



Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*)

David E. Rothstein^{a,*}, Bert M. Cregg^{b,1}

^aDepartment of Forestry & Department of Horticulture, Michigan State University, East Lansing, MI 48824-1222, USA

^bDepartment of Horticulture & Department of Forestry, Michigan State University, East Lansing, MI 48824-1325, USA

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Abstract

Shade-tolerant conifer species are generally thought to prefer NH_4^+ as a source of inorganic N and may perform poorly on recently disturbed sites or agricultural soils with high rates of nitrification. Fraser fir (*Abies fraseri*) is a highly shade-tolerant climax species characteristic of acidic forest soils that is now planted on a wide range of soils for Christmas tree production. We hypothesised that poor N nutrition of Fraser fir Christmas trees on calcareous soils resulted from: (1) an inability to take up and assimilate N in the form of NO_3^- and (2) a specific antagonism between uptake of Mg^{2+} and NH_4^+ . Four-year-old Fraser fir seedlings were grown for 80 days in sand culture with nutrient solutions varying in $\text{NH}_4^+:\text{NO}_3^-$ ratio (100:0, 75:25, 50:50, 25:75, 0:100) and Mg:Ca ratio (22:78, 33:67, 44:56). The $\text{NH}_4^+:\text{NO}_3^-$ ratio of nutrient solution strongly affected virtually every measure of plant performance, whereas Mg:Ca ratio exerted significant effects only on base cation nutrition. Contrary to our original hypothesis, Fraser fir was able to utilize NO_3^- as a sole N source and typically performed better under NO_3^- dominated N supply compared to NH_4^+ dominated N supply. Also contrary to our original hypothesis, we found no evidence for a specific antagonism between NH_4^+ and Mg in Fraser fir nutrition. Foliar nutrition, uptake of N, P, K, Ca and Mg, and photosynthetic capacity all decreased with increasing proportion of NH_4^+ in nutrient solutions. In vivo NO_3^- reductase activity was induced in Fraser fir roots exposed to NO_3^- demonstrating that this species is capable of taking up and assimilating NO_3^- . Our results clearly demonstrate that Fraser fir seedlings are able to utilize NO_3^- , and plants grown on 75–100% NO_3^- outperformed those grown on high NH_4^+ concentrations in terms of growth, nutrition and photosynthesis. This suggests that commercial production of Fraser fir in high nitrifying soils is not likely to be limited an inability to acquire N in the form of NO_3^- .

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1. Introduction

There has been a long history of research investigating the physiological and ecological con-

* Corresponding author. Tel.: +1 517 432 3353;

fax: +1 517 432 1143.

E-mail address: rothste2@msu.edu (D.E. Rothstein).

¹ Tel.: +1 517 355 5191x1335; fax: +1 517 353 0890.

sequences of plant species' preference for acquiring nitrogen (N) as nitrate (NO_3^-) or ammonium (NH_4^+) (Stewart et al., 1974; Lee and Stewart, 1978; Pate, 1983; Raven et al., 1992; Kronzucker et al., 1997). In general, plant species adapted to open, disturbed sites are believed to prefer NO_3^- , whereas late successional, shade-tolerant species are thought to take up predominantly NH_4^+ . Conifers, in particular, are viewed as strongly adapted to utilize NH_4^+ (Smirnoff et al., 1984; Lavoie et al., 1992) presumably because they are typically associated with acidic soils, with low rates of nitrification. More recently, an inability to utilize NO_3^- has been hypothesised as a key factor driving regeneration failure of late-successional conifers following disturbance (Kronzucker et al., 1997, 2003). Strong preferences for NH_4^+ as an N source have important implications for management when conifers are brought into cultivation since disturbance and fertilization tend to promote nitrification (Vitousek et al., 1982; Hanson et al., 2002).

Fraser fir (*Abies fraseri*) is a shade-tolerant conifer species with a highly restricted native range, occurring on very acidic soils (pH ca. 3.5) at high elevations in the southern Appalachian Mountains, USA (Beck, 1990). Because of its excellent form, color, fragrance and needle retention, planting of Fraser fir for Christmas tree production is spreading rapidly throughout the eastern USA (Alm et al., 1994; Koelling et al., 1998). In the upper Midwest, Fraser fir is often planted on soils derived from highly calcareous glacial till with much higher pH than that found in its native environment (Rothstein and Lisuzzo, in press). In a recent survey of foliar nutrition of Fraser fir in Michigan, we observed a strong inverse relationship between foliar N levels and soil pH with plants appearing N deficient on high pH soils (>6.0) (Rothstein and Lisuzzo, in press). Strong preference for NH_4^+ by Fraser fir could explain the occurrence of N limitation at high pH despite high soil N availability and widespread N fertilization. In this case, high pH soils would promote the activity of nitrifying bacteria and accelerate the conversion of NH_4^+ to NO_3^- . On these same high pH soils, we observed that foliar N levels declined as magnesium (Mg) saturation of the cation exchange complex increased suggesting competition between Mg^{2+} and NH_4^+ for root uptake (MehneJakobs and Gulpen,

1997). In contrast to Mg, we observed no apparent relationship between foliar N levels and soil calcium (Ca) availability. A better understanding of N nutrition of Fraser fir, as well as potential antagonisms between N and other mineral nutrients, is essential as production of this species expands onto calcareous soils of the Upper Midwest. We investigated the effects of varying $\text{NH}_4^+:\text{NO}_3^-$ ratios and varying Mg:Ca ratios on foliar nutrition, nutrient uptake and photosynthetic performance of Fraser fir seedlings to test the following hypotheses: (1) Fraser fir is adapted to utilize N predominantly as NH_4^+ and (2) high Mg availability interferes with NH_4^+ uptake by Fraser fir.

