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A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species

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ABSTRACT

$^{13}NO_3^-$ and $^{13}NH_4^+$ compartmental analyses were carried out in seedling roots of trembling aspen (*Populus tremuloides* Michx.), lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) and interior Douglas-fir (*Pseudotsuga menziesii* var. glauca [Beissn.] Franco) at 0·1 and 1·5 mol m^{-3} external NO_3^- or NH_4^+ concentrations ($[NO_3^-]_o$ or $[NH_4^+]_o$, respectively). At the lower $[NO_3^-]_o$, the capacities and efficiencies of acquisition and accumulation of NO_3^-, based upon NO_3^- fluxes and cytoplasmic NO_3^- concentrations ($[NO_3^-]_c$), were in the order aspen >> Douglas-fir > pine. At 1·5 mol m^{-3} $[NO_3^-]_o$, the NO_3^- influx increased 18-fold in pine, four-fold in Douglas-fir and approximately 1·4-fold in aspen; in fact, at 1·5 mol m^{-3} $[NO_3^-]_o$, the NO_3^- influx in pine was higher than in aspen. However, at high $[NO_3^-]_o$, efflux also increased in the two conifers to a much greater extent than in aspen. In aspen, at both $[NO_3^-]_o$, approximately 30% of the ^{13}N absorbed was translocated to the shoot during 57 min of ^{13}N loading and elution, compared with less than 10% in the conifers. At 0·1 mol m^{-3} $[NH_4^+]_o$, influx and net flux were in the order: aspen > pine > Douglas-fir but the differences were much less than in NO_3^- fluxes. At 1·5 mol m^{-3} $[NH_4^+]_o$, NH_4^+ influx, efflux and $[NH_4^+]_c$ greatly increased in aspen and Douglas-fir and, to a much lesser extent, in pine. In aspen, 29 and 12% of the ^{13}N absorbed was translocated to the shoot at 0·1 and 1·5 mol m^{-3} $[NH_4^+]_o$, respectively, compared with 5 to 7% in the conifers at either $[NH_4^+]_o$. These patterns of nitrogen (N) uptake, particularly in the case of NO_3^-, and the observed concentration responses of NO_3^- uptake, reflect the availability of N in the ecological niches, to which these species are adapted.

Key-words: ammonium fluxes; compartmental analysis; cytoplasmic ammonium; cytoplasmic nitrate; Douglas-fir; lodgepole pine; nitrate fluxes; nitrogen nutrition; trembling aspen.

INTRODUCTION

Plant species show marked genetic differences in their ability to absorb and utilize particular inorganic nutrients for growth (Glass 1989). In the cases of N sources, for example NO_3^- or NH_4^+, these differences appear to have arisen as adaptations to the availability of particular N forms in particular habitats (Kronzucker, Siddiqi & Glass 1997). It is therefore no coincidence that their distribution in space and time reflects these N-form preferences and the temporal changes of the predominant N forms which occur in particular ecosystems (e.g. Bledsoe & Rygiewicz 1986; Chapin, Moilanen & Kielland 1993; Kielland 1994; Kronzucker *et al*. 1997; Min *et al*. 1998). In temperate and boreal forest ecosystems, the soils under late-successional communities are typically high in NH_4^+ and low in NO_3^- (Van Cleve *et al*. 1983; Chapin, Van Cleve & Tyron 1986; Stark & Hart 1997; see also Min *et al*. 1998). Following disturbance, the soil $[NO_3^-]$ generally increases (e.g. Likens, Bormann & Johnson 1969; Lodhi 1978; Walley, Van Kessel & Pinnock 1996; Prescott 1997; see also Min *et al*. 1998); in some cases soil $[NH_4^+]$ may also increase (e.g. Schmidt, MacDonald & Rothwell 1996). It has been argued that appropriate physiological adaptations to efficiently acquire and utilize NH_4^+ or NO_3^- are key factors in the success of a particular species in a seral sequence (Kronzucker *et al*. 1997). For example, white spruce, a late-successional conifer, demonstrated low rates of NO_3^- uptake and accumulation, and a clear preference for NH_4^+ over NO_3^- (Kronzucker, Glass & Siddiqi 1995a; Kronzucker, Siddiqi & Glass 1995b, c, d, e, 1996, 1997). By contrast, trembling aspen, an early-successional broad-leaved species, exhibited high rates of NO_3^- uptake at low (0·1 mol m^{-3}) and high (1·5 mol m^{-3}) external $[NO_3^-]$ ($[NO_3^-]_o$) (Min *et al*. 1998). However, lodgepole pine, an early-successional conifer that is particularly adapted to colonizing forest sites following fires, showed high rates of NO_3^- uptake only at high (1·5 mol m^{-3}) $[NO_3^-]_o$ (Min *et al*. 1998).

The activities of the enzymes responsible for immediate metabolism of NO_3^- and NH_4^+, that is nitrate reductase (NR) and glutamine synthetase (GS), respectively, are dependent upon the concentrations of their respective substrates (Oaks 1994). On the basis of the cytoplasmic localization of NR and GS in root tissues, NO_3^- and/or NH_4^+ concentrations in the cytoplasm are extremely important for the activities of these enzymes. In the case of plastidic GS, it is likely that $[NH_4^+]$ in the plastids is dependent upon cytoplasmic $[NH_4^+]$ ($[NH_4^+]_c$). The primary aim of the present study was to investigate interspecific differ-

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ences in component fluxes and compartmentation of NO_3^- and NH_4^+ at the subcellular level in trembling aspen, a broad-leaved early-successional species and two early-successional conifers, lodgepole pine and Douglas-fir, under well-defined nutrient regimes. The plants were therefore grown hydroponically and were devoid of any mycorrhizal infections (Min *et al*. 1998; see also Kronzucker *et al*. 1997).

MATERIALS AND METHODS

Plant culture

Seeds of trembling aspen (*Populus tremuloides* Michx., seedlot 42307), lodgepole pine (*Pinus contorta* Dougl. ex Loud. var *latifolia* Engelm., seedlot 3847) and interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco, seedlot 33124) were obtained from the Tree Seed Center, the Ministry of Forests, Surrey, B.C., Canada. Douglas-fir seeds were stratified at 4 °C for 3 weeks prior to germination. Seeds of the other species did not require stratification. Seeds were germinated in styrofoam blocks in a 2 : 1 (v/v) peat/perlite mixture also containing 4·3 kg m^{-3} dolomite. Seedlings were maintained in the peat/perlite mixture for approximately 3 months and then transferred to hydroponic tanks (8 L capacity) containing aerated, modified 0·1 strength Johnson's solution with NO_3^- or NH_4^+ provided as $Ca(NO_3)_2$ and $(NH_4)_2SO_4$, respectively (Min *et al*. 1998). Solutions were buffered at pH 6·4–6·8 by adding excess powdered $CaCO_3$. The plants were maintained in a controlled environment room at 20 ± 2 °C, 70% relative humidity and 16 h light : 8 h dark photoperiod. 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 Corporation, Fairfield, NJ, USA).

NO_3^- and NH_4^+ pretreatments

In NO_3^- experiments, 0·1 or 1·5 mol m^{-3} NO_3^- was provided during the entire hydroponic culture period of 20 d. In NH_4^+ experiments, the plants were grown in low NO_3^- (< 25 mmol m^{-3}) for 15 d and then transferred to 0·1 or 1·5 mol m^{-3} NH_4^+ 5 d prior to experiments. Ammonium was omitted from the culture solution for the first 15 d because an appreciable amount of NO_2^- was detected in the culture solution when NH_4^+ was present for 10 d or longer, presumably due to the activity of nitrifying micro-organisms carried over from the peat/perlite growth medium. A time-course study showed that NH_4^+ fluxes had reached a steady value after 4 to 5 d of NH_4^+ pre-treatment (our unpublished results, see also Kronzucker *et al*. 1996). Thus, a 5 d NH_4^+ pre-treatment offered a means of avoiding the accumulation of NO_2^- without sacrificing apparent steady-state conditions with respect to N.

