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Compartmentation and flux characteristics of ammonium in spruce

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Abstract. Using $^{13}NH_4^+$ as a tracer, compartmental analyses for NH_4^+ were performed in non-mycorrhizal roots of intact *Picea glauca* (Moench) Voss. seedlings at four different concentration regimes of external NH_4^+ ($[NH_4^+]_o$), i.e. 0, 10, 100, and 1500 μM. Three kinetically distinct compartments were identified, with half-lives of exchange of approximately 2 s, 30 s, and 14 min, assumed to represent surface adsorption, Donnan free space, and cytoplasm, respectively. No significant differences were found in half-lives of exchange with changes in $[NH_4^+]_o$. Influx was calculated to be 0.96 $\mu mol \cdot g^{-1} \cdot h^{-1}$ in N-deprived plants (measured at 10 μM $[NH_4^+]_o$), while under steady-state conditions it was 0.21 $\mu mol \cdot g^{-1} \cdot h^{-1}$ at 10 μM $[NH_4^+]_o$, 1.96 $\mu mol \cdot g^{-1} \cdot h^{-1}$ at 100 μM $[NH_4^+]_o$, and 6.45 $\mu mol \cdot g^{-1} \cdot h^{-1}$ at 1.5 mM $[NH_4^+]_o$. Efflux measured over the same range constituted approximately 9% of influx in N-deprived plants, 10% at 10 μM, 28% at 100 μM, and 35% at 1.5 mM $[NH_4^+]_o$. Cytoplasmic $[NH_4^+]$ was estimated at 6 mM in N-deprived plants, 2 mM at 10 μM $[NH_4^+]_o$, 14 mM at 100 μM, and 33 mM at 1.5 mM. Free-space $[NH_4^+]$ was 84 μM, 50 μM, 700 μM, and 8 mM, respectively. In comparison with previously published data on fluxes and compartmentation of NO_3^- in white-spruce seedlings, results of this study identify a pronounced physiological preference of this species for NH_4^+ over NO_3^- as an inorganic N source in terms of uptake and intracellular accumulation. The significant ecological importance of this N-source preference is discussed.

Key words: Ammonium – Compartmental analysis – Nitrogen-13 – *Picea*

Abbreviations and symbol: $[NH_4^+]_{cyt}$ = cytoplasmic NH_4^+ concentration; $[NH_4^+]_{free\ space}$ = NH_4^+ concentration in the Donnan free space; $[NH_4^+]_o$ = concentration of NH_4^+ in the external solution; ϕ = NH_4^+ flux (for subscripts, see *Materials and methods*)

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Introduction

Soil habitats vary greatly not only in their total nitrogen contents but also in the chemical speciation of nitrogen in the soil solution. Inorganic nitrogen can be available to plants in the oxidized form, as NO_3^-, or in the reduced form as NH_4^+. Well-aerated agricultural or ruderal soils are generally considered rich in NO_3^- and poor in NH_4^+, whereas mature forest soils, particularly in ecosystems dominated by coniferous species, are often characterized by the reverse situation (Smith et al. 1968; Rice and Pancholy 1972; Lodhi 1978; Klingensmith and Van Cleve 1993). This difference in the chemical nature of the soil N pool is believed to be a result of a multifactorial suppression of nitrification in later successional forest soils, caused by such factors as low average soil temperatures, low pH, and allelopathic soil conditions. While usually only residual NO_3^- production of a heterotrophic nature is found in such habitats (see reviews by Runge 1983, and Attiwell and Adams 1993), nitrification can proceed at high rates in agricultural, perturbed or early successional soils (Likens et al. 1969; see also Vitousek et al. 1989 for references). For this reason, species indigenous to later successional forest habitats have been assumed to preferentially utilize ammonium as their inorganic nitrogen source, whereas species indigenous to agricultural, perturbed or early successional forest soils are believed to prefer nitrate (Smirnoff and Stewart 1985; Stewart et al. 1990). While the assumption that a poor utilization capacity for nitrate is a characteristic trait of woody as opposed to herbaceous species represents an incorrect generalization (Stadler and Gebauer 1992), a pronounced preference for ammonium over nitrate does appear to be systemic in many coniferous species (Kronzucker et al. 1995a, and references therein). Interestingly, this preference seems to be shared by the ectomycorrhizal fungi normally colonizing the roots of these species in the field (Littke 1982; Littke et al. 1984; Rygiewicz et al. 1984a, b; Lang and Jagnow 1986).

In previous communications, we have investigated in detail the physiological nature of this poor adaptation to nitrate in non-mycorrhizal white-spruce seedlings by determining nitrate fluxes and compartmentation in the

roots using the technique of efflux analysis (Kronzucker et al. 1995a, b). We have shown that rates of the very first steps in NO_3^- utilization, i.e. NO_3^- influx and NO_3^- reduction to NO_2^-, are inherently low and undoubtedly limiting for growth when NO_3^- is the sole N source available to the plants (Kronzucker et al. 1995a, b).

An inherent physiological preference for ammonium over nitrate in certain conifer species may have considerable ecological and economic consequences, given that major reforestation failures of such 'NH_4^+ species' have been observed on 'NO_3^- soils' (see Kronzucker et al. 1995a, b). Since physiological information on NH_4^+ fluxes in conifer roots is sparse, we have applied the technique of compartmental analysis to investigate the flux characteristics and the subcellular compartmentation of ammonium in roots of intact white-spruce seedlings. With a view to the ecological implications advanced above, the design of these experiments on ammonium was identical to that of our earlier nitrate study (Kronzucker et al. 1995a), to allow for direct comparisons in terms of N-source adaptation. The study provides estimates for cytoplasmic and cell wall ammonium concentrations as well as values for unidirectional influx and efflux of ammonium under varying external ammonium concentrations. In juxtaposition to our earlier study on nitrate, the work demonstrates that the capacities for ammonium and nitrate utilization differ very substantially in white spruce at the levels of ion uptake across the plasmalemmata, intracellular accumulation and physiological processing.