2. Methods

2.1. Plant materials and experimental treatments

Four-year-old (plug + 2), bare-root Fraser fir seedlings (Mount Rogers seed source) were obtained from a commercial nursery. On April 28, 2003 seedlings were potted in commercial-grade fine sand in 3.8 L tree pots (bulk density = 1.43) and placed in a greenhouse maintained at a 20/27 °C diurnal temperature regime. Individual seedlings were randomly assigned to one of 15 treatments in a complete block design with 5 levels of N ratio, 3 levels of Mg:Ca ratio and 6 replicate seedlings per treatment. The N ratio treatments consisted of 0, 25, 50, 75 and 100% NH_4^+ (with the balance as NO_3^-) at a constant total N concentration of 5 mM. Magnesium:Ca ratio treatments consisted of 22, 33 and 44% Mg (molar %, with the balance as Ca) at a constant Ca + Mg concentration of 6.9 mM. The particular source compounds used in each treatment were manipulated in order to maintain uniform concentrations of all other macro- and micronutrients (Table 1). The pH of nutrient solutions was maintained between 5.4 and 5.6 across all treatments; this range is considered optimal for Fraser fir production (Koelling, 2002). Plants were watered daily with approximately 250 mL of nutrient solution and allowed to drain freely. Gravimetric moisture content of the potting medium ranged from 20 to 14% between waterings. The position of blocks within the greenhouse was rotated monthly throughout the experiment.

Table 1
Chemical composition of nutrient solutions

Element	Element concentration	Source	Compound concentration
Macronutrients (mmoles/L)			
N	5.0	Ca(NO ₃) ₂	0–2.5
		(NH ₄) ₂ SO ₄	0–2.5
P	1.0	KH ₂ PO ₄	1.0
K	1.5	KCl	0.5
		KH ₂ PO ₄	1.0
Ca	3.9–5.4	Ca(NO ₃) ₂	0–2.5
		CaCl ₂	0.7–5
		CaSO ₄	0–1
Mg	1.5–3.1	MgCl	0–3.0
		MgSO ₄	0–1.9
S	2.5	(NH ₄) ₂ SO ₄	0–2.5
		CaSO ₄	0–1
		MgSO ₄	0–1.9
Na	0.4	NaCl	0.4
Micronutrients (μmoles/L)			
Fe	12	Fe EDTA	12
Mn	4.7	MnSO ₄	4.7
Cu	0.3	CuSO ₄	0.3
Zn	0.7	ZnSO ₄	0.7
Mo	0.07	(NH ₄) ₆ Mo ₇ O ₂₄	0.01
B	2.4	H ₃ BO ₃	2.4

2.2. Physiological measures

In order to assess the effects of N form and Mg:Ca ratio on foliage health and photosynthetic performance we measured chlorophyll fluorescence and CO₂ exchange on days 76 and 77 of the study (July 13 and 14, 2003). Chlorophyll fluorescence measurements were made on three randomly-selected needles per tree using a portable fluorometer (PEA, Hansatech Instruments, UK). For each needle we measured the parameter F_v/F_m , or the ratio of variable fluorescence (F_v) to maximum total fluorescence (F_m), after a 15 min dark incubation. F_v/F_m provides an estimate of the photochemical efficiency of photosystem II, which is sensitive to nutrient and other environmental stresses (Mohammed et al., 1995).

Light saturated photosynthetic rate (A_{\max}) and dark respiration (R_d) were determined on one shoot from the topmost whorl on each seedling using a

portable gas exchange system (LI-6400, Li-Cir, Inc., Lincoln, NE) equipped with a 0.15 L conifer cuvette. Preliminary photosynthetic light response curves indicated that photosynthesis of the seedlings was light saturated at PPFD greater than 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For our A_{\max} measurements ambient light in the greenhouse was supplemented with a high-pressure sodium lamp to ensure that PPFD remained above light saturation (mean PPFD = 1305 $\mu\text{mol m}^{-2} \text{s}^{-2}$ (± 26.1)). Air temperature in the conifer cuvette was maintained at 30.6 °C (± 0.4) by means of integrated Peltier coolers on the chamber block. The CO₂ concentration of the air entering the conifer chamber was maintained at 400 ppm. Dark respiration was measured after the A_{\max} measurement by completely darkening the leaf chamber with foil and allowing the CO₂ exchange rate to equilibrate (usually ca. 5 min). Immediately after the gas exchange measurements, the portion of the shoot enclosed in the conifer chamber was excised from the plant and placed into a labeled plastic bag for cold storage. The projected shoot area of each shoot was determined using a photoelectric leaf area meter (LI-3100, Li-Cor, Inc. Lincoln, NE). All gas exchange measurements are presented on a projected shoot area basis.