Compartmental (efflux) analysis

The procedure for efflux analysis employed in this study was essentially as described by Siddiqi, Glass & Ruth (1991) and Kronzucker *et al*. (1995a). In brief, intact seedling roots were exposed to $^{13}NO_3^-$-labelled or $^{13}NH_4^+$-labelled solutions for 35 min (NO_3^--experiments) or 45 min (NH_4^+-experiments). These exposures brought the specific activities of cytoplasmic compartments (S_c) to > 90% of external solution for both $^{13}NO_3^-$and $^{13}NH_4^+$, based upon preliminary estimates of the half-lives of exchange for these ions (see also Tables 1 and 3). Therefore, all calculations were based upon corrected values of S_c. After loading, the seedlings were removed from the radioactive solutions, held upright for 10 s to drain excess radiolabelled surface solution and then transferred to a funnel fitted with a clamped drainage tube. The roots were then subjected to 24 successive elutions (for periods ranging from 5 s to 2 min over a total of 22 min) with either 20 or 60 cm^3 aliquots (depending on root mass) of an identical but non-radioactive solution. During elution, the solution bathing the roots was well aerated and mixed by bubbling with compressed air. Subsequently, roots were excised from shoots and their fresh weights determined. Radioactivities (^{13}N counts per min) of wash solutions as well as those of roots and shoots were measured in a γ-counter (Packard, Minaxi δ, Auto-γ 5000 series Canberra Packard Canada, Mississauga, ON, Canada).

Treatment of the data

Semi-logarithmic plots of the rate of release of $^{13}NO_3^-$ or $^{13}NH_4^+$ (log cpm min^{-1}) versus time of elution were constructed. The data were analysed by a microcomputer-based method to objectively determine the break-points for

Species	$[NO_3^-]_o$ (mM)	Compartment I $t_{0.5}$ (s)	Compartment II $t_{0.5}$ (s)	Compartment III $t_{0.5}$ (min)
Trembling aspen	0·1	3·07 ± 0·80	29·57 ± 6·18	9·94 ± 0·21
	1·5	3·10 ± 0·96	27·30 ± 7·27	9·96 ± 1·01
Lodgepole pine	0·1	3·62 ± 0·31	32·80 ± 6·20	7·05 ± 0·27
	1·5	4·92 ± 0·23	42·07 ± 1·93	7·28 ± 0·32
Douglas-fir	0·1	4·12 ± 0·24	29·72 ± 2·18	8·11 ± 0·38
	1·5	2·03 ± 0·14	38·14 ± 0·66	6·85 ± 0·25

Table 1. Half-lives of exchange ($t_{0.5}$) for NO_3^- of compartment I (surface film), II (apparent free space), and III (cytoplasm) at 0·1 and 1·5 mol m^{-3} $[NO_3^-]_o$ in the roots of trembling aspen, lodgepole pine and Douglas-fir (mean ± SE, n = 3–5)

linear regressions (Rygiewicz, Bledsoe & Glass 1984). Various fluxes, the half-time of exchange for cytoplasm and apparent free space (AFS), and concentrations of NO_3^- and NH_4^+ in these compartments were estimated according to Lee & Clarkson (1986) and Siddiqi *et al*. (1991). The symbols used for various parameters are:

ϕ_{oc}, the influx from external solution into cytoplasm;
ϕ_{co}, the efflux from cytoplasm to external solution;
ϕ_{net}, the net flux from external solution to cytoplasm.
ϕ_{net} consists of flux to metabolism (ϕ_{met}), net flux to vacuole (ϕ_{vac}) and flux to xylem (ϕ_{cx}); the former two cannot be separated by the current procedure and are expressed as $\phi_{met+vac}$ (Siddiqi *et al*. 1991; Kronzucker *et al*. 1995a). Since ^{13}N metabolites may also be translocated to the shoot during the experimental period, we have denoted ϕ_{cx} as ϕ_x* to identify the latter as flux of N (NO_3^- or NH_4^+ plus N metabolites).
$[NO_3^-]$ or $[NH_4^+]$: NO_3^- or NH_4^+ concentration with subscripts c = cytoplasm, AFS, apparent free space, i = inside, o = outside.
$t_{0\cdot5}$, half-time of exchange.
S, the specific activity of the isotope (cpm μmol^{-1}), with subscripts o = external solution, c = cytoplasm, v = vacuole.

In the estimation of various parameters, the following assumptions have been made: (i) as roots were exposed to the radioactive solution for four to five $t_{0\cdot5}$ of cytoplasm, S_c closely approximates S_o at the end of the loading periods; (ii) as the time of exposure to the isotope is very short in relation to $t_{0\cdot5}$ of the vacuole (e.g. 16 h or longer for NO_3^-, see Belton, Lee & Ratcliffe 1985; Lee & Clarkson 1986), S_v must have been extremely low and ^{13}N released from the vacuole during elution can therefore be considered negligible; (iii) cytoplasmic and AFS volumes were taken as 0·05 and 0·15 cm^3 g^{-1} root fresh weight, respectively (Lee & Ratcliffe 1983; Lee & Clarkson 1986; Siddiqi *et al*. 1991; Kronzucker *et al*. 1995a, 1998).

The results are presented as means ± standard errors. In addition, *t*-tests, analyses of variances (ANOVA) and Duncan's multiple range tests were performed. The differences between treatments described as significant are those where probability (*P*) was < 0·05.

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

The ^{13}N 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, B.C. Canada. The protocol for removal of radiocontaminants was essentially the same as described by Kronzucker *et al*. (1995a), except that 5 cm^3 of 100 mmol 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.

The $^{13}NH_4^+$ was obtained by reduction of $^{13}NO_3^-$ using Devarda's alloy (Wang *et al*. 1993). In brief, $^{13}NO_3^-$ solution was introduced into a round-bottom flask containing approximately 10 g of Devarda's alloy (50% Cu, 45% Al, 5% Zn). The flask was connected to an Erlenmeyer flask containing approximately 50 cm^3 of trapping solution, which was identical to the loading solution except that it was acidified by adding 0·125 cm^3 of 2N H_2SO_4 (pH 1–2). The $^{13}NO_3^-$ reduction to $^{13}NH_3$ was initiated by adding 20 cm^3 of 1N NaOH into the round-bottom flask, maintained in a water bath at 75 °C. Gaseous $^{13}NH_3$ was trapped as $^{13}NH_4^+$ in the acidic solution in the Erlenmeyer flask, which was connected to a vacuum pump. When $^{13}NH_4^+$ trapping was complete, pH was restored to neutral by adding 2N KOH.