Materials and methods

Plant culture. Several-months-old seedlings of white spruce (*Picea glauca* (Moench) Voss., provenance 29170 from the Prince George region in British Columbia, Canada) were used. Seedlings were grown for a minimum of $3\frac{1}{2}$ months in a 3:1 peat/perlite mixture in styrofoam boxes in an outdoor nursery located on the University of British Columbia campus. Seedlings were then transferred indoors and, after gentle removal of the rooting medium, adapted to hydroponic culture in 24-dm^3 Plexiglas tanks. The tanks contained 1/10-strength N-free Johnson's solution mixed with analytical-grade chemicals in distilled-deionized water (for a detailed description of growth conditions and for exact solution composition, see Kronzucker et al. 1995a). Seedling roots in hydroponic solution were non-mycorrhizal, as determined by microscopic examination. Plants were adapted in the tanks for a period of three weeks prior to the experiments. For the concentration-dependence experiments, NH_4^+ was added as $(NH_4)_2SO_4$ at the desired concentrations (i.e. 10, 100, or 1500 μM) 4 d prior to efflux analyses, to provide steady-state conditions with regard to NH_4^+. Powdered $CaCO_3$ was added to the tanks to buffer against acidification caused by plant NH_4^+ uptake (pH was kept constant around 6.5). Seedlings were maintained in a 16 h/8 h photoperiod, 70% relative humidity, and at 20 ± 2°C. Light was provided at approximately 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level by fluorescent tubes with spectral composition similar to sunlight.

Production of $^{13}NH_4^+$. Nitrogen-13 ($t_{1/2}$ = 9.96 min) was produced by proton irradiation of H_2O (Meeks 1993) at the TRIUMF cyclotron on the University of British Columbia (UBC) campus in Vancouver, Canada. Since this irradiation procedure generates primarily $^{13}NO_3^-$ (see Kronzucker et al. 1995a), a chemical conversion was necessary to obtain $^{13}NH_4^+$. The chemical purification and conversion procedure was carried out in a lead-shielded fumehood at the UBC Botany Department and was as follows (in modification of Vaalburg et al. 1975, Meeks et al. 1978, and Wang et al. 1993a): Approximately 15 cm^3 of irradiated target solution were taken up by a 20-cm^3 syringe and passed twice through a SEP-PAC Alumina-N cartridge (Waters Associates, Milford, Mass., USA) to remove the radiocontaminant ^{18}F. To remove residual volume, 5 cm^3 of 2.5 μM $Ca(NO_3)_2$ was used to rinse the cartridge. The sample was then introduced into a 500-cm^3 round-bottom flask containing approximately 10 g of Devarda's alloy (50% Cu, 45% Al, 5% Zn). The flask was connected to two 100-cm^3 Erlenmayer flasks arranged in series and containing a total of 100 cm^3 'trapping solution' (same composition as in labelling solution) acidified by addition of 1 cm^3 of 1 N HCl. Reduction of $^{13}NO_3^-$ to $^{13}NH_4^+$ was then initiated by transferring the round-bottom flask to a 70°C water-bath and adding 20 cm^3 of 1 N NaOH, according to the following reaction (shown is the reaction of a putative Na-salt of $^{13}NO_3^-$ with the Devarda's alloy component Zn; similar hydroxo-complex formation and concomitant $^{13}NH_3$ generation occurs with Cu and Al under alkaline conditions):

$$Na^{13}NO_3 + 4\ Zn + 3\ NaOH + 6\ H_2O \rightarrow$$
$$4\ Na[Zn(OH)_3] + {}^{13}NH_3\uparrow.$$

Gaseous $^{13}NH_3$ was separated from remaining chemical species by steam distillation on the water-bath, and ionic $^{13}NH_4^+$ was trapped in the acidic solution contained in the Erlenmayer flasks, which were connected to a suction pump to facilitate directional gas flow. Under these conditions, most of the ^{13}N contained in the original sample solution was transferred to the trapping solution in less than 5 min. The efficiency of transfer was checked by means of a Geiger-Müller counter. The pH of the $^{13}NH_4^+$-labelled trapping solution was then neutralized by adding 1 cm^3 of 1 N NaOH and added to the actual loading solution (composition of the latter as described above for growth solutions).

Efflux analysis. Efflux experiments were carried out essentially as described before (Kronzucker et al. 1995a). In brief, roots of intact spruce seedlings were equilibrated in non-labelled preloading solution for a duration of 5 min and then transferred to $^{13}NH_4^+$-labelled loading solution for 45–60 min. Seedlings were then taped to 'efflux funnels' and the roots eluted with 60- to 100-cm^3 aliquots of non-radioactive solution (of a chemical composition otherwise identical to that of the loading solution) after varying time intervals. These time intervals ranged from 5 s to 2 min, over an experimental duration of 22 min. Eluates from a total of 25 time intervals were collected separately, and the radioactivity of 20 cm^3 subsamples from each eluate was determined in a gamma-counter (Minaxi δ, Auto-γ 5000 Series; Packard, Downers Grove, Ill., USA). After the final elution, roots and shoots were excised, introduced into scintillation vials and also counted for γ-activity.