In order to determine the potential for Fraser fir to assimilate NO₃⁻, we measured in vivo activity of the enzyme NO₃⁻ reductase in needles and fine-roots at the time of final harvest (days 79 and 80). Nitrate reductase catalyzes the reduction of NO₃⁻ to NO₂⁻, the first and rate limiting step in the assimilation of NO₃⁻ (Beevers and Hageman, 1969). Approximately, 0.5 g fresh weight of leaf or root tissue was suspended in 7.5 mL of an assay medium consisting of 0.1 M NaH₂PO₄ (pH 7.5), 0.04 M KNO₃, 5% propanol and 0.5 mg/mL chloramphenicol (Jaworski, 1971). The tissue was vacuum infiltrated and then incubated in the dark at 25 °C. After 1 h of incubation, a 1 mL aliquot was removed and immediately analyzed for nitrite (NO₂⁻) by colorimetric reaction with sulfanilimide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Following the incubation, root and needle samples were dried at 65 °C to determine the oven-dry mass of tissue in each incubation. Nitrate reductase activity was calculated as nmoles of NO₂⁻ produced per gram dry weight of tissue per hour ($\text{nmol g}^{-1} \text{h}^{-1}$).

2.3. Biomass, foliar nutrition and nutrient uptake

On July 15 and 16, 2003 plants were harvested by block, their root systems washed free of sand with deionized water and dried to a constant mass at 65 °C. Each dried plant was separated into current-year needles, older needles, twigs, stems, coarse roots and fine-roots (<2 mm diameter). Each tissue sample was weighed, ground coarsely with a Wiley mill (10 mesh), and then a representative subsample was ground to a fine powder using a ball mill. Nitrogen concentration was determined by dry combustion using a Carlo Erba NA 1500 elemental analyzer (Carlo-Erba, Milan, Italy), whereas P, K, Ca and Mg concentrations were determined by nitric acid digestion followed by analysis with a dual-coupled plasma atomic emission spectrometer (SMI Inc.). Reference standard pine needles (#1575) from the National Institute of Standards and Technology were run with samples for all procedures to ensure accuracy of results within $\pm 10\%$ CV. Total nutrient content of each plant at the time of harvest was determined by summing the product of mass \times nutrient concentration for all tissues. Masses of needles and fine-roots for each seedling were adjusted to account for tissue removed at harvest for measurement of NO_3^- reductase activity (described above).

In order to determine nutrient uptake over the course of the experiment we used an indirect method to estimate the initial content of N, P, K, Ca and Mg of each tree in the experiment. At the time of potting, all trees were measured for aboveground height and basal diameter; however, an additional random subsample of 20 plants was measured, immediately oven dried (65 °C), and then processed for nutrient analysis as described above. This initial harvest was used to determine the relationships among initial tree size, initial biomass and initial nutrient content. Relationships for each nutrient are as follows:

$$\text{Initial biomass (g)} = -27.80 + 43.22 \times \text{BD} + 0.37 \times H \quad (R^2 = 0.784).$$

$$\text{Total N (mg)} = -319.33 + 461.41 \times \text{BD} + 6.68 \times H \quad (R^2 = 0.807).$$

$$\text{Total P (mg)} = -45.79 + 59.33 \times \text{BD} + 1.13 \times H \quad (R^2 = 0.725).$$

$$\text{Total K (mg)} = -167.42 + 226.52 \times \text{BD} + 3.15 \times H \quad (R^2 = 0.782).$$

$$\text{Total Ca (mg)} = -106.11 + 122.54 \times \text{BD} + 3.45 \times H \quad (R^2 = 0.672).$$

$$\text{Total Mg (mg)} = -30.90 + 37.95 \times \text{BD} + 1.03 \times H \quad (R^2 = 0.664).$$

where BD is the basal diameter in cm and H is the height in cm. Relative nutrient uptake (mg/g) was calculated for each harvested plant by subtracting the estimate of its initial nutrient content from its final nutrient content and dividing by the estimate of its initial dry mass (mg/g). Root specific nutrient uptake was calculated for each harvested plant subtracting the estimate of its initial nutrient content from its final nutrient content and dividing by its final fine-root mass (mg/g).

2.4. Data analysis

Differences in nutrient uptake, foliar nutrition, gas exchange, nitrate reductase activity and biomass distribution among treatments were analyzed using a two-way analysis of variance (ANOVA) with main effects of $\text{NH}_4^+:\text{NO}_3^-$ ratio and Mg:Ca ratio. Because chlorophyll fluorescence data could not be adequately transformed to meet assumptions of normality and homogeneity of variance, these data were analyzed using a non-parametric Kruskal–Wallis test. We used stepwise multiple regression analyses (backward elimination) to investigate the influence of foliar nutrient concentrations on photosynthetic rates. All statistical analyses were conducted using SYSTAT for personal computers. Statistical significance was accepted at an $\alpha = 0.05$.