RESULTS

$^{13}NO_3^-$ efflux analyses

Log_{10} of rates of release of $^{13}NO_3^-$ [log_{10} cpm min^{-1} g^{-1} root fresh weight (FW)] was plotted versus time for trembling aspen, lodgepole pine and Douglas-fir seedlings pretreated with 0·1 mol m^{-3} or 1·5 mol m^{-3} NO_3^- for 20 d. Figure 1 shows a representative plot for trembling aspen. In all the species, and at both NO_3^- concentrations, three distinct compartments with markedly different $t_{0\cdot5}$-values were recognized (Table 1). These compartments were assigned to superficial solution (compartment I), AFS (compartment II) and cytoplasm (compartment III), respectively, based on earlier findings (Siddiqi *et al*. 1991; Kronzucker *et al*. 1995c; see Discussion). The $t_{0\cdot5}$ for cytoplasm was longer in trembling aspen (≈ 10 min) than in lodgepole pine and Douglas-fir (≈ 7–8 min). Nitrate concentration of the media (0·1 or 1·5 mol m^{-3}) had no significant effect on the $t_{0\cdot5}$ of cytoplasmic exchange in trembling aspen and lodgepole pine and only a slight effect in Douglas-fir (Table 1). By contrast, although the $t_{0\cdot5}$ of exchange for AFS (30–33 s) was similar among all species at low $[NO_3^-]_o$, at higher $[NO_3^-]_o$ $t_{0\cdot5}$-values for the two conifers (38–42 s) increased significantly compared with trembling aspen (27 s). The $t_{0\cdot5}$ for superficial solution

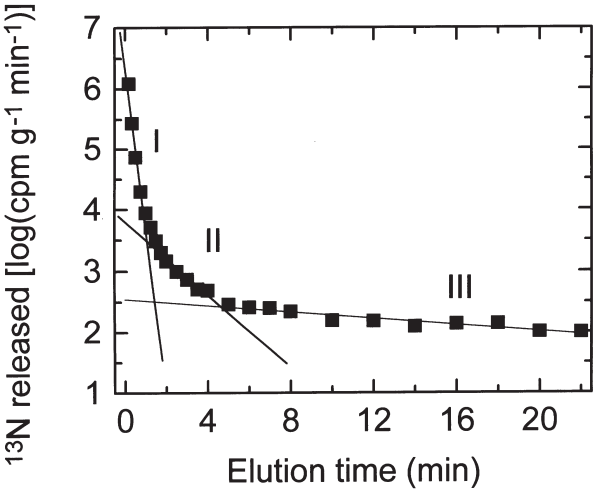

Figure 1. Representative semi-logarithmic plot of the rate of release of ^{13}N [log (cpm released) g^{-1} min^{-1}] versus time of elution for intact roots of trembling aspen at 1·5 mol m^{-3} NO_3^-. Three distinct compartments are indicated by Roman numerals.

showed only minor differences among the species and between NO_3^- treatments.

At 0·1 mol m^{-3} NO_3^-, influx of NO_3^- into the roots of trembling aspen was four-fold higher than Douglas-fir and eight-fold higher than lodgepole pine (Fig. 2). By contrast, the efflux as percentage of influx was in the order: lodgepole pine > Douglas-fir > trembling aspen, with the result that, compared with influx, the net flux in trembling aspen was even higher (approximately five-fold and 12-fold, respectively) than in Douglas-fir and lodgepole pine (Fig. 2). When $[NO_3^-]_o$ was increased from 0·1 to 1·5 mol m^{-3}, the influx in Douglas-fir and lodgepole pine increased approximately three-fold and 18-fold, respectively. At the same time, however, the efflux also increased approximately 31-fold in lodgepole pine and approximately 10-fold in Douglas-fir at the higher $[NO_3^-]_o$. By contrast, in trembling aspen, increases in influx and efflux, in going from 0·1 to 1·5 mol m^{-3} $[NO_3^-]_o$, were relatively small. Thus, at 1·5 mol m^{-3} $[NO_3^-]_o$, although NO_3^- influxes in Douglas-fir and lodgepole pine were similar to or even slightly higher than trembling aspen, the net fluxes were still significantly lower in the former two species than in trembling aspen (Fig. 2).

At 0·1 mol m^{-3} $[NO_3^-]_o$, the $[NO_3^-]_c$ of trembling aspen was significantly higher (11-fold and five-fold, respectively), than in lodgepole pine and Douglas-fir (Fig. 3). When $[NO_3^-]_o$ was increased to 1·5 mol m^{-3}, the $[NO_3^-]_c$ increased 18-fold, three-fold and 1·3-fold, respectively, in lodgepole pine, Douglas-fir and trembling aspen; all increases being statistically significant (Fig. 3). As a consequence, $[NO_3^-]_c$ of lodgepole pine and trembling aspen became similar, but approximately double that of Douglas-fir in plants grown at 1·5 mol m^{-3} $[NO_3^-]_o$. In all three species, $[NO_3^-]_{AFS}$ was close to the ambient $[NO_3^-]_o$ (Table 2).

In trembling aspen, approximately 30% of the ^{13}N absorbed was translocated to the shoot at both low and high $[NO_3^-]_o$ during the experimental period, consisting of 35 min of $^{13}NO_3^-$ labelling and 22 min of elution. In the two coniferous species, by contrast, only a very small amount of the absorbed isotope was translocated to the shoot during the same time period (Fig. 4).

NH_4^+ efflux analyses

NH_4^+ efflux analyses in trembling aspen, lodgepole pine and Douglas-fir were also carried out at 0·1 and 1·5 mol m^{-3} $[NH_4^+]_o$ under steady-state conditions. A representative plot of $\log_{10}$ ^{13}N cpm min^{-1} g^{-1} root FW versus time is presented in Fig. 5 for roots of trembling aspen. As in the case of NO_3^- efflux analyses, three distinct phases were recognized: compartment I (surface film), compartment II (AFS), and compartment III (cytoplasm), respectively (see Discussion). Generally, external $[NH_4^+]$ had only minor effects on estimates of $t_{0·5}$ of exchange for the various compartments (Table 3). Among the species, the $t_{0·5}$ of exchange for cytoplasmic NH_4^+ were not significantly different. The $t_{0·5}$ for AFS of trembling aspen was longer than those of lodgepole pine and Douglas-fir (Table 3). There were only minor differences in $t_{0·5}$ for compartment I among these species and between $[NH_4^+]_o$.

At 0·1 mol m^{-3} $[NH_4^+]_o$, trembling aspen had higher values of influx than lodgepole pine (1·6-fold) and Douglas-fir (three-fold). At the same time, efflux (as a percentage of influx) was substantially lower in aspen than in the conifer-

