to elute residual $^{13}NO_3^-$ from the SEP-PAC Alumina-N cartridge (Min *et al*. 1998). Briefly, ^{18}F was removed by passing the sample through a SEP-PAC Alumina-N cartridge (Waters, Milford, Massachusetts, USA) twice. The sample was then alkalinized by adding KOH and boiled for 2·5 min to volatilize $^{13}NH_3$. Subsequently, $^{13}NO_2^-$ was oxidized by adding H_2SO_4 and H_2O_2 and boiling for another 2·5 min. The solution was cooled to room temperature, neutralized and excess H_2O_2 reduced by adding catalase.

For NH_4^+ experiments, $^{13}NO_3^-$ was reduced to $^{13}NH_3$ by adding Devarda's alloy at pH 12 at 75 °C. The resulting $^{13}NH_3$ gas was trapped in an acidified solution and thus converted to $^{13}NH_4^+$ (Wang *et al*. 1993a; Kronzucker *et al*. 1995b). The solution was then neutralized by adding KOH.

Influx measurements

Influx of NO_3^- or NH_4^+ was measured from modified 0·1 strength Johnson's nutrient solution containing appropriate concentrations of ^{13}N-labelled NO_3^- or NH_4^+. Seedlings were transferred from hydroponic tanks to a non-radioactive solution, identical in composition (except for isotope) to the uptake solution, for 5 min; they were then transferred to ^{13}N-labelled uptake solution for 10 min. Immediately following isotope loading, roots were dipped into a non-radioactive solution for 5 s, and then transferred to a non-radioactive solution, which was otherwise identical to the uptake solution, for 2 min to remove $^{13}NO_3^-$ and for 3 min to remove $^{13}NH_4^+$ from the free space (Kronzucker *et al*. 1995a,d). Following desorption, roots were excised from shoots, spun for 45 s in a low-speed centrifuge to remove surface solution, and fresh weights determined. Radioactivities of the shoots and roots were measured with a Packard-γ-counter (Minaxi, Auto-gamma 5000) and NO_3^- and NH_4^+ fluxes thus determined were expressed as μmol g^{-1} root fresh weight h^{-1}.

All experiments were performed 3–5 times; within each experiment, each treatment was replicated 3–4 times.

RESULTS AND DISCUSSION

Kinetic analyses (Figs 1–4) revealed that in both trembling aspen and lodgepole pine, influxes of NO_3^- and NH_4^+ are mediated by distinct high- and low-affinity transport systems (HATS and LATS, respectively). This is consistent with earlier reports regarding the uptake of NO_3^-, NH_4^+ and several other ions, in other species ranging from unicellular organisms to higher plants (e.g. Epstein 1976; Serra *et al*. 1978; Kochian & Lucas 1982; Siddiqi *et al*. 1990; Aslam *et al*. 1992; Wang *et al*. 1993b; Kronzucker *et al*. 1995b, 1996; see also Glass & Siddiqi 1995 and Crawford & Glass 1998 for reviews). Below approximately 0·5 mol m^{-3}, saturable NO_3^- and NH_4^+ influxes occurred via their respective HATS (Figs 1 & 2), while at high $[N]_o$, NO_3^- and NH_4^+ influxes