3. Results

The $\text{NH}_4^+:\text{NO}_3^-$ ratio of the nutrient solution strongly affected virtually every measure of plant performance, whereas Mg:Ca ratio affected only base cation nutrition. To ease interpretation of the effects of $\text{NH}_4^+:\text{NO}_3^-$ ratio, all figures present data pooled across Mg:Ca treatments whenever P -values for the Mg:Ca main effect, and for the interaction term, were greater than 0.1. Complete ANOVA results for all parameters are presented in Table 2. There was a trend toward lower total plant biomass with increasing NH_4^+ concentration in the nutrient solution (Table 2; Fig. 1); however, differences among treatments were not

Table 2
Analysis of variance results

	NH ₄ ⁺ :NO ₃ ⁻ effect		Mg:Ca effect		Interaction	
	F _{4,75}	P	F _{2,75}	P	F _{8,75}	P
Final biomass						
Total plant	2.19	0.079	0.007	0.993	1.43	0.199
Current needles	1.00	0.411	0.505	0.606	1.01	0.434
Older needles	0.43	0.788	0.23	0.793	1.26	0.280
Twigs	4.27	0.004	0.48	0.618	0.99	0.447
Stems	1.64	0.173	0.11	0.893	1.63	0.131
Coarse roots	1.75	0.173	0.11	0.893	1.63	0.131
Fine-roots	4.51	0.003	0.77	0.468	0.69	0.700
Foliar nutrition						
N	3.65	0.009	0.16	0.853	0.37	0.931
P	3.06	0.023	0.93	0.400	0.13	0.997
K	2.92	0.027	3.94	0.024	0.94	0.493
Ca	18.63	< 0.001	5.64	0.005	0.80	0.608
Mg	2.59	0.044	8.27	< 0.001	1.02	0.427
Whole-plant nutrient uptake						
N	1.77	0.144	1.55	0.220	1.45	0.190
P	7.66	< 0.001	1.95	0.15	1.36	0.230
K	4.46	0.003	1.55	0.219	1.43	0.198
Ca	22.83	< 0.001	9.25	< 0.001	1.76	0.099
Mg	6.71	< 0.001	4.33	0.017	1.14	0.348
Specific root uptake						
N	0.789	0.536	0.571	0.568	0.852	0.561
P	2.219	0.076	0.850	0.432	0.820	0.587
K	0.737	0.570	0.677	0.512	0.855	0.559
Ca	6.495	< 0.001	1.736	0.184	0.570	0.799
Mg	1.322	0.271	3.711	0.029	0.613	0.764
NO ₃ ⁻ reductase	17.47	< 0.001	0.18	0.839	0.69	0.768
A _{max}	2.62	0.042	0.794	0.456	0.735	0.660
Dark respiration	1.90	0.119	0.168	0.846	0.99	0.444
Stomatal condition	5.95	< 0.001	0.44	0.643	0.78	0.625

All effects significant at $P < 0.1$ are highlighted in bold.

statistically significant. This pattern was driven by significant declines in fine-root and twig biomass, whereas there were no significant effects of NH₄⁺:NO₃⁻ ratio on biomass of new needles, older needles, stem tissue, or coarse roots (Table 2). Variation in the Mg:Ca ratio of the nutrient solution had no effect on plant biomass pools (Table 2).

Concentrations of all foliar nutrients varied significantly among NH₄⁺:NO₃⁻ treatments (Table 2, Fig. 2). Foliar N concentrations were greater at intermediate NH₄⁺:NO₃⁻ ratios than with either pure NO₃⁻ or pure NH₄⁺. Foliar P concentrations were greatest at 25% NH₄⁺, K concentrations varied inconsistently with NH₄⁺:NO₃⁻ ratio, and Ca and

Mg concentrations decreased as the proportion of NH₄⁺ in nutrient solutions increased. Varying the Mg:Ca ratio of the nutrient solution had no effect on foliar N and P levels; however, foliar Mg levels increased, and foliar K and Ca decreased, as the percentage of Mg increased.

Results for nutrient uptake largely reflected patterns of foliar nutrition, in that NH₄⁺:NO₃⁻ ratio exerted strong effects on most nutrients, whereas Mg:Ca ratio only affected uptake of Ca and Mg (Table 2; Fig. 3). Interestingly, N uptake was not significantly affected by the NH₄⁺:NO₃⁻ ratio of the nutrient solution, although the trend was towards peak uptake by plants receiving only 25% of their N as

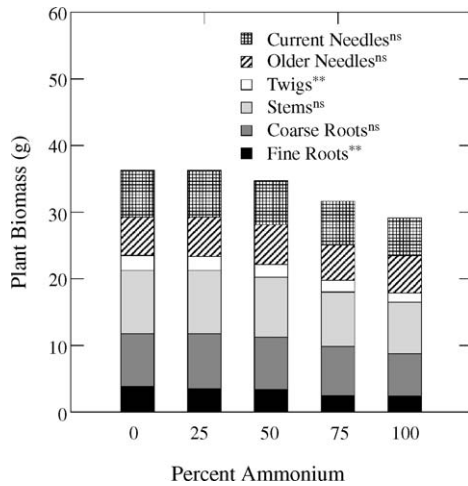


Fig. 1. Component and total biomass of Fraser fir seedlings grown under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. There was no effect of Mg:Ca, nor any interaction between $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca, therefore data are pooled across Mg:Ca treatments. Significance levels for main effects are noted as follows: ns, $P > 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

NH_4^+ . Uptake of P and K followed a similar pattern across N ratio treatments with uptake being greatest in plants receiving 0–25% of their N as NH_4^+ and decreasing with increasing NH_4^+ concentration.