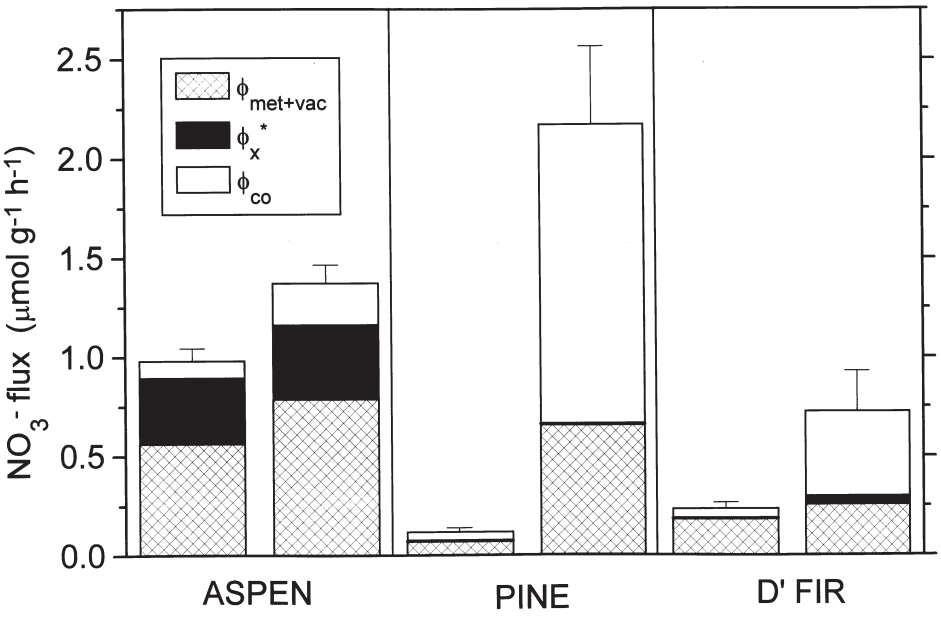

Figure 2. Influx (ϕ_{oc}), combined flux to metabolism and vacuole ($\phi_{met+vac}$), flux to xylem (ϕ_x*), and efflux (ϕ_{co}) (μmol g^{-1} h^{-1}) for NO_3^- at 0·1 mol m^{-3} (left) and 1·5 mol m^{-3} (right) $[NO_3^-]_o$ in trembling aspen, lodgepole pine and Douglas-fir as determined by efflux analysis. Standard errors (vertical lines above the means) are shown for influx only. Note: net flux (ϕ_{net}) = ($\phi_{met+vac}$ + ϕ_x*) and (ϕ_{oc}) = (ϕ_{net} + ϕ_{co}).

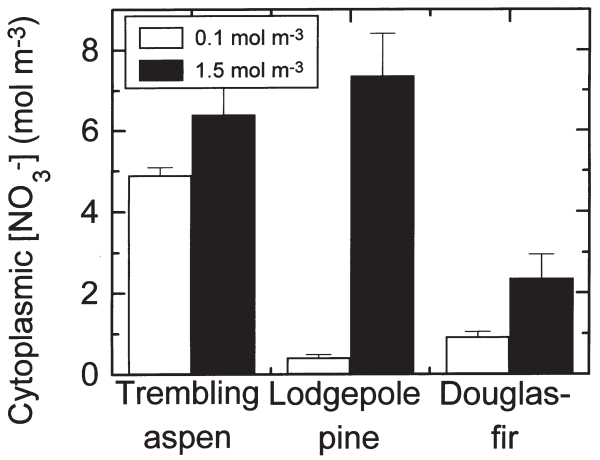

Figure 3. Cytoplasmic [NO_3^-] (mol m^{-3}) in the roots of trembling aspen, lodgepole pine and Douglas-fir at two external concentrations as estimated by compartmental analysis. Standard errors are shown as vertical lines above the means (bars).

ous species, resulting in even greater differences in the net fluxes among these species (Fig. 6). When $[NH_4^+]_o$ was increased to 1·5 mol m^{-3}, NH_4^+ influx increased approximately four-fold in lodgepole pine, approximately 10-fold in aspen and approximately 20-fold in Douglas-fir (Fig. 6). At this $[NH_4^+]_o$, the efflux relative to influx was high in all the species, ranging from 73% in lodgepole pine to 86% in Douglas-fir. Among the species, all fluxes were higher in trembling aspen than in the two conifers (Fig. 6).

At 0·1 mol m^{-3} $[NH_4^+]_o$, trembling aspen and lodgepole pine had similar $[NH_4^+]_c$ (12–13 mol m^{-3}), which were significantly higher than that of Douglas-fir (3·6 mol m^{-3}) (Fig. 7). When $[NH_4^+]_o$ was increased to 1·5 mol m^{-3}, $[NH_4^+]_c$ increased approximately 10-fold in trembling aspen, approximately four-fold in lodgepole pine and approximately 20-fold in Douglas-fir. $[NH_4^+]_{AFS}$ was six-fold to 13-fold higher than the ambient $[NH_4^+]$ in these species (Table 4).

In trembling aspen, substantial amounts of the absorbed ^{13}N were translocated to the shoot during 45 min of $^{13}NH_4^+$ loading followed by 22 min of elution, amounting to 29 and 12% of the respective influxes at 0·1 and 1·5 mol m^{-3} $[NH_4^+]_o$. In lodgepole pine and Douglas-fir the proportions of ^{13}N translocated to the shoot were 5 to 7% of influx at both $[NH_4^+]_o$ (Fig. 8).

DISCUSSION

Compartmental analyses of $^{13}NO_3^-$ and $^{13}NH_4^+$ at 0·1 and 1·5 mol m^{-3} $[N]_o$ revealed three distinct compartments in all species examined (Figs 1, 5). These phases, separated by very different $t_{0·5}$ of exchange (3–5 s, 30–60 s and 7–13 min, respectively), were recognized as: surface film (compartment I), AFS (compartment II) and cytoplasm (compartment III) (Tables 1, 3). These phases and their observed $t_{0·5}$-values in the present study agree well with those observed in white spruce (Kronzucker *et al*. 1995a, c, d, e) and in cereals such as barley (Deane-Drummond & Glass 1982; Lee & Clarkson 1986; Siddiqi *et al*. 1991), rice (Wang *et al*. 1993; Kronzucker *et al*. 1998), wheat (Devienne, Mary & Lamaze 1994a, b), and maize (Presland & McNaughton 1984). The latter studies made use of a variety of radiotracers, i.e. ^{13}N, ^{15}N and $^{36}ClO_3^-$. The identities of these compartments as surface film, AFS and cytoplasm have been confirmed by using pretreatments such as high temperature, sodium dodecylsulfate, H_2O_2, 2-chloro-ethanol, MSO, and α-KG (Siddiqi *et al*. 1991; Kronzucker *et al*. 1995c) and, perhaps most convincingly in the case of NH_4^+ exchange, by varying $[Ca^{2+}]_o$, $[H^+]_o$ within the physiological range as well as $[Al^{3+}]_o$ (Kronzucker *et al*. 1995e). Furthermore, using ion exchange resins, the studies cited above also established that the effluxing ^{13}N during the 22 min elution period was indeed in the form of $^{13}NO_3^-$ or $^{13}NH_4^+$ (≈ 99% purity).

Surface film and apparent free space (AFS) (compartments I and II)

Only small differences in the $t_{0·5}$-values for compartment I (considered to represent the surface film) were observed between NO_3^- and NH_4^+ treatments, or between concen-

Table 2. NO_3^- concentration (mol m^{-3}) in compartment II (apparent free space) ($[NO_3^-]_{AFS}$) at 0·1 and 1·5 mol m^{-3} $[NO_3^-]_o$ in the roots of trembling aspen, lodgepole pine and Douglas-fir (mean ± SE, n = 3–5)

	$[NO_3^-]_o$ (mM)	
Species	0·1	1·5
Trembling aspen	0·12 ± 0·05	1·45 ± 0·58
Lodgepole pine	0·10 ± 0·001	2·36 ± 0·39
Douglas-fir	0·12 ± 0·01	1·15 ± 0·36

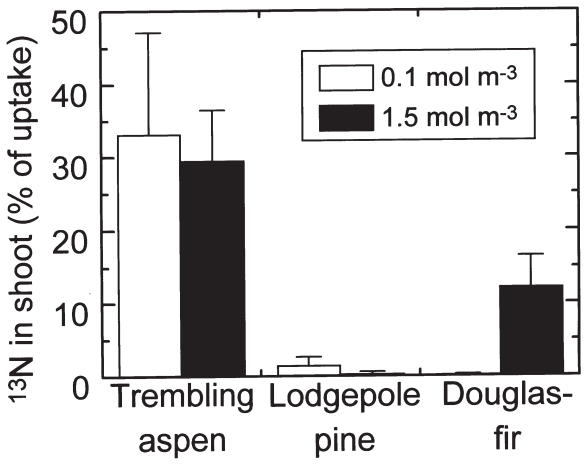

Figure 4. Translocation of ^{13}N to the shoot (% of total ^{13}N absorbed) during 35 min loading with $^{13}NO_3^-$ followed by 22 min washing in trembling aspen, lodgepole pine and Douglas-fir at two external concentrations. Standard errors are shown as vertical lines above the means (bars).