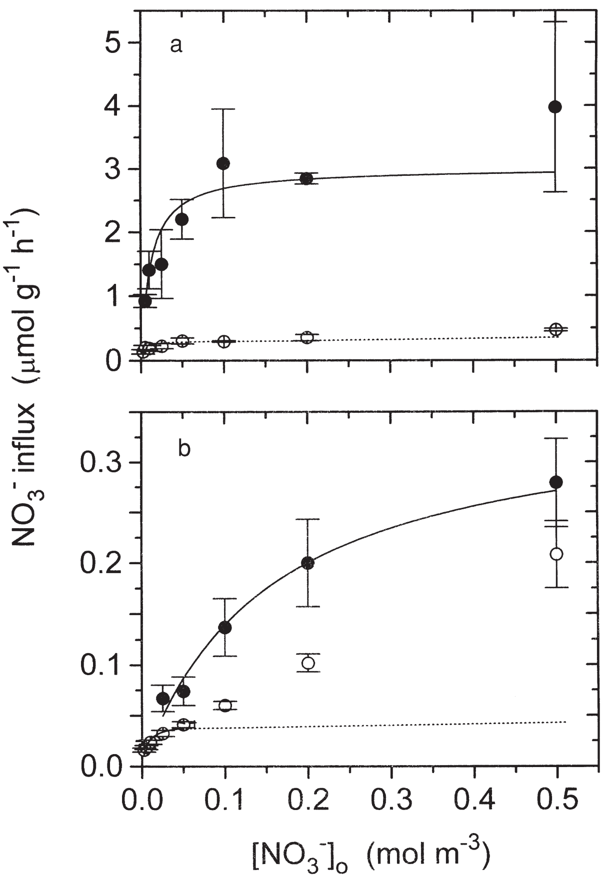

Figure 1. Nitrate influx (means ± SE; n = 8–12) into non-induced (open symbols and dashed lines) or 0·1 mol m^{-3} NO_3^--induced (closed symbols and solid lines) seedling roots of trembling aspen (a) and lodgepole pine (b) in the external concentration range of 0·005–0·5 mol m^{-3} NO_3^-. The lines were drawn using a non-linear curve-fitting model describing a hyperbola (Michaelis–Menten kinetics). In non-induced lodgepole pine (b, dotted line), HATS saturated at < 0·05 mol m^{-3} NO_3^-; thus, the line representing the HATS is based on data from 0·005–0·05 mol m^{-3} NO_3^-; however, the points above 0·05 mol m^{-3} are also shown.

occurred via their respective LATS. The latter showed no indication of saturation, being linearly dependent on $[N]_o$ up to 40 mol m^{-3} $[N]_o$ (Figs 3 & 4). However, both HATS and LATS showed substantial quantitative differences between the two species, between the two ions and between N-pre-treated and untreated plants, as discussed below.

HATS for NO_3^- and NH_4^+ uptake

Consistent with the data for other species (Glass & Siddiqi 1995 for review), NO_3^- influx into the roots of trembling aspen and lodgepole pine by HATS was inducible. In plants grown at 0·1 mol m^{-3} NO_3^- (induced), V_{max} values for NO_3^- influxes were 8-fold (pine) to 10-fold higher (aspen) than those of non-induced plants, i.e. plants grown without NO_3^- (Fig. 1, Table 1). Fluxes measured in non-induced plants at low external $[NO_3^-]$ result from the activities of constitutive high-affinity transport system (CHATS; Aslam *et al.* 1992; King *et al.* 1992). It has been argued that CHATS is genetically distinct from the inducible high-affinity transport system (IHATS), and their simultaneous existence in induced barley and white spruce seedlings has been demonstrated (Aslam *et al.* 1992; Kronzucker *et al.* 1995b; Glass & Siddiqi 1995). Indeed, Wang & Crawford (1996) have identified a mutant of *Arabidopsis thaliana* (*chl8*) that appears to be defective in this CHATS activity

In agreement with our earlier results (Min *et al.* 1998, 1999), at low $[NO_3^-]_o$, aspen seedlings showed a much higher capacity for NO_3^- uptake than the pine seedlings: V_{max} for influxes in aspen were approximately 9-fold (IHATS) and approximately 7-fold (CHATS) higher than pine in induced and non-induced plants, respectively. Further, the K_m for NO_3^- influx was approximately 13-fold higher in pine than in aspen. This implies that both at high and low $[NO_3^-]_o$, aspen would absorb NO_3^- by HATS activ-