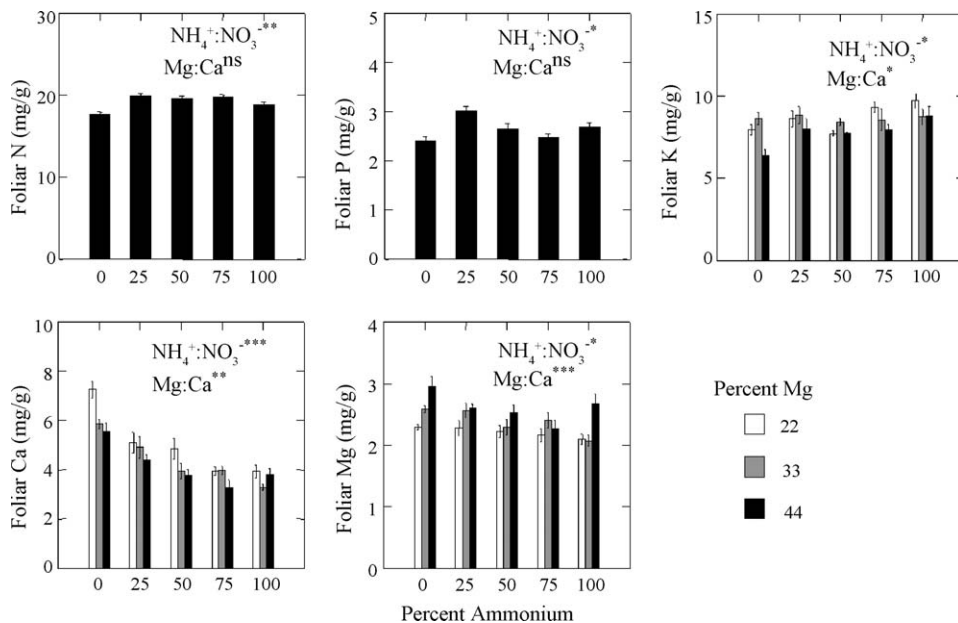


Fig. 2. Concentrations of N, P, K, Ca and Mg in current-year needles of Fraser fir under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. Magnesium:Ca ratio had no effect on foliar N or P concentrations, therefore data for these elements are pooled across Mg:Ca treatments. Values are treatment means \pm 1 S.E. Symbols denoting significance levels are as in Fig. 1. There were no significant interactions between $\text{NH}_4^+:\text{NO}_3^-$ and Ca:Mg effects.

Uptake of Ca decreased markedly with increasing proportion of NH_4^+ or Mg in the nutrient solution. Magnesium depression of Ca uptake was pronounced at low $\text{NH}_4^+:\text{NO}_3^-$ ratios, whereas at NH_4^+ concentrations greater than 50% Ca uptake varied little across Mg:Ca treatments; however, the interaction term was not statistically significant ($P = 0.099$). Uptake of Mg generally decreased with increasing NH_4^+ concentration and increased with increasing Mg concentration. When nutrient uptake was expressed on a fine-root biomass basis, differences among $\text{NH}_4^+:\text{NO}_3^-$ treatments were always reduced, and in the case of K and Mg, disappeared entirely (Table 2; Fig. 4).

We were unable to detect any NO_3^- reductase activity in Fraser fir needle tissue, irrespective of treatment. In contrast, root NO_3^- reductase increased markedly with increasing NO_3^- concentration in the nutrient solution (Table 2; Fig. 5). Magnesium:Ca ratio had no effect on NO_3^- reductase activity. We found no differences among treatments in rates of needle dark respiration; however, the trend was toward increased respiration at high NH_4^+ concentrations (Table 2; Fig. 6). In contrast to respiration, plants in the 100% NO_3^- treatment had the highest photosynthetic capacity and stomatal conductance, and