In experiments designed to determine the electrical charge of the ^{13}N-labelled compound(s) eluted from the roots, 100-cm^3 eluates were collected at 10, 20, and 30 min following plant transfer into non-labelled 'elution solution'. Subsamples (20 cm^3) from these eluates were then passed through anion- and cation-exchange columns (analytical grade AG 1-X 8 anion exchange resin, 200–400 mesh, acetate form, and analytical grade AG 50 W-X 8 cation exchange resin, 200–400 mesh, Na^+-form BIO-RAD Laboratories, Richmond, Calif., USA). The proportion of label contained in each fraction was determined by counting the radioactivities of resins and of resin filtrates in the γ-counter.

Plants received steady-state conditions with regard to nutrient concentrations throughout experiments (i.e. throughout growth, pretreatment, prelabelling, labelling, and elution). Only 'N-deprived plants' were maintained without nitrogen during the entire growth and pretreatment periods, and NH_4^+ was then added at 10 μM only for the actual efflux analysis to make the monitoring of fluxes and the estimations of compartmental concentrations possible, while still keeping $[NH_4^+]_o$ close to zero. Even in putative '0 μM' NH_4^+ solutions, however, trace amounts of NH_4^+ (≤ 2 μM) could always be detected by chemical analysis; this was probably due to a chemical equilibration reaction of nutrient solutions with NH_3 gas contained in the ambient air.

Treatment of data. Treatment of efflux data was as outlined by Siddiqi et al. (1991) and was based on the theoretical considerations of Lee and Clarkson (1986). All experiments were performed using two replicates and were repeated at least three times. A representative experiment was chosen for the semi-logarithmic plot of the rate of release of ^{13}N versus time of elution. All other data displayed in figures and tables represent the means of several experiments ($\pm$ standard errors SE, as indicated). Calculations of fluxes and compartmental NH_4^+ concentrations were as described for NO_3^- by Siddiqi et al. (1991). All fluxes are expressed in $\mu mol\ NH_4^+ \cdot g^{-1}$ (root FW)$\cdot h^{-1}$. Symbols used for fluxes are as follows:

ϕ_{co} = efflux from the cytoplasm, obtained from the rate of ^{13}N release from the cytoplasm at time zero divided by the specific activity of the loading solution;
ϕ_{net} = net flux, obtained directly from the accumulation of ^{13}N in the plants at the end of the elution period;
ϕ_{oc} = unidirectional influx, calculated from $\phi_{net} + \phi_{co}$;
ϕ_{xylem} = flux of ^{13}N to the shoot, obtained directly from count accumulation in the shoot at the end of the elution period;
$\phi_{vac./ass.}$ = combined fluxes to ammonium assimilation and to the vacuole, resulting from $\phi_{net} - \phi_{xylem}$.

Independent flux measurements. Independent of compartmental analysis, ϕ_{oc} was determined by exposing seedling roots to $^{13}NH_4^+$-labelled solutions of varying $[NH_4^+]_o$ for 10 min. Seedlings were equilibrated in non-labelled 'prewashing' solution of the same composition as the loading solution for 5 min and desorbed in the same solution for a period of 3 min following loading. Prior to desorption, a short 5-s dip in non-labelled solution was used in order to prevent excessive carry-over of surface label into the desorption solution. Immediately following desorption, seedlings were cut into roots and shoots, the roots spun for 45 s in a slow-speed centrifuge (International Chemical Equipment, Boston, Mass., USA) to remove surface liquid adhering to the roots, and the plant organs then counted in a γ-counter.

Concomitantly, ϕ_{oc} was also determined by counting the radioactivities of 1-cm^3 samples withdrawn from the labelling solution at various times during the 10-min labelling period. Rates of ^{13}N-depletion, representing ϕ_{oc} of NH_4^+ into seedling roots, were calculated.

In separate experiments, ϕ_{net} was measured by monitoring the disappearance of $^{14}NH_4^+$ from uptake solutions over a period of 60 min. Solution samples were taken at different times during this 1-h uptake period. The $[NH_4^+]$ was assayed colorimetrically according to Solorzano (1969), and the difference in $[NH_4^+]$ was used to calculate ϕ_{net}.

Results

Phase regression. Figure 1 shows a representative semi-logarithmic plot of ^{13}N efflux versus elution time for white-spruce seedlings, determined at 100 μM external NH_4^+ ($[NH_4^+]_o$). Assuming first-order kinetics for the loss of tracer from subcellular compartments, we identified three kinetically distinct phases with high r^2 values (0.91–0.99) in linear regression of elution data such as seen in Fig. 1. The plot shown includes linear regression lines for each of the three phases as well as the respective linear equations. By analyzing the slopes of these lines (i.e. the kinetic constants k), half-lives of exchange ($t_{1/2}$) for the phases could be derived. These were 2.3 s (for the fastest exchanging phase), 33.7 s (for the intermediate phase), and 14.1 min (for the slowest exchanging phase) (Table 1). The subcellular and/or extracellular compartments that were tentatively assigned to these phases were the film of liquid adhering to the root surface, the cell wall free space, and the cytoplasm, respectively. No significant difference was found in these $t_{1/2}$ values with changing $[NH_4^+]_o$. Analyses of the electrical charge of the ^{13}N species released from seedling roots by means of anion and cation exchange columns (as outlined in *Materials and methods*) revealed that $\geq 96.3\%$ of the radioactive compounds effluxing from the roots were positively charged. Since the concentration of positively charged amino acids in the root cell cytoplasm of plants is usually negligible (see Wang et al. 1993b for references), ^{13}N release was assumed to be in the form of NH_4^+.