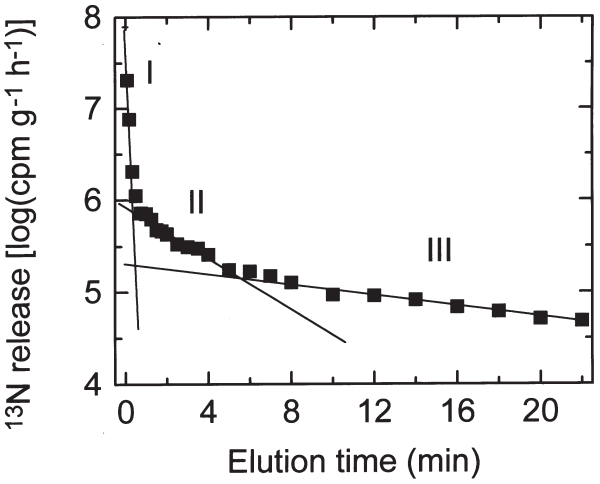

Figure 5. Representative semi-logarithmic plot of the rate of release of ^{13}N [log(cpm released) g^{-1} min^{-1}] versus time of elution for intact roots of trembling aspen at 1·5 mol m^{-3} NH_4^+. Three distinct compartments are indicated by Roman numerals.

trations of these ions (0·1 versus 1·5 mol m^{-3}), or among species. This result was anticipated because compartment I has been shown to represent the surface film of solution adhering to the roots. The $t_{0·5}$-values are similar to those observed in other species (Siddiqi *et al*. 1991; Wang *et al*. 1993; Kronzucker *et al*. 1995c, d, e, 1998). It has been suggested that compartment I may also include the water free space (WFS) component of the AFS and that these two components may act as a single phase (Kronzucker *et al*. 1995e). The failure to detect a fourth component which might represent the WFS as distinct from the Donnan free space (DFS) argues in favour of this suggestion (see also Siddiqi *et al*. 1991; Wang *et al*. 1993; Kronzucker *et al*. 1995c, d, e, 1998). Interestingly, the $t_{0·5}$-values for NO_3^- and NH_4^+ exchange in the AFS (30–40 s) were similar in lodgepole pine and Douglas-fir (Tables 1 and 3). Kronzucker *et al*. (1995c, d) also found no significant difference between $t_{0·5}$ of NO_3^- and NH_4^+ exchange in the AFS of white spruce. It is generally believed that cation binding in the AFS is strong because of the preponderance of negatively charged sites which constitute the DFS. In all species examined, the apparent $[NH_4^+]_{AFS}$ was several-fold higher than the ambient $[NH_4^+]$ (Table 4, see also Wang *et al*. 1993; Kronzucker *et al*. 1995d, 1998), as might be expected of a DFS with a strong capacity for cation binding. By contrast, the apparent $[NO_3^-]_{AFS}$ was similar to the ambient $[NO_3^-]$.

The cytoplasmic compartment (compartment III)

Our results show that, at relatively low $[NO_3^-]_o$, trembling aspen was by far the most, and lodgepole pine the least competent species in the acquisition and utilization of NO_3^- (Fig. 2a; Min *et al*. 1998). At 0·1 mol m^{-3} $[NO_3^-]_o$ where influx is mainly mediated by a high-affinity transport system (HATS) (our unpublished results; see Glass & Siddiqi 1995 for a general review), influx and net flux into the roots of aspen were several-fold higher than in Douglas-fir and lodgepole pine (Min *et al*. 1998). Between the latter two, fluxes into Douglas-fir roots were significantly higher than those in lodgepole pine.

At the same low $[N]_o$, NH_4^+ fluxes were higher than NO_3^- fluxes in all species examined (Figs 2a & 6a). However, the preference for NH_4^+ over NO_3^- was much more pronounced in lodgepole pine (≈ 16-fold) and Douglas-fir (> four-fold) than in trembling aspen (two- to three-fold) (see also Rygiewicz & Bledsoe 1986; Kamminga-van Wijk & Prins 1993; Min *et al*. 1998). A preference for NH_4^+ over NO_3^- has been reported in several other coniferous species, e.g. *Pinus sylvestris* (Boxman & Roelofs 1988), *Pinus pinaster* (Scheromm & Plassard 1988), *Picea abies* (Marschner, Häussling & George 1991), and *Picea glauca* (Kronzucker *et al*. 1997).

Compared with influx at 0·1 mol m^{-3}, the capacity for NO_3^- uptake at 1·5 mol m^{-3} $[NO_3^-]_o$, increased greatly in lodgepole pine (18-fold) and to a lesser degree in Douglas-fir (three-fold), but relatively little in trembling aspen (1·3-fold) (Fig. 2). These increases of NO_3^- fluxes, are indicative of the relative contributions of the low-affinity transport system (LATS) for NO_3 at 1·5 mol m^{-3} $[NO_3^-]_o$ in these species (Fig. 2; see also Min *et al*. 1998; our unpublished results). These observations suggest that in lodgepole pine a highly developed LATS represents a valuable adaptation to soils which have relatively high $[NO_3^-]$; the latter condition may result from site disturbance by fire (e.g. Klinka *et al*. 1990; Brayshaw 1996). Compared to aspen, efflux in the two coniferous species was also high at 1·5 mol m^{-3} $[NO_3^-]_o$. Thus, net flux was still substantially higher in aspen than in the other two species (Fig. 2). It is noteworthy that NO_3^- fluxes at 1·5 mol m^{-3} $[NO_3^-]_o$ were

Species	$[NH_4^+]_o$ (mM)	Compartment I $t_{0·5}$ (s)	Compartment II $t_{0·5}$ (s)	Compartment III $t_{0·5}$ (min)
Trembling aspen	0·1	3·94 ± 0·83	49·60 ± 10·38	9·13 ± 1·74
	1·5	3·17 ± 0·17	61·03 ± 3·29	9·33 ± 0·50
Lodgepole pine	0·1	5·57 ± 0·65	40·75 ± 4·29	12·87 ± 2·41
	1·5	2·97 ± 0·57	37·19 ± 7·24	9·76 ± 1·14
Douglas-fir	0·1	2·83 ± 0·34	35·29 ± 3·60	7·82 ± 0·66
	1·5	2·62 ± 0·21	38·62 ± 5·41	7·26 ± 0·85

Table 3. Half-lives of exchange ($t_{0·5}$) for NH_4^+ of compartment I (surface film), compartment II (apparent free space), and compartment III (cytoplasm) at 0·1 and 1·5 mol m^{-3} $[NH_4^+]_o$ in the roots of trembling aspen, lodgepole pine and Douglas-fir (mean ± SE, n = 4)