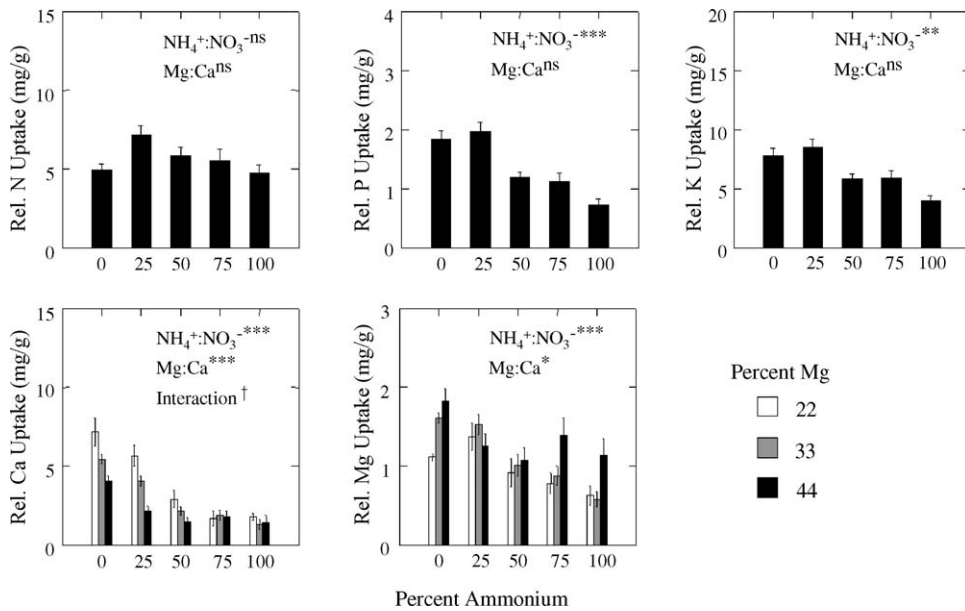


Fig. 3. Relative uptake of N, P, K, Ca and Mg by Fraser fir seedlings under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. Relative uptake for each seedling was calculated as mg of each nutrient element taken up per g of initial biomass. Magnesium:Ca ratio had no effect on N, P or K uptake, therefore data for these elements are pooled across Mg:Ca treatments. Values are treatment means \pm 1S.E. Symbols denoting significance levels are as in Fig. 1. There were no significant interactions between $\text{NH}_4^+:\text{NO}_3^-$ and Ca:Mg effects for N, P, K or Mg uptake.

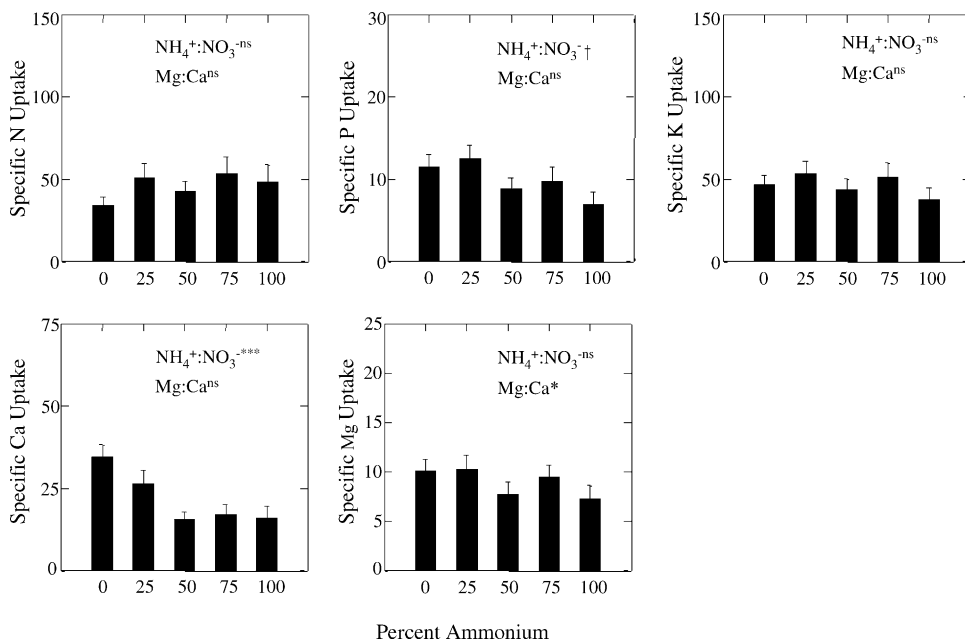


Fig. 4. Specific root uptake of N, P, K, Ca and Mg by Fraser fir seedlings grown under varying $\text{NH}_4^+:\text{NO}_3^-$ ratios. Specific uptake for each seedling was calculated as mg of each nutrient element taken up per g of final fine-root biomass. Data are pooled across all Mg:Ca treatments. Values are treatment means \pm 1S.E. Symbols denoting significance levels are as in Fig. 1.

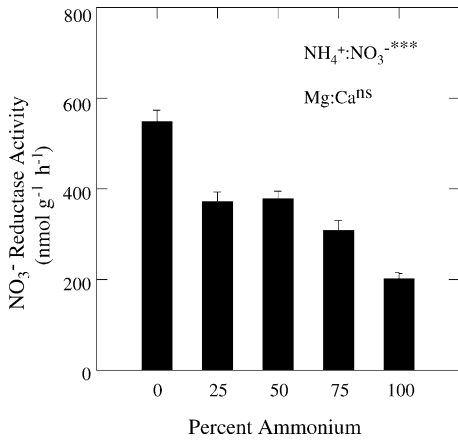


Fig. 5. Nitrate reductase activity in fine-roots of Fraser fir seedlings under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. There was no effect of Mg:Ca, nor any interaction between $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca, therefore data are pooled across Mg:Ca treatments. Values are treatment means \pm 1 S.E. Symbols denoting significance levels are as in Fig. 1.

A_{\max} and g decreased significantly as NH_4^+ concentration increased (Fig. 6). Stepwise multiple regression analysis demonstrated that there were no statistically significant relationships between foliar nutrition variables (singly or in combination) and A_{\max} (data not shown). In contrast, stomatal conductance was an excellent predictor of A_{\max} : $A_{\max} = -0.60 + 129.05 \times g - 338.92 \times g^2$; $R^2 = 0.896$; $P < 0.001$. Finally, there were no significant differences in F_v/F_m across either $\text{NH}_4^+:\text{NO}_3^-$ (Kruskal–Wallis Test Statistic = 0.769; $P = 0.943$), or Mg:Ca (Kruskal–Wallis Test Statistic = 0.342; $P = 0.843$), treatments.