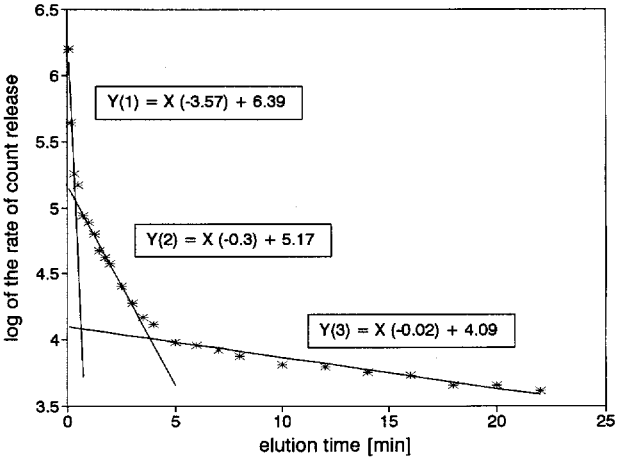

Fig. 1. Representative semi-logarithmic plot of the rate of release of ^{13}N [log (cpm released)$\cdot g^{-1} \cdot min^{-1}$] versus time of elution for intact roots of white spruce at 100 μM $[NH_4^+]_o$. The plot includes linear regression lines and equations for the three phases resolved in efflux analysis.

Table 1. Half-lives of exchange ($t_{1/2}$) for NH_4^+ of compartments I, II, and III (assumed to represent surface film, cell wall free space, and cytoplasm, respectively) at four different external concentrations of NH_4^+ as estimated from compartmental analysis. Data $\pm$ SE (n = 3–9)

(Pre)treatment	Compartment I $t_{1/2}$(s)	Compartment II $t_{1/2}$(s)	Compartment III $t_{1/2}$(min)
0 μM NH_4^+ ('0–10')	1.67 $\pm$ 0.37	36.24 $\pm$ 5.82	13.67 $\pm$ 1.5
10 μM NH_4^+	2.61 $\pm$ 0.17	33.11 $\pm$ 4.84	17.47 $\pm$ 1.41
100 μM NH_4^+	2.15 $\pm$ 0.31	34.41 $\pm$ 3.56	14.74 $\pm$ 1.41
1500 μM NH_4^+	2.67 $\pm$ 0.05	28.53 $\pm$ 1.25	10.53 $\pm$ 0.39

Table 2. Ammonium fluxes as estimated from compartmental analysis (for symbols see text). Plants were grown in N-free solution for three weeks and then exposed to the indicated NH_4^+ concentrations for 4 d. Efflux analysis was conducted at the same NH_4^+ concentration, with the exception of '0 μM NH_4^+ plants', which were grown and pretreated at 0 μM $[NH_4^+]_o$, but measured at 10 μM $[NH_4^+]_o$.
Data ± SE (n = 3–9).

(Pre) treatment	NH_4^+ fluxes (μmol·g^{-1}·h^{-1})				
	ϕ_{oc}	ϕ_{co}	ϕ_{net}	$\phi_{vac./ass.}$	ϕ_{xylem}
0 μM NH_4^+ ('0–10')	0.9639 ± 0.0814	0.0839 ± 0.0132	0.8786 ± 0.095	0.8771 ± 0.0944	0.0015 ± 0.0011
10 μM NH_4^+	0.2129 ± 0.0171	0.0214 ± 0.0009	0.1915 ± 0.0179	0.1848 ± 0.0149	0.0068 ± 0.003
100 μM NH_4^+	1.9626 ± 0.2091	0.5512 ± 0.1699	1.4114 ± 0.1226	1.3698 ± 0.1126	0.0418 ± 0.0133
1500 μM NH_4^+	6.4584 ± 0.2589	2.2458 ± 0.4295	4.2126 ± 0.6133	3.9671 ± 0.6125	0.2455 ± 0.001

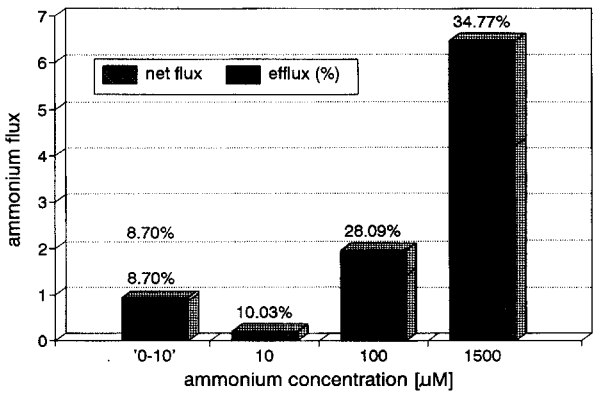

Fig. 2. Concentration dependence of net flux, influx and efflux [μmol·g^{-1}·h^{-1}] for NH_4^+ in white spruce as determined by efflux analysis. *Dashed-line segments* in stacked bar graph represent net flux, *dark-filled segments* represent efflux (percentage of influx indicated above bar). Influx results from the sum of the former (see Table 2).