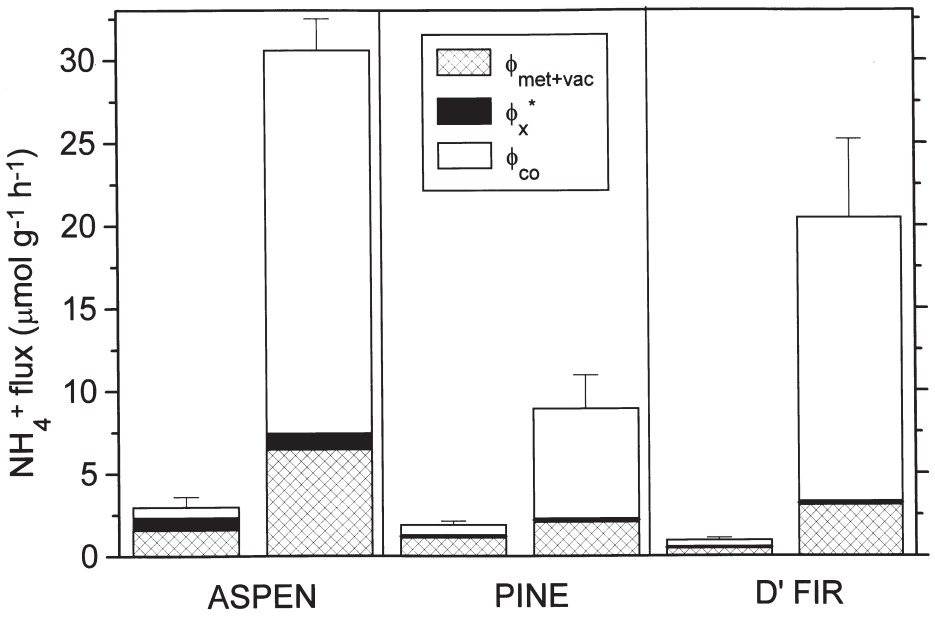

Figure 6. Influx (ϕ_{oc}), combined flux to metabolism and vacuole ($\phi_{met+vac}$), flux to xylem (ϕ_x*), and efflux (ϕ_{co}) (μmol g^{-1} h^{-1}) for NH_4^+ at 0·1 mol m^{-3} (left) and 1·5 mol m^{-3} (right) $[NH_4^+]_o$ in trembling aspen, lodgepole pine and Douglas-fir as determined by efflux analysis. Standard errors (vertical lines above the means) are shown for influx only. Note: net flux (ϕ_{net}) = ($\phi_{met+vac}$ + ϕ_x*) and (ϕ_{oc}) = (ϕ_{net} + ϕ_{co}).

significantly lower than NH_4^+ fluxes at 0·1 mol m^{-3} $[NH_4^+]_o$ (Figs 2b & 6a).

At high $[NH_4^+]_o$, both influx and efflux increased very substantially in trembling aspen and Douglas-fir (Fig. 6). Such large increases in NH_4^+ fluxes have also been observed in barley (our unpublished results). In lodgepole pine, the increase in influx was relatively modest but the percentage efflux was still as high as in other species. We can only speculate that these pronounced changes in fluxes at high $[NH_4^+]_o$ are characteristic of nitrophilous species. By contrast, in plants which are adapted to relatively high soil $[NH_4^+]$, e.g. white spruce and rice (Wang *et al*. 1993; Kronzucker *et al*. 1995d, 1996, 1998), influx and efflux do not increase to such high values.

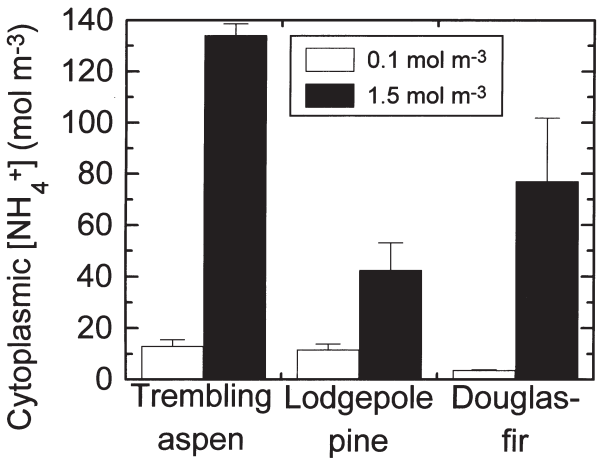

Figure 7. Cytoplasmic $[NH_4^+]$ (mol m^{-3}) in the roots of trembling aspen, lodgepole pine and Douglas-fir at two external concentrations as estimated by compartmental analysis. Standard errors are shown as vertical lines above the means (bars).

Cytoplasmic $[NO_3^-]$ and $[NH_4^+]$

Interestingly, cytoplasmic concentrations of the two ions reflected the patterns of their net uptake. For example in lodgepole pine grown at 0·1 mol m^{-3}, cytosolic $[NO_3^-]$, commensurate with the low value of NO_3^- uptake rate, was extremely low. By contrast, calculated values for net NH_4^+ uptake and cytoplasmic $[NH_4^+]$, for the same species grown at 0·1 mol m^{-3} NH_4^+, were approximately 10 times larger. This correspondence between net uptake and cytoplasmic ion concentration is also illustrated in the data for plants grown at 1·5 mol m^{-3} (Figs 2, 3, 6 & 7). Cytoplasmic $[NO_3^-]$ may represent an important determinant of rates of NO_3^- reduction by the nitrate reductase enzymes (e.g. King, Siddiqi & Glass 1992). This would apply under the present conditions, i.e. in lodgepole pine, maintained on 0·1 mol m^{-3} $[NO_3^-]_o$, but not at 1·5 mol m^{-3} $[NO_3^-]_o$, when NO_3^- influx and $[NO_3^-]_c$ increased approximately 10-fold. By contrast, in Douglas-fir and aspen, $[NO_3^-]_c$-values were probably sufficiently high, that NO_3^- reduction was not NO_3^- limited, even at low $[NO_3^-]_o$ (see also Min *et al*. 1998). Interestingly, despite a 15-fold increase in external NO_3^- concentration, $[NO_3^-]_c$ in Douglas-fir and aspen increased only modestly (2·5 and 1·3-fold, respectively). Clearly, aspen was able to obtain sufficient NO_3^- even from 0·1 mol m^{-3} $[NO_3^-]_o$ to sustain its $[NO_3^-]_c$ at a level comparable to that at high $[NO_3^-]_o$. We here propose that with respect to $[NO_3^-]_o$, lodgepole pine, Douglas-fir and

trembling aspen may be classified as non-competent/responsive, moderately competent/responsive, and competent/non-responsive, respectively.

In NH_4^+-fed plants, the $[NH_4^+]_c$ was higher than the corresponding $[NO_3^-]_c$-values in NO_3-fed plants in all species (Fig. 7, see also Kronzucker *et al*. 1995a, c, d for white spruce). Our measured $[NH_4^+]_c$ values are much higher than the reported K_m values for the enzyme glutamine synthetase (GS) (e.g. Stewart, Mann & Fentem 1980; Vega, Gotor & Menacho 1987). This may result from exposures to ambient $[NH_4]$ that are higher than are typical for these plants, or that irradiance levels (250 μE m^{-2} s^{-1}) may have imposed light-limitation on NH_4^+ assimilation. In contrast to the GS enzyme, glutamate dehydrogenase (GDH) has a low affinity for NH_4^+ (e.g. Bhadula & Shargool 1995; Stewart *et al*. 1980; Florencio, Marques & Candau 1987), and a role for this enzyme in primary NH_4^+ assimilation has frequently been suggested (Singh 1995 for refs.).

However, the consensus of opinion, including information derived from studies using conifers (Joy, Vogel & Thorpe 1997), is that the primary pathway for NH_4^+ assimilation is via GS-GOGAT (e.g. Yoneyama & Kumazawa 1974; Skokut *et al*. 1978; Kumar & Abrol 1990; Lea, Robinson & Stewart 1990).