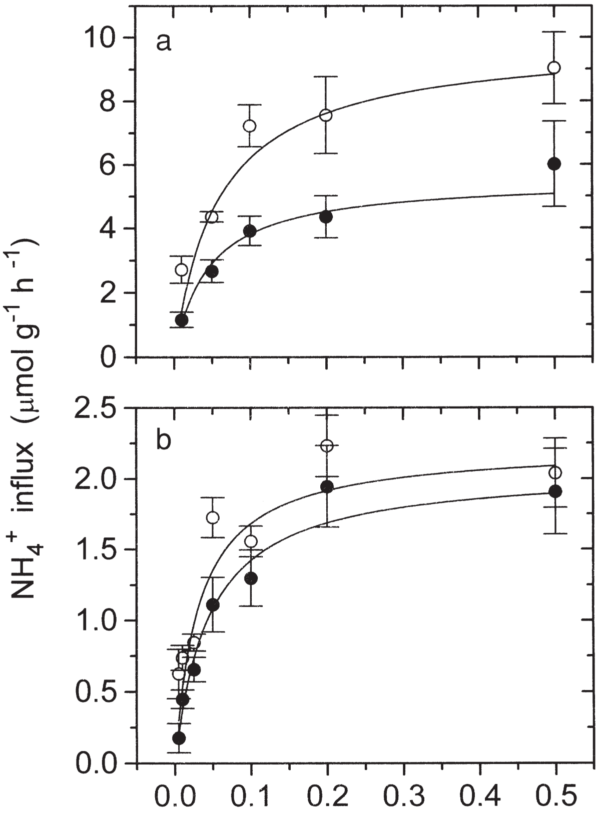

Figure 2. Ammonium influx (means ± SE, n = 8–12) into untreated (open symbols and dashed lines) or 0·1 mol m^{-3} NH_4^+-pre-treated (closed symbols and solid lines) seedling roots of trembling aspen (a) and lodgepole pine (b) in the external concentration range of 0·005–0·5 NH_4^+ (high-affinity transport system, HATS). The lines were drawn using a non-linear curve-fitting model describing a hyperbola (Michaelis–Menten kinetics).

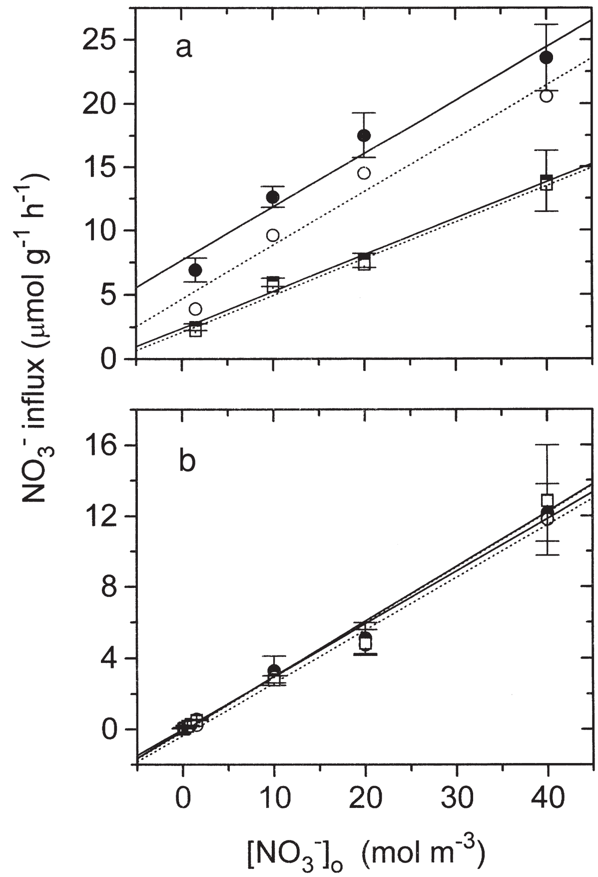

Figure 3. Nitrate influx (means ± SE, n = 8–12) into non-induced (squares) or 0·1 mol m^{-3} NO$_3^-$-induced (circles) seedling roots of trembling aspen (a) and lodgepole pine (b) in the external concentration range of 1·5–40 mol m^{-3} NO$_3^-$ (low-affinity transport system, LATS). Fluxes were plotted before subtracting (closed symbols and solid lines) and after subtracting V_{max} for the respective high-affinity system (open symbols and dotted lines). The lines represent linear regressions of the data. See Table 2 for significance of differences between slopes and intercepts.