Ammonium fluxes. Ammonium fluxes, as determined by compartmental analysis, changed significantly as a function of $[NH_4^+]_o$. The value of ϕ_{oc} in N-deprived plants ($[NH_4^+]_o$ = 0 μM) was measured at 10 μM $[NH_4^+]_o$ (indicated in Figures and Tables as '0–10') and was 0.96 μmol·g^{-1}·h^{-1}. Under steady-state conditions, ϕ_{oc} was 0.21 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 1.96 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$, and 6.46 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$ (Table 2). All plants were exposed to the respective concentrations for 4 d. Measured under the same conditions, ϕ_{co} was 0.08 μmol·g^{-1}·h^{-1} in N-deprived plants and was 0.02 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 0.55 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$, and 2.25 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$. Thus, expressed in percent of influx, efflux increased from 8.7% to 10%, 28.1% and 34.8%, respectively (Fig. 2). The value of ϕ_{net} therefore was 0.88 μmol·g^{-1}·h^{-1} in N-deprived plants, 0.19 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 1.41 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$ and 4.21 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$. Most of the ^{13}N taken up by the roots went to NH_4^+-assimilation or to the vacuoles in the root cells ($\phi_{vac./ass.}$ in Table 2), while only small rates of translocation to the stele were observed over the duration of the experiments, which, including labelling and elution, totalled 67 min in most experiments. The value of ϕ_{xylem} was less than 0.2% of ϕ_{oc} in N-deprived plants, while it constituted 2.1–3.8% in plants grown and measured under steady-state conditions with regard to $[NH_4^+]_o$ (see Table 2).

Flux estimates from methods independent of compartmental analysis were close in magnitude to the ones arrived at by analyzing efflux plots (Table 3). ϕ_{oc}, as determined by ^{13}N accumulation in seedling roots after exposure to isotopically labelled solution for 10 min, was 0.57 μmol·g^{-1}·h^{-1} in N-deprived plants, 0.41 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 1.28 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$ and 6.72 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$. By contrast, ϕ_{oc} estimates based on ^{13}N depletion over the same time period tended to be somewhat higher. They were 1.09 μmol·g^{-1}·h^{-1} in N-deprived plants, 0.49 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 2.34 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$ and 6.26 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$. A direct measurement of ϕ_{net} based on the chemical depletion of $^{14}NH_4^+$ from solution over a time period of 60 min yielded values very similar to ϕ_{net}-estimates from efflux analysis (see Tables 2, 3). Net flux obtained by these measurements was 0.25 μmol·g^{-1}·h^{-1} at 10 μM $[NH_4^+]_o$, 1.44 μmol·g^{-1}·h^{-1} at 100 μM $[NH_4^+]_o$ and 3.42 μmol·g^{-1}·h^{-1} at 1.5 mM $[NH_4^+]_o$.

Compartmental NH_4^+ concentrations. With increasing $[NH_4^+]_o$, $[NH_4^+]_{cyt}$ increased substantially (Table 4). It rose almost eightfold from 1.75 mM at 10 μM $[NH_4^+]_o$ to 13.7 mM at 100 μM $[NH_4^+]_o$ and, by more than a further twofold, to 32.7 mM at 1.5 mM $[NH_4^+]_o$. In N-deprived seedlings measured at 10 μM ('0–10'), $[NH_4^+]_{cyt}$ was 6.02 mM, significantly higher than in seedlings measured at 10 μM $[NH_4^+]_o$ in the steady state. The value of $[NH_4^+]_{free\ space}$ was estimated to be 84.01 μM in N-deprived plants in the '0–10' design, and 50.1 μM at 10 μM $[NH_4^+]_o$ in the steady state. At 100 μM $[NH_4^+]_o$ the estimate was 690 μM, and at 1.5 mM $[NH_4^+]_o$ it was 8.1 mM (Table 4).

Discussion

Half-lives of exchange. The assignment of particular cellular entities, e.g. cell wall and cytoplasm, to the phases identified by the present efflux analyses of spruce roots is

Table 3. Ammonium influx and net flux as determined by methods independent of compartmental analysis. Influx was measured at the same time by accumulation of ^{13}N in roots after loading in labelled solution for 10 min and by depletion of ^{13}N from the loading solution during this time. Net flux was determined from depletion of $^{14}NH_4^+$ over a period of 60 min. Data ± SE (n = 3–4).

(Pre) treatment	Method of measurement		
	^{13}N accumulation	^{13}N depletion	^{14}N depletion
0 μM NH_4^+ ('0–10')	0.57 ± 0.03	1.09 ± 0.04	N.D.
10 μM NH_4^+	0.41 ± 0.13	0.49 ± 0.06	0.25 ± 0.05
100 μM NH_4^+	1.28 ± 0.02	2.34 ± 0.37	1.44 ± 0.27
1500 μM NH_4^+	6.72 ± 0.47	6.26 ± 0.58	3.42 ± 0.54

Table 4. Compartment concentrations of NH_4^+ as a function of $[NH_4^+]_o$ as estimated from compartmental analysis. Tissue volume was assumed to be 5% for cytoplasm and 10% for free space. Treatment of plants was as indicated in Table 2. Data ± SE (n = 3–9).