Translocation of ^{13}N to the shoot

N is translocated to the shoot either as NO_3^- *per se* (in NO_3^- -fed plants) or as amino acids; generally, very little NH_4^+ *per se* is translocated to the shoot, regardless of whether N is supplied as NH_4^+ or NO_3^- (e.g. Wang *et al*. 1993 and references therein). In aspen, when N was supplied as NO_3^-, there was a substantial flux of ^{13}N (approximately 30% of influx) to the shoot (see also Min *et al*. 1998). Based upon leaf NR induction studies, Min *et al*. (1998) argued that in aspen a substantial portion of ^{13}N translocated was as $^{13}NO_3^-$. In sharp contrast to aspen, however, very little ^{13}N appeared in the shoot in pine and Douglas-fir (see also Min *et al*. 1998). Thus, in the latter species, even the translocation of amino acids was extremely low or perhaps delayed.

Interestingly in aspen, despite several-fold increases of influx, net flux and $[NH_4^+]_c$ at 1·5 mol m^{-3} $[NH_4^+]_o$ compared with 0·1 mol m^{-3}, the ^{13}N flux to the xylem increased only marginally (1·3-fold), consistent with the general assumption that amino N rather than NH_4^+ *per se* is translocated to the shoot (e.g. Wang *et al*. 1993). It is noteworthy that in the two conifers, ^{13}N flux to the xylem was higher in NH_4^+-fed plants than in NO_3^--fed plants.

Table 4. NH_4^+ concentration (mol m^{-3}) in compartment II (apparent free space) ($[NH_4^+]_{AFS}$) at 0·1 and 1·5 mol m^{-3} $[NH_4^+]_o$ in the roots of trembling aspen, lodgepole pine and Douglas-fir (mean ± SE, n = 4)

	$[NH_4^+]_o$ (mM)	
Species	0·1	1·5
Trembling aspen	0·56 ± 0·15	19·53 ± 2·51
Lodgepole pine	0·88 ± 0·15	8·76 ± 2·56
Douglas-fir	1·09 ± 0·27	17·04 ± 0·81

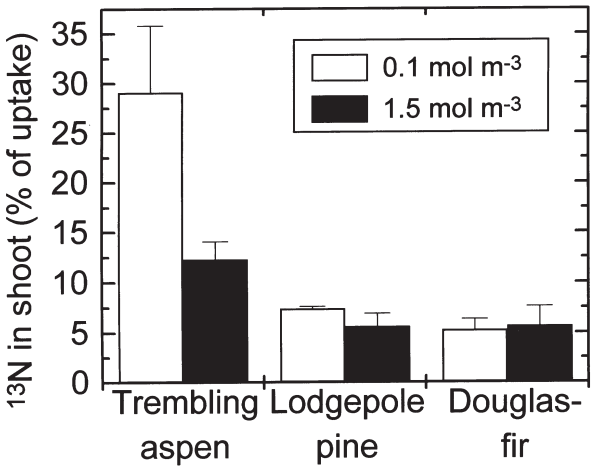

Figure 8. Translocation of ^{13}N to the shoot (% of total ^{13}N absorbed) during 45 min loading with $^{13}NH_4^+$ followed by 22 min washing in trembling aspen, lodgepole pine and Douglas-fir at two external concentrations. Standard errors are shown as vertical lines above the means (bars).

In summary, with respect to NO_3^- utilization, trembling aspen, Douglas-fir and lodgepole pine were characterized as a competent non-responder, a moderately competent responder, and a non-competent responder, respectively. In these species, $[NO_3^-]_c$ appeared to depend upon the rates of NO_3^- uptake. At low $[NO_3^-]_o$, the two conifers had lower $[NO_3^-]_c$ than trembling aspen; indeed, particularly in lodgepole pine, $[NO_3^-]_c$ might well have been rate-limiting for the NR enzyme(s). At high $[NO_3^-]_o$, by contrast, rates of NO_3^- uptake and $[NO_3^-]_c$ of lodgepole pine were close to those of aspen. Moreover, compared with aspen, the two conifers showed much lower rates of N translocation to the shoot as well as lower levels of leaf NRA, further limiting their capacities for NO_3^- utilization. By contrast, the capacities for NH_4^+ utilization were not as different among these species as those for NO_3^- utilization. The present study confirms our earlier conclusions (Min *et al*. 1998) that N-source availability may be an important determinant of species distribution in temperate and boreal ecosystems and that these differences in the patterns of N-utilization may be a factor in niche separation among these species.

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REFERENCES

Belton P.S., Lee R.B. & Ratcliffe R.G. (1985) A ^{14}N nuclear magnetic resonance study of inorganic nitrogen metabolism in barley, maize and pea roots. *Journal of Experimental Botany* **36,** 190–210.

Bhadula S.K. & Shargool P.D. (1995) Glutamate dehydrogenase: purification, properties and regulation. In *Nitrogen Nutrition in Higher Plants* (eds H.S. Srivastava & R.P. Singh), pp. 205–217. Associated Publishers, New Delhi, India.

Bledsoe C.S. & Rygiewicz P.T. (1986) Ectomycorrhizas affect ionic balance during ammonium uptake by Douglas-fir roots. *New Phytologist* **106,** 271–283.

Boxman A.W. & Roelofs J.G.M. (1988) Some effects of nitrate versus ammonium nutrition on the nutrient fluxes in *Pinus sylvestris* seedlings: effects of mycorrhizal infection. *Canadian Journal of Botany* **66,** 1091–1097.

Brayshaw T.C. (1996) *Trees and Shrubs of British Columbia*. UBC Press, Vancouver.

Chapin F.S. III, Moilanen L. & Kielland K. (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. *Nature* **361,** 150–153.

Chapin F.S. III, Van Cleve K. & Tyron P.R. (1986) Relationship of ion absorption to growth rate in taiga trees. *Oecologia* **69,** 238–242.

Deane-Drummond C.E. & Glass A.D.M. (1982) Nitrate uptake into barley (*Hordeum vulgare*) plants. A new approach using $^{36}ClO_3^-$ as an analogue for NO_3^-. *Plant Physiology* **70,** 50–54.

Devienne F., Mary B. & Lamaze T. (1994a) Nitrate transport in intact wheat roots. I. Estimation of cellular fluxes and NO_3^- distribution using compartmental analysis from data of $^{15}NO_3^-$ efflux. *Journal of Experimental Botany* **45,** 667–676.

Devienne F., Mary B. & Lamaze T. (1994b) Nitrate transport in intact wheat roots. II. Long-term effects of NO_3^- concentration in the nutrient solution on NO_3^- unidirectional fluxes and distribution within the tissues. *Journal of Experimental Botany* **45,** 677–684.

Florencio F.J., Marques S. & Candau P. (1987) Regulation by ammonia of glutamine synthetase from unicellular cyanobacteria. In *Inorganic Nitrogen Metabolism* (eds W.R. Ullrich, P.J. Aparicio, P.J. Syrett & F. Castillo), pp. 144–147. Springer-Verlag, Berlin.

Glass A.D.M. (1989) Physiological mechanisms involved with genotypic differences in ion absorption and utilization. *Hortscience* **24,** 559–564.

Glass A.D.M. & Siddiqi M.Y. (1995) Nitrogen absorption in higher plants. In *Nitrogen Nutrition in Higher Plants* (eds H.S. Srivastava & R.P. Singh), pp. 21–55. Associated Publishers, New Delhi, India.

Joy R.W.I.V., Vogel H.J. & Thorpe T.A. (1997) Inorganic nitrogen metabolism in embryogenic white spruce cultures: a nitrogen 14/15 NMR study. *Journal of Plant Physiology* **151,** 306–315.

Kamminga-van Wijk C. & Prins H.B.A. (1993) The kinetics of NH_4^+ and NO_3^- uptake by Douglas-fir from single N-solutions and from solutions containing both NH_4^+ and NO_3^-. *Plant and Soil* **151,** 91–96.