ity more effectively than pine. Furthermore, the two species differed with respect to the concentration ranges of their respective HATS for NO$_3^-$ influx. In both induced and non-induced aspen seedlings, NO$_3^-$ influx was saturable up to 0·2 mol m^{-3} [NO$_3^-$]$_o$ and LATS was evident at ≥ 0·5 mol m^{-3} [NO$_3^-$]$_o$ (Fig. 1a). In non-induced pine, on the other hand, influx was saturable only up to 0·05 mol m^{-3} [NO$_3^-$]$_o$ and a linear system was evident at approximately 0·05 mol m^{-3} [NO$_3^-$]$_o$ (Fig. 1b). In non-induced barley seedlings also, a linear LATS was evident at low [NO$_3^-$]$_o$ (Siddiqi *et al.* 1990). In aspen, exposure to NO$_3^-$ caused the K_m for NO$_3^-$ influx to increase approximately 3-fold (Table 1). However, this decrease of affinity is more than compensated by the increase of V_{max}. In pine, the decrease of affinity associated with induction was much larger (approximately 26-fold) probably having a significant impact upon influx. Aslam *et al.* (1992) and Kronzucker *et al.* (1995b) also reported an increase in K_m of a similar magnitude following induction in barley and white spruce, respectively. An examination of Fig. 1(b) suggests, however, that the observed V_{max} and K_m values for NO$_3^-$ influx by HATS in the induced pine seedlings may have been overestimated, because of the relatively high contribution to measured fluxes from the LATS. This presumes that influx by LATS was similar in induced and non-induced plants (Fig. 3b; see also Siddiqi *et al.* 1990). By virtue of a high LATS contribution to total NO$_3^-$ uptake, the difference between induced and non-induced plants is less in pine (Fig. 1b). Indeed, this may account for the fact that average tissue [NO$_3^-$] in induced and non-induced pine seedlings were similar (Min *et al.* 1998) and [NO$_3^-$]$_{cyt}$ in 0·1 mol m^{-3} NO$_3^-$-grown plants was also very low (Min *et al.* 1999).

In contrast to NO$_3^-$ influx, NH$_4^+$ influx was not inducible (Fig. 2, Table 1). Whereas in pine both V_{max} and K_m of HATS for NH$_4^+$ influx were similar between NH$_4^+$-