(Pre) treatment	$[NH_4^+]_{cytoplasm}$ (mM)	$[NH_4^+]_{free\ space}$ (μM)
0 μM NH_4^+ ('0–10')	6.02 ± 1.01	84.01 ± 17.61
10 μM NH_4^+	1.75 ± 0.26	50.06 ± 2.33
100 μM NH_4^+	13.66 ± 1.77	690.03 ± 159.57
1500 μM NH_4^+	32.7 ± 2.31	8098.01 ± 793.72

based upon the effects of various treatments, including high temperature, SDS, H_2O_2, and 2-chloro-ethanol, designed to distinguish membrane-bound/metabolic (intracellular) compartments from non-membrane-bound/non-metabolic (extracellular) compartments (see Siddiqi et al. 1991, and Kronzucker et al. 1995a for discussion). These tests, in combination with an in-series assumption for the compartments revealed, support the belief that compartments I, II, and III represent a film of solution adhering to the root surface, the root apparent free space, and the root cell cytoplasm, respectively.

Interestingly, the $t_{1/2}$ values for NH_4^+ exchange in the cell wall of spruce were not significantly different from those reported for NO_3^- exchange in the same species (Kronzucker et al. 1995a). Half-lives of similar magnitude have been reported for NO_3^- exchange in corn (Presland and McNaughton 1986; Cooper et al. 1989; Devienne et al. 1994), and in barley (Lee and Clarkson 1986), as well as for NH_4^+ exchange in rice (Wang et al. 1993a). Nevertheless, the quantity of the NH_4^+ bound in the wall fraction was around three- to fivefold larger than for NO_3^- (see Kronzucker et al. 1995a), consistent with the large excess of negative charge in the Donnan free space. In contrast to the earlier suggestion by Wang et al. (1993a) that compartment II be a combination of water free space and Donnan free space, we consider it more reasonable that the water free space may exchange with a $t_{1/2}$ closer to that of the 'surface film' (i.e. compartment I) and may therefore not be sufficiently different to be distinguished. Evidence for a rapid water-free-space equilibration was provided in the kinetics of apoplasmic 'bypass flow' of $^{13}NO_3^-$ in barley roots (Siddiqi et al. 1991).

The $t_{1/2}$ values for cytoplasmic NH_4^+ exchange in spruce ($\approx$ 14 min) were longer than reported for rice ($\approx$ 8 min; Wang et al. 1993a), but rather close to unpublished values for barley (14–18 min). Macklon et al. (1990), in studies of $^{15}NH_4^+$ exchange in excised root segments of onion, reported $t_{1/2}$-values for the cytoplasm of 68.6 to 82.4 min, which is significantly longer than any of the estimates reported by other workers, including the present study. Because of this striking disagreement with the values found by others, and since there were no tests of compartment identity conducted by Macklon and coworkers, we question the validity of the assignment of efflux phases to subcellular compartments as advanced in that study (see also Kronzucker et al. 1995a, and later discussion).

Ammonium fluxes. Fluxes of NH_4^+ in white spruce as estimated by compartmental analysis were considerably higher than fluxes of NO_3^- measured under comparable conditions by the same technique (Kronzucker et al. 1995a, b). In fact, ϕ_{oc} of NH_4^+ at 10 μM $[NH_4^+]_o$ in the steady state was approx. twofold higher than ϕ_{oc} of NO_3^- after exposure to 10 μM $[NO_3^-]_o$ for 3 d (i.e. in a state of full induction for NO_3^- transport; see Kronzucker et al. 1995a), it was approx. fourfold higher with an external concentration of 100 μM for the two N species, and approx. fivefold higher at 1.5 mM. Since NO_3^- influx is substrate-inducible, i.e. enhanced by the presence of external NO_3^- (see Kronzucker et al. 1995b for references),

while NH_4^+ influx in most plant systems is down-regulated rather than enhanced by the presence of external NH_4^+ (see Wang et al. 1993b). The most dramatic difference for ϕ_{oc} for the two N species was found in the '0–10' μM design, i.e. with plants not previously exposed to their respective N source and then measured at an external concentration of 10 μM. Under those conditions, ϕ_{oc} for NH_4^+ was almost 75 times larger than ϕ_{oc} for NO_3^-, and, due to a low efficiency of NO_3^- utilization in white spruce in this state (evident as a relatively high efflux percentage; see Kronzucker et al. 1995b), the difference for ϕ_{net} was 100-fold. Similar differences in flux magnitude for NH_4^+ and NO_3^- were also observed when methods independent of compartmental analysis were used in our plant system (see Table 3 and Kronzucker et al. 1995b), and they are in general agreement with results of longer-term net flux estimations by other workers (see Kronzucker et al. 1995a for references).

Flux estimates from compartmental analysis were compared to those determined by unidirectional NH_4^+ influx, by 'quasi steady' flux to the vacuole (cf. Cram 1968), and by depletion of $^{14}NH_4^+$ (to estimate ϕ_{net}) (Table 3). The close agreement among these different methods provides good support for the methodology of compartmental analysis and the validity of the calculations reported in this paper.

The fluxes for NH_4^+ show a clear increase with increasing $[NH_4^+]_o$ (see Table 2). Interestingly, estimates for ϕ_{oc} and ϕ_{net} were ≈ 4.5 times higher in the '0–10' treatments than under steady-state conditions of 10 μM $[NH_4^+]_o$ (Fig. 2). This is in contrast to the situation for NO_3^-, where, with 10 μM treatment, ϕ_{oc} was more than 7-fold and ϕ_{net} close to 11-fold higher than in the '0–10' design (Kronzucker et al. 1995a, b). This is probably attributable to the induction of NO_3^- uptake by external NO_3^- (see above). With NH_4^+, a down-regulation was evident (Fig. 2). The ratio of ϕ_{co}/ϕ_{oc} (i.e. the percentage of efflux) appeared to be relatively unaffected in this down-regulation under steady-state $[NH_4^+]_o$ conditions, but, in absolute terms, the reduction in ϕ_{oc} (and ϕ_{net}) was almost fivefold. Since $[NH_4^+]_{cyt}$ was also significantly higher in the '0–10' experiments (see Table 4 and Fig. 3), the factor responsible for the observed negative feedback upon NH_4^+ uptake is probably not the cytoplasmic NH_4^+ pool under these conditions, but rather one or several down-stream assimilation product(s) produced by the flux through glutamine synthetase/glutamate synthase (GS/GOGAT). This conclusion agrees with that of other workers (Wang et al. 1993b, and references therein).