Kielland K. (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* **75,** 2373–2383.

King B.J., Siddiqi M.Y. & Glass A.D.M. (1992) Studies of the uptake of nitrate in barley. V. Estimation of root cytoplasmic nitrate concentration using nitrate reductase activity – implications for nitrate influx. *Plant Physiology* **99,** 1582–1589.

Klinka K., Feller M.C., Green R.N., Meidinger D.V., Pojar J. & Worrall J. (1990) Ecological principles: applications. In *Regenerating British Columbia's Forests* (eds D.P. Lavender, R. Parish, C.M. Johnson, G. Montgomery, A. Vyse, R.A. Willis, & D. Winston), pp. 55–72. University of British Columbia Press, Vancouver.

Kronzucker H.J., Glass A.D.M. & Siddiqi M.Y. (1995a) Nitrate induction in spruce: an approach using compartmental analysis. *Planta* **196,** 683–690.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995b) Kinetics of NO_3^- influx in spruce. *Plant Physiology* **109,** 319–326.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995c) Compartmentation and flux characteristics of nitrate in spruce. *Planta* **196,** 674–682.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995d) Compartmentation and flux characteristics of ammonium in spruce. *Planta* **196,** 691–698.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1995e) Analysis of $^{13}NH_4^+$ efflux in spruce roots: a test case for phase identification in compartmental analysis. *Plant Physiology* **109,** 481–490.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1996) Kinetics of NH_4^+ influx in spruce. *Plant Physiology* **110,** 773–779.

Kronzucker H.J., Siddiqi M.Y. & Glass A.D.M. (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature* **385,** 59–61.

Kronzucker H.J., Kirk G.J.D., Siddiqi M.Y. & Glass A.D.M. (1998) Effects of hypoxia on $^{13}NH_4^+$ fluxes in rice roots. Kinetics and compartmental analysis. *Plant Physiology* **116,** 581–587.

Kumar P.A. & Abrol Y.P. (1990) Ammonia assimilation in higher plants. In *Nitrogen in Higher Plants* (ed. Y.P. Abrol), pp. 159–180. John Wiley and Sons, New York.

Lea P.J., Robinson S.A. & Stewart G.R. (1990) The enzymology and metabolism of glutamine, glutamate and asparagine. In *The Biochemistry of Plants. A Comprehensive Treatise. Intermediary Nitrogen Metabolism* (eds B.J. Mifflin & P.J. Lea), Vol. 16, pp. 121–159. Academic Press, New York.

Lee R.B. & Clarkson D.T. (1986) Nitrogen-13 studies of nitrate fluxes in barley roots. I. Compartmental analysis from measurements of ^{13}N efflux. *Journal of Experimental Botany* **37,** 1753–1767.

Lee R.B. & Ratcliffe R.G. (1983) Phosphorus nutrition and the intracellular distribution of inorganic phosphate in pea root tips: quantitative study using ^{31}P-NMR. *Journal of Experimental Botany* **34,** 1222–1244.

Likens G.E., Bormann F.H. & Johnson N.M. (1969) Nitrification: importance to nutrient losses for a cutover forest ecosystem. *Science* **163,** 1205–1206.

Lodhi M.A.K. (1978) Inhibition of nitrifying bacteria, nitrification and mineralization of spoil soils as related to their successional stages. *Bulletin of Torrey Botanical Club* **106,** 284–289.

Marschner H.H., Häussling M. & George E. (1991) Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst.]. *Trees* **5,** 14–21.

Min X., Siddiqi M.Y., Guy R.D., Glass A.D.M. & Kronzucker H.J. (1998) Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine. *Plant, Cell and Environment* **21,** 1039–1046.

Oaks A. (1994) Primary nitrogen assimilation in higher plants and its regulation. *Canadian Journal of Botany* **72,** 739–750.

Prescott C. (1997) Effects of clearcutting and alternative silvicultural systems on rates of decomposition and nitrogen mineralization in a coastal montane coniferous forest. *Forest Ecology and Management* **95,** 253–260.

Presland M.R. & McNaughton G.S. (1984) Whole plant studies using radioactive 13-nitrogen. II. A compartmental model for the uptake and transport of nitrate ions by *Zea mays*. *Journal of Experimental Botany* **35,** 1277–1288.

Rygiewicz P.T. & Bledsoe C.S. (1986) Effects of pretreatment conditions on ammonium and nitrate uptake by Douglas-fir seedlings. *Tree Physiology* **1,** 145–150.

Rygiewicz P.T., Bledsoe C.S. & Glass A.D.M. (1984) A comparison of methods for compartmental analysis of Rb efflux from barley and Douglas-fir roots. *Plant Physiology* **76,** 913–917.

Scheromm P. & Plassard C. (1988) Nitrogen nutrition of non-mycorrhized maritime pine (*Pinus pinaster*) grown on nitrate or ammonium. *Plant Physiology and Biochemistry* **26,** 261–269.

Schmidt M.G., MacDonald S.E. & Rothwell R.L. (1996) Impacts of harvesting and mechanical site preparation on soil chemical properties of mixed-wood boreal forest sites in Alberta. *Canadian Journal of Soil Science* **76,** 531–540.

Siddiqi M.Y., Glass A.D.M. & Ruth T.J. (1991) Studies of the uptake of nitrate in barley. III. Compartmentation of NO_3^-. *Journal of Experimental Botany* **42,** 1455–1463.

Singh R.P. (1995) Ammonia assimilation. In *Nitrogen Nutrition in Higher Plants* (eds H.S. Srivastava & R.P. Singh), pp. 189–203. Associated Publishers, New Delhi, India.

Skokut T.A., Wolk C.P., Thomas J., Meeks J.C. & Shaffer P.W. (1978) Initial organic products of assimilation of [^{13}N] ammonium and [^{13}N] nitrate by tobacco cells cultured on different sources of nitrogen. *Plant Physiology* **62,** 299–304.

Stark J.M. & Hart S.C. (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* **385,** 61–64.

Stewart G.R., Mann A.F. & Fentem P.A. (1980) Enzymes of glutamate formation: glutamate dehydrogenase, glutamine synthetase, and glutamate synthase. In *The Biochemistry of Plants*, Vol. 5*: Amino Acids and Derivatives* (ed. B.J. Miflin), pp. 271–327. Academic Press, New York.

Van Cleve K., Oliver L., Schlentner R., Viereck L.A. & Dyrness C.T. (1983) Productivity and nutrient cycling in taiga forest ecosystems. *Canadian Journal of Forestry Research* **13,** 747–766.

Vega J.M., Gotor C. & Menacho A. (1987) Enzymology of the assimilation of ammonium by the green alga *Chlamydomonas reinhardtii*. In *Inorganic Nitrogen Metabolism* (eds W.R. Ullrich, P.J. Syrett & F. Castillo), pp. 132–136. Springer-Verlag, Berlin.

Walley F.L., van Kessel C. & Pennock D.J. (1996) Landscape-scale variability of N mineralization in forest soils. *Soil Biology and Biochemistry* **28,** 383–391.

Wang M.Y., Siddiqi M.Y., Ruth T.J. & Glass A.D.M. (1993) Ammonium uptake by rice roots. I. Fluxes and subcellular distribution of $^{13}NH_4^+$. *Plant Physiology* **103,** 1249–1258.

Yoneyama T. & Kumazawa K. (1974) A kinetic study of the assimilation of ^{15}N-labelled ammonium in rice seedling roots. *Plant and Cell Physiology* **15,** 655–661.

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