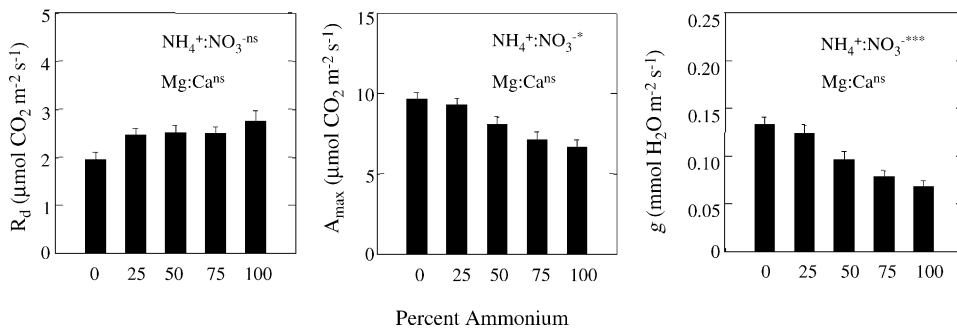


Fig. 6. Dark respiration (R_d), maximal photosynthetic capacity (A_{\max}) and stomatal conductance (g) in current-year needles of Fraser fir seedlings under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. There were no significant effects of Mg:Ca, nor any interactions between $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca, therefore data are pooled across Mg:Ca treatments. Values are treatment means \pm 1 S.E. Symbols denoting significance levels are as in Fig. 1.

4. Discussion

Contrary to our original hypothesis, Fraser fir was able to utilize NO_3^- as a sole N source and typically performed better under NO_3^- dominated N supply compared to NH_4^+ dominated N supply. Poor nutrition and plant performance at high NH_4^+ concentrations appeared to be driven by NH_4^+ effects on fine-root production, and possible effects of NH_4^+ on water transport. Also contrary to our original hypothesis, we found no evidence for a specific antagonism between NH_4^+ and Mg in Fraser fir nutrition. In fact, the strongest antagonism we observed was between NH_4^+ and Ca. Results from this study indicate that inability to utilize NO_3^- cannot be generalized to all shade-tolerant conifers, and that poor N nutrition of Fraser fir on high pH agricultural soils cannot be explained solely by an inability to utilize N in the form of NO_3^- .

The effects of N form on conifer N acquisition can be viewed as a tradeoff between the positive effects of NH_4^+ on specific uptake rates (i.e. uptake per unit root biomass) and negative effects of NH_4^+ on root production. Most conifer species exhibit far greater specific uptake rates for NH_4^+ compared to NO_3^- (e.g. Rygielwicz et al., 1984a,b; Kamminga-van Wijk and Prins, 1993; Buchmann et al., 1995; Kronzucker et al., 1997), yet many studies have also demonstrated that high ammonium concentrations inhibit conifer fine-root production (Van Dijk et al., 1990; Boxman et al., 1991; Olsthoorn et al., 1991; Ohlund and Nasholm, 2001). Our data indicate that high $\text{NH}_4^+:\text{NO}_3^-$ ratios inhibited fine-root production (Table 2; Fig. 1), but we

did not observe a compensatory increase in specific N uptake rates under NH_4^+ nutrition (Fig. 4). Instead we observed significantly higher foliar N concentrations, and a non-significant increase in total N uptake in mixed NH_4^+ and NO_3^- treatments relative to pure NH_4^+ or NO_3^- (Table 2; Fig. 3). These results are consistent with previous studies demonstrating improved N nutrition when both NH_4^+ and NO_3^- are present in the growth medium (Britto and Kronzucker, 2002), and suggest a synergistic effect of NH_4^+ and NO_3^- on plant N uptake. One potential mechanism driving this response may be differential effects of NH_4^+ versus NO_3^- nutrition on solution pH. Uptake of NH_4^+ tends to acidify the growing medium which can then facilitate NO_3^- uptake through H^+/NO_3^- cotransport (Mengel and Kirkby, 2001). While we maintained all nutrient solutions at a constant pH, it is possible that changes in solution pH in the period between daily waterings may have facilitated improved N uptake in mixed solutions relative to pure NH_4^+ or NO_3^- treatments.

Whereas $\text{NH}_4^+:\text{NO}_3^-$ ratio had relatively small effects on N nutrition, increasing the proportion of NH_4^+ in the growing medium strongly depressed uptake of P, K, Ca and Mg. In general, the maintenance of charge balance across the plasma-membrane is one of the key mechanisms by which $\text{NH}_4^+:\text{NO}_3^-$ ratio is thought to influence the uptake of other nutrient elements (Mengel and Kirkby, 2001). Thus, uptake of N as NH_4^+ should promote the uptake of anions and inhibit uptake of other cations. The fact that we observed strong declines in uptake of all nutrients with increasing NH_4^+ concentration, irrespective of charge, suggests that depression of root production at high $\text{NH}_4^+:\text{NO}_3^-$ ratios may have been the overriding factor. The fact that differences in specific nutrient uptake rates among N form treatments (Fig. 4) were small in comparison to total plant uptake (Fig. 3) provides further evidence that poor plant nutrition at high $\text{NH}_4^+:\text{NO}_3^-$ ratios is driven primarily by NH_4^+ effects on fine-root production.