Notably, ϕ_{xylem} remained unaffected by this down-regulation of NH_4^+ transport. In fact, ϕ_{xylem} was significantly lower in the '0–10' experiments than it was at 10 μM $[NH_4^+]_o$ in the steady state. While constituting only 0.16% of ϕ_{oc} in the '0–10' treatment, ϕ_{xylem} increased almost fivefold to 3.2% of ϕ_{oc} at 10 μM $[NH_4^+]_o$. This trend of an apparent 'induction' of translocation to the stele is in marked contrast to the trend exhibited by all other fluxes estimated by compartmental analysis. A similar proportional increase in ϕ_{xylem} for NO_3^- was observed under steady-state exposure to external NO_3^- (see Kronzucker et al. 1995a, b, and Siddiqi et al. 1991), where, in analogy to the induction of NO_3^- influx at the plasmalemma, it may be attributed to the induction of a NO_3^--translocation system by external NO_3^-. Since, however, in conifer species, NO_3^- reduction usually occurs quantitatively in the roots (Bollard 1957; Pate 1980; Martin et al. 1981; Schmidt et al. 1991), so that only reduced N can be expected to be available to long-distance translocation, and since free NH_4^+ is usually not detected at any appreciable concentration in the xylem stream (Flaig and Mohr 1992; see also Wang et al. 1993a), the underlying mechanism of the observed increase in ϕ_{xylem} after prolonged exposure to the respective N species may be identical under NO_3^- and NH_4^+ nutrition. It may be that a certain threshold concentration of NH_4^+-assimilation products has to be reached in the cytoplasm before transport to the stele becomes significant. Clearly, the restriction of N translocation to the shoot in N-deprived plants has adaptive value as a means of optimizing root growth, enabling plants to intercept fresh sources of N.

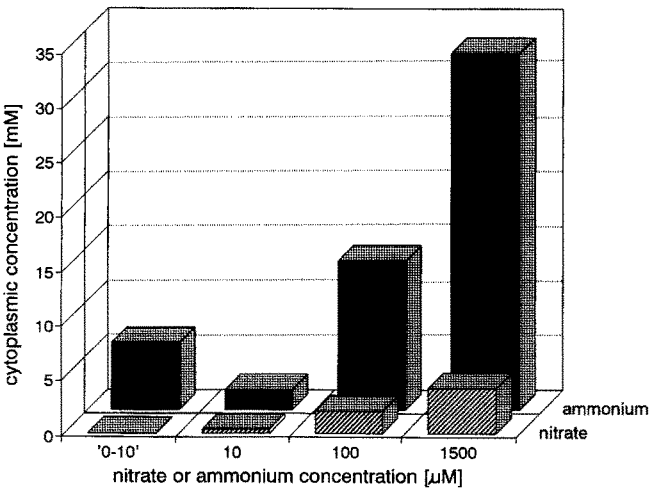

Fig. 3. Juxtaposition of the differential magnitude of cytoplasmic accumulation (in mM) of NH_4^+ (*dark-filled bars*) and NO_3^- (*dashed-line bars*) in roots of intact white-spruce seedlings at four different regimes of external concentration of the respective N species. The NO_3^- data are redrawn from Kronzucker et al. 1995a, b.

Compartmental ammonium concentrations. Few studies have been undertaken to determine $[NH_4^+]_{cyt}$ in plant roots, and no data are available for $[NH_4^+]_{cyt}$ in roots of tree species. The only higher plant studies we are aware of have relied on compartmental analyses with the isotopes ^{15}N and ^{13}N (Cooper et al. 1989; Macklon et al. 1990; Wang et al. 1993a), on mathematical modelling of ^{15}N-labelling data (Fentem et al. 1983), on ^{14}N-nuclear magnetic resonance (^{14}N-NMR; Lee and Ratcliffe 1991) and on a combination of ^{31}P- and ^{13}C-NMR (Roberts and Pang 1992). However, a direct comparison of the $[NH_4^+]_{cyt}$ values presented in our study with those reported by other groups proves problematic.

Using in-vivo ^{14}N-NMR, Lee and Ratcliffe (1991) determined values of 3–8 mM for $[NH_4^+]_{cyt}$ for maize root segments grown at 1.5 mM $[NH_4^+]_o$, and values as high as 90 mM after pretreatment of plants with the GS/GOGAT-inhibitor methionine sulphoximine (MSO).

Interestingly, in spruce, $[NH_4^+]_{cyt}$ estimates obtained by efflux analysis at 1.5 mM $[NH_4^+]_o$ and after pretreatment of plants with 1 mM MSO were in a similar range (50–70 mM; unpublished results). Roberts and Pang (1992) used NMR-signals originating from [^{31}Pi]- and [^{13}C]malate to monitor the pH changes accompanying NH_4^+ uptake, and derived $[NH_4^+]_{cyt}$ values of 3–438 μM at $[NH_4^+]_o$ up to 10 mM. However, the study employed excised root tips of maize, which were perfused with an NH_4^+-free solution for 3 h. During this rather long experimental duration, substantial NH_4^+ efflux and metabolism may have occurred, which may explain the much lower values for $[NH_4^+]_{cyt}$ than were obtained in the study by Lee and Ratcliffe (1991).