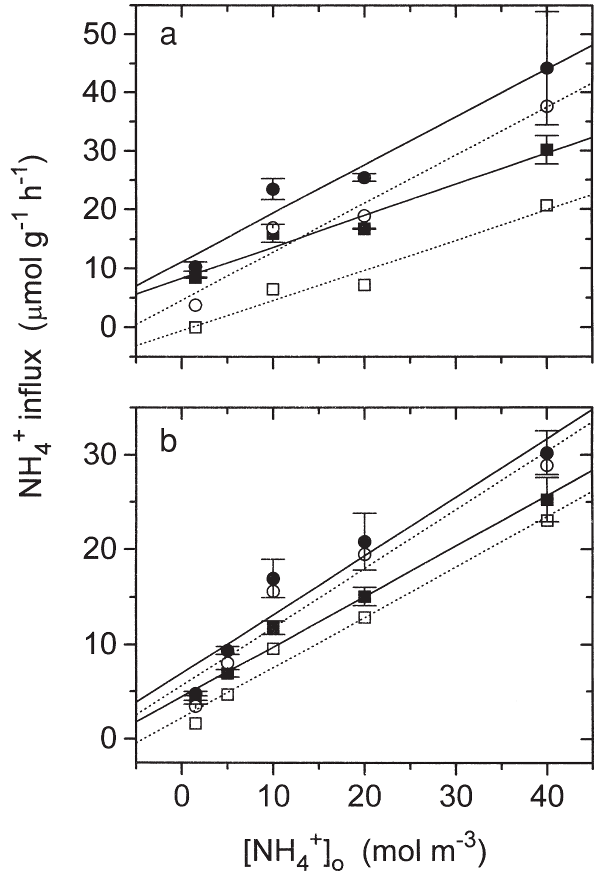

Figure 4. Ammonium influx (means ± SE, n = 8–12) into untreated (squares) or 0·1 mol m^{-3} NH$_4^+$-pre-treated (circles) seedling roots of trembling aspen (a) and lodgepole pine (b) in the external concentration range of 1·5–40 mol m^{-3} NH$_4^+$ (low-affinity transport system, LATS). Fluxes were plotted before subtracting (closed symbols and solid lines) and after subtracting V_{max} for the respective high-affinity system (open symbols and dotted lines). The lines represent linear regressions of the data. See Table 2 for significance of differences between slopes and intercepts.

Table 1. Kinetic parameters (means ± SD, $n = 3$) of high-affinity NO_3^- and NH_4^+ transport systems (HATS) of trembling aspen and lodgepole pine grown without or with 0·1 mol m^{-3} NO_3^- or NH_4^+ (see text)

	V_{max} (μmol g^{-1} h^{-1})		K_m (mmol m^{-3})	
	Minus N	Plus N	Minus N	Plus N
Nitrate				
Aspen	0·305 ± 0·013	3·00 ± 0·10	3·43 ± 1·14	11·69 ± 1·90
Pine	0·042 ± 0·003	0·354 ± 0·080	5·91 ± 1·37	153·4 ± 59·2
Ammonium				
Aspen	9·88 ± 1·27	5·53 ± 0·86	59·5 ± 15·1	45·1 ± 17·5
Pine	2·22 ± 0·18	2·07 ± 0·25	32·1 ± 6·6	45·4 ± 10·5

V_{max} and K_m were determined by a non-linear curve-fitting model describing a hyperbola (Michaelis–Menten kinetics).

pre-treated and untreated plants (Fig. 2b, Table 1), in aspen NH_4^+ pre-treatment reduced (down-regulated) V_{max} to approximately 50% (Glass & Siddiqi 1995) but had little effect on K_m (Fig. 2a, Table 1). This down-regulation of NH_4^+ influx has been demonstrated to result from effects of root glutamine, acting upon *AMT1* transcript level in *Arabidopsis thaliana* (Rawat *et al*. 1999). Consistent with our earlier results (Min *et al*. 1999), aspen showed a higher capacity for NH_4^+ uptake than pine, but the differences (2·7-fold between N-treated plants) were not as large as the corresponding differences in NO_3^- influx by HATS (≥ 10-fold).

Clearly, at low $[NO_3^-]_o$ (HATS range), aspen has a high capacity for both NO_3^- and NH_4^+ uptake (V_{max} 3·0 and 5·53 μmol g^{-1} h^{-1}, respectively). By contrast, pine appears to have a much lower capacity for NO_3^- uptake than NH_4^+ uptake (V_{max} 0·354 and 2·07 μmol g^{-1} h^{-1}, receptively). Indeed, in pine, the differences between NO_3^- uptake and NH_4^+ uptake increased with decreasing $[N]_o$; the K_m for NO_3^- uptake was approximately 3-fold higher than that for NH_4^+ (Table 1). Several other coniferous species show the same preference for NH_4^+ over NO_3^- (e.g. Boxman & Roelofs 1988; Marschner, Häussling & George 1991; Kronzucker *et al*. 1997). In aspen, by contrast, the K_m for NH_4^+ uptake was approximately 4-fold higher than that for NO_3^- uptake. We have suggested that, in pine, unlike aspen, NO_3^- uptake from 0·1 mol m^{-3} $[NO_3^-]_o$ was not adequate to maintain a $[NO_3^-]_{cyt}$ that is optimal for metabolism (Min *et al*. 1999). By comparison, $[NH_4^+]_{cyt}$ at 0·1 mol m^{-3} $[NH_4^+]_o$ were similar between the two species (Min *et al*. 1999). The same study established that at higher $[NO_3^-]_o$ (1·5 mol m^{-3}) fluxes and $[NO_3^-]_{cyt}$ of the two species were similar (Min *et al*. 1999).