Fentem et al. (1983) used mathematical modelling of ^{15}N-labelling data to identify several distinct subcellular 'NH_4^+ pools'. These included a 'metabolic' NH_4^+ pool of 1.37 $\mu mol \cdot g^{-1}$ for basal root segments and one of $\leq$ 0.1 $\mu mol \cdot g^{-1}$ for root tips. These pool sizes, if confined to the cytoplasm, would correspond to $[NH_4^+]_{cyt}$ of 27.4 mM in basal root cells and $\approx$ 200 μM in apical root cells, if one assumes cytoplasmic volumes of $\approx$ 5% and $\approx$ 50%, respectively. Considering that these pool-size estimates were obtained at 40 μM $[NH_4^+]_o$, the values, especially for basal root cells, appear high by comparison with other reports and our values. However, it is clear that the approach by Fentem and coworkers is highly derivative, and therefore the potential for error could be considerable.

Compartmental analysis, similar to the present study, was used to provide $[NH_4^+]_{cyt}$ estimates by Cooper et al. (1989), Macklon et al. (1990) and Wang et al. (1993a). As in our study, Cooper et al. (1989) used ^{13}N, and their reported steady-state values of 40 mM for $[NH_{4+}]_{cyt}$ at 500 μM $[NH_4^+]_o$ in roots of wheat plants are close to our values in spruce. Macklon et al. (1990) reported a value of 75.8 mM for $[NH_4^+]_{cyt}$ at 2 mM $[NH_4^+]_o$ in onion slices. However, the assignment of phases in their ^{15}N-efflux analyses did not include testing for their subcellular identity. We believe that, without rigorous testing of phase identification as attempted in efflux analyses of NO_3^- by Siddiqi et al. (1991) and Kronzucker et al. (1995a, b), calculations of compartmental pool sizes are questionable.

For methodological reasons, only the study by Wang et al. (1993a), which used ^{13}N-efflux analysis and non-excised root material, is directly comparable to the present study. The values reported for roots of intact rice plants under steady-state $[NH_4^+]_o$ were 3.72 mM at 2 μM $[NH_4^+]_o$, 20.55 mM at 100 μM $[NH_4^+]_o$, and 38.08 mM at 1 mM $[NH_4^+]_o$, and are thus slightly higher than, but in the same range as our values for white spruce. This observation is consistent with rice also being a species known to prefer NH_4^+ over NO_3^-, and occurring in soils where NH_4^+ predominates. Interestingly, barley, a typical 'NO_3^- plant' (Huffaker and Rains 1978), accumulates NO_3^- in the cytoplasm to values very similar to $[NH_4^+]_{cyt}$ in rice and spruce (Siddiqi et al. 1991): $\approx$ 1 mM for N-deprived plants, 12 mM at 10 μM $[NO_3^-]_o$, 20 mM at 100 μM $[NO_3^-]_o$, and 37 mM at 1 mM $[NO_3^-]_o$ after 4 d of exposure to these respective concentrations. Values for $[NO_3^-]_{cyt}$ in spruce under conditions comparable to those of the present NH_4^+ study were, by contrast, much lower than both the reported $[NO_3^-]_{cyt}$ levels in barley (Siddiqi et al. 1991) and the $[NH_4^+]_{cyt}$ levels in rice and in spruce (Wang et al. 1993a). A direct graphical juxtaposition of the cytoplasmic accumulation data for NO_3^- versus NH_4^+ in spruce illustrates the dramatic preference for NH_4^+ over NO_3^- in this species (Fig. 3).

Conclusion

In summary, the present study has established that cytoplasmic $[NH_4^+]$ increased from $\approx$ 2 mM in white-spruce seedlings grown at low $[NH_4^+]_o$ to $\approx$ 33 mM at 1.5 mM. Likewise, the efflux of NH_4^+, (ϕ_{co}) increased under these conditions, indicating that $[NH_4^+]_{cyt}$ is the probable determinant of this flux. Interestingly, the $t_{1/2}$ values for $^{13}NH_4^+$ exchange were independent of $[NH_4^+]_o$ provided during the prior growth period. Given the substantial increase of $[NH_4^+]_{cyt}$, this indicates that there must be compensatory changes in all other fluxes (in addition to ϕ_{co}). These include ϕ_{oc}, $\phi_{vac./ass.}$, and ϕ_{xylem} (of NH_4^+ assimilates). The value of ϕ_{oc} was clearly increased by elevated $[NH_4^+]_o$, notwithstanding the apparent negative feedback from tissue N. Fluxes of N to the xylem appear to be extremely low in plants deprived of N, and are increased approx. tenfold under steady-state conditions, suggesting an apparent 'induction' of the shoot translocation term, presumably initiated by NH_4^+-assimilation products. These also appear to be responsible for the onset of negative feedback on NH_4^+ uptake, which is evident in the transition from the N-deprived state to that of steady-state supply of $[NH_4^+]_o$. Finally, the present study permits direct comparisons of parameters of NH_4^+ and NO_3^- assimilation as obtained by the same methodology (i.e. ^{13}N-compartmental analysis) in white-spruce seedlings and establishes the substantially greater efficiency of NH_4^+ assimilation in this conifer species.

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