LATS for NO_3^- and NH_4^+ uptake

In aspen, NO_3^- influx by the LATS was higher in induced than non-induced seedlings even after subtracting the V_{max} for HATS (Fig. 3a). By contrast, in pine, NO_3^- pre-treatment had no effect on influx by LATS (Fig. 3b). The latter pattern conforms to that observed in several other species where LATS for NO_3^- has been shown to be constitutive, e.g. barley (Siddiqi *et al*. 1990; Glass *et al*. 1990) and white spruce (Kronzucker *et al*. 1995b). In the cases of barley and white spruce, NO_3^- influx by LATS (after subtracting the V_{max} for HATS) was higher in non-induced plants than plants grown with NO_3^-, indicating some down-regulation of LATS by accumulated N. Likewise, Touraine & Glass (1997) demonstrated that LATS activity in roots of *Arabidopsis thaliana* was partially down-regulated by growth on NH_4NO_3 but not by growth on KNO_3. In contrast to the apparent constitutive nature of LATS at the physiological level, Tsay *et al*. (1993) demonstrated that the *NRT1* gene (formerly *CHL1*), considered to encode the LATS in this species, was inducible by exposure to NO_3^-. Notwithstanding the apparent (but relatively small) increase of LATS activity associated with provision of NO_3^- in aspen, it is unclear whether this represents a real 'induction' phenomenon or simply an expression of increased growth arising from improved N supply.

Interestingly, while the HATS capacity of pine for NO_3^- was low (see above), the capacity of its LATS for NO_3^- uptake was substantial (Fig. 3). Although NO_3^- influxes by LATS were still higher in aspen than pine (Fig. 3a,b; Table 2), the differences were only approximately 2-fold or less compared to ≥ 10-fold differences for the HATS. This high LATS activity in pine may represent an important adaptation for colonizing sites which have high soil $[NO_3^-]$.

In the case of NH_4^+, both species showed higher LATS fluxes in NH_4^+-pre-treated than untreated plants, although the differences in pine were relatively small (Fig. 4a,b). This is consistent with the results reported for rice (Wang *et al*. 1993b) and *Arabidopsis* (Rawat *et al*. 1999). Lower fluxes in untreated plants in the rice studies were attributed to an N-deficiency (Wang *et al*. 1993b). In the study by Kronzucker *et al*. (1996), however, fluxes by LATS of untreated white spruce seedlings were higher than those of NH_4^+-pre-treated seedlings. It may be that in the latter, N-deficiency was not as severe as in the relatively fast-growing aspen.

As noted earlier, we have characterized the kinetics of NO_3^- and NH_4^+ uptake in aspen and lodgepole pine seedling roots without any confounding effects of mycorrhizae. We emphasize that the lack of mycorrhization does not detract from the ecological significance of the present

Table 2. Comparison of slopes and intercepts for LATS in Figs 3 & 4

	Nitrate		Ammonium	
Comparison	Slope	*y* intercept	Slope	*y* intercept
LATS (+ HATS)				
Pine: –N versus +N	NS	NS	NS	*
Aspen: –N versus +N	*	***	NS	*
–N: pine versus aspen	NS	***	NS	**
+N: pine versus aspen	NS	***	NS	**
LATS (– HATS)				
Pine: –N versus +N		NS		**
Aspen: –N versus +N		**		**
–N: pine versus aspen		**		NS
+N: pine versus aspen		**		NS

t tests were performed for pair-comparisons and levels of significance for the differences are given here (NS, not significant; $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$). For LATS (– HATS), $V_{\max}$ values for HATS were subtracted from the observed influx values in the LATS region (i.e. LATS + HATS); slope values therefore remain unchanged.

study for the following reasons. (a) NO_3^- uptake is not affected significantly by the presence of mycorrhizae (Rygiewicz *et al*. 1984; Plassard *et al*. 1993) and therefore their presence is not likely to improve the NO_3^- acquisition capacity of lodgepole pine. (b) In the case of NH_4^+, roots of both species show relatively high capacities for NH_4^+ uptake even from low external concentrations although the presence of mycorrhizae may further enhance NH_4^+ uptake capacity. In fact, in the case of NH_4^+, its exclusion rather than its enhanced abstraction may be ecologically more important, especially in the light of potential toxicity of the ion (Kronzucker *et al*. 1995d, 1996; Gerendas *et al*. 1997; Min *et al*. 1999). Moreover, mycorrhization is inhibited with increasing nutrient status of soil including that of NH_4^+ (e.g. Haug 1990; Marschner *et al*. 1991; de Graaf *et al*. 1998 and references therein).

It should be pointed out that the $[NO_3^-]_o$ and $[NH_4^+]_o$ we have used for kinetic determinations in the present study, as well as in our recently published studies (Kronzucker *et al.* 1995a, b,c,d; 1996, 1997; Min *et al*. 1998, 1999), are ecologically relevant. In temperate and boreal forest ecosystems, exchangeable soil NH_4^+–N concentrations range from 1·5–100 mg kg^{-1} (e.g. Vitousek *et al*. 1982); free NH_4^+, which may vary with soil type, is estimated to be approximately 10% of the exchangeable value, translating into approximately 0·054–3·5 mol m^{-3} (based on an average 20% water content). These estimates agree well with the few published values for free NH_4^+ concentrations in forest soil solutions (e.g. Vogt & Edmonds 1982; Gijsman & De Willigen 1991). Nitrate-N concentrations generally range from undetectable levels to approximately 12 mg kg^{-1} soil (e.g. Vitousek *et al*. 1982). In contrast to NH_4^+, however, NO_3^- is not bound and therefore these figures closely approximate the free NO_3^- concentrations. Generally, in late-successional coniferous forests, soil N is dominated by NH_4^+, while NO_3^- may be extremely low (e.g. Lodhi & Killingbeck 1980; Kronzucker *et al*. 1997 and references therein). Following disturbance, soil $[NH_4^+]$ and $[NO_3^-]$ may vary from low micromolar to several millimolar (Vitousek *et al*. 1982; Prescott 1997), depending on a host of environmental and biotic factors, even among clear-cut sites. However, it is well established that during the first few years after clear-cut, when seedling establishment takes place, rates of nitrification increase and NH_4^+/NO_3^- ratios decline (Lodhi & Killingbeck 1980; Vitousek *et al*. 1982; Gijsman & De Willigen 1991; Truax *et al*. 1994). For example, in a clear-cut, Truax *et al*. (1994) found soil $[NH_4^+]$ and $[NO_3^-]$ to be approximately 0·05 and 1·3 mol m^{-3}, respectively, yielding an NH_4^+/NO_3^- ratio of 0·038. Where total inorganic N is low, it is likely that $[NO_3^-]$ may be in the micromolar range, and $[NH_4^+]$ may be almost negligible (see e.g. Vitousek *et al*. 1982). In the case of fire disturbance, however, both $[NO_3^-]$ and $[NH_4^+]$ may increase; but rates of nitrification also increase and NH_4^+/NO_3^- ratios still decline (e.g. Raison 1979).

In summary, aspen represents a generalist species with respect to soil N, equally capable of thriving on low and high NO_3^- or NH_4^+. By contrast, kinetic parameters for N acquisition in lodgepole pine identify it as a specialist species particularly well adapted to high soil N concentrations characteristic of forest soils after fire (Raison 1979; Klinka *et al*. 1990; Brayshaw 1996), and at some such sites it may persist as an edaphic climax species (e.g. Pfister & Daubenmire 1975, Lotan & Critchfield 1990). The exclusion of species such as aspen and lodgepole pine from late-successional stands where soils are dominated by NH_4^+ (e.g. Kronzucker *et al*. 1997) may possibly be related to toxicity associated with high soil $[NH_4^+]$ (Givan 1979; Magalhaes *et al*. 1992) and/or other environmental factors that remain to be resolved.

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