Comparisons of foliar nutrient concentrations (Fig. 2) and whole-plant nutrient uptake (Fig. 3) demonstrate the danger of relying solely on foliar nutrition data to infer patterns of nutrient uptake. Notice that foliar concentrations of P, K and Mg show relatively weak response to $\text{NH}_4^+:\text{NO}_3^-$ ratio, whereas uptake of all three nutrients declined markedly with

increasing NH_4^+ concentration. Calcium was the only nutrient for which foliar concentrations reflected the effects of $\text{NH}_4^+:\text{NO}_3^-$ ratio on total plant uptake. Because P, K and Mg are all mobile within the plant and Ca is not (Mengel and Kirkby, 2001), these data suggest that discrepancies between foliar concentrations and estimates of whole-plant uptake reflect retranslocation of mobile nutrients to new foliage.

Contrary to our hypothesis of a specific antagonism between Mg^{2+} and NH_4^+ uptake, we found much stronger evidence for an antagonism between Ca^{2+} and NH_4^+ uptake. Ammonium concentration in the nutrient solution had a much stronger effect on Ca uptake than it did on either K or Mg uptake (Table 2; Fig. 3). Both foliar Ca and Ca uptake declined with increasing Mg:Ca ratio, and the response of whole-plant Ca uptake to Mg:Ca ratio appeared to be conditional on NH_4^+ concentration (Table 2; Fig. 3). In this case, increased Mg:Ca ratio strongly depressed Ca uptake at low $\text{NH}_4^+:\text{NO}_3^-$ ratio but had no effect at high NH_4^+ . This suggests that the NH_4^+ inhibition of Ca uptake overwhelms effects of Mg:Ca ratio at high NH_4^+ concentration. It is important to note that we cannot infer a specific antagonism between Ca and Mg because increases in Mg concentration in our treatments were accompanied by decreases in Ca and vice versa. Thus, decreased Ca uptake at high Mg:Ca ratio could have resulted solely from a decline in solution Ca concentration.

Gas exchange data provide further evidence that Fraser fir is able to utilize NO_3^- as an N source, in that A_{max} increased as the proportion of NO_3^- in nutrient solutions increased (Fig. 6). Greater photosynthetic rates at low NH_4^+ concentrations appear to be driven by changes in plant water relations, rather than any changes in foliar nutrition. Evidence for this conclusion includes: (1) no concomitant effects of $\text{NH}_4^+:\text{NO}_3^-$ ratio on rates of dark respiration (Fig. 6) or chlorophyll fluorescence (Fig. 7); (2) no significant relationship between foliar nutrient concentrations and photosynthetic rates and (3) the strong relationship between A_{max} and stomatal conductance. These results are consistent with other studies documenting decreased stomatal conductance and water uptake under NH_4^+ nutrition in a variety of plant species (Pill and Lambeth, 1977; Adler et al., 1996; Guo et al., 2002; Lu et al., 2005). Increasing root:shoot ratios under NH_4^+ nutrition may play a role in decreased

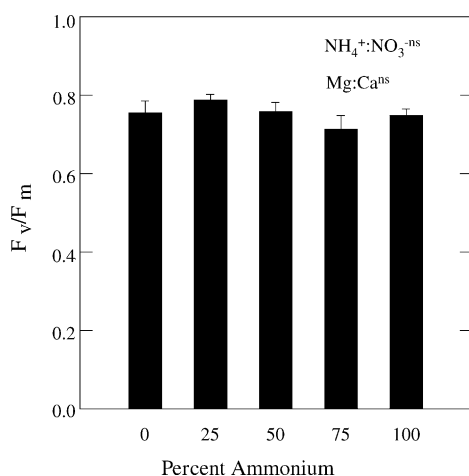


Fig. 7. Maximum quantum yield (F_v/F_m) in current-year needles of Fraser fir seedlings under varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. There were no effects of Mg:Ca, nor any interactions between $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca, therefore data are pooled across Mg:Ca treatments. Values are treatment means \pm 1 S.E. Symbols denoting significance levels are as in Fig. 1.

stomatal conductance at high NH_4^+ concentrations; however, decreased root hydraulic conductivity (Adler et al., 1996; Guo et al., 2002) and elevated abscisic acid levels under NH_4^+ nutrition have also been implicated (Peuke et al., 1994; Jeschke and Hartung, 2000). Regardless of mechanism, it is clear that photosynthetic performance of Fraser fir seedlings declines markedly under NH_4^+ nutrition relative to NO_3^- nutrition.

Contrary to current thinking that shade-tolerant conifers are unable to effectively utilize NO_3^- , results from the NO_3^- reductase assay clearly show that Fraser fir is capable of assimilating NO_3^- . In the 100% NH_4^+ treatment root NO_3^- reductase activity was at the low end of the range reported for woody perennials (100–4000 $\text{nmol g}^{-1} \text{h}^{-1}$; Al Gharbi and Hipkin, 1984; Downs et al., 1993; Knoepp et al., 1993; Truax et al., 1994) and likely represents constitutive activity. However, NO_3^- reductase was clearly induced by the presence of NO_3^- in the rooting medium, allowing Fraser fir to maintain adequate N nutrition in the absence of NH_4^+ . Note that foliar N concentrations in the 0% NH_4^+ treatment were well above the sufficiency threshold of 15 mg/g for Fraser fir Christmas trees (Fisher, 1996). Our data are consistent with a large body of work demonstrating that conifers

typically assimilate NO_3^- in the roots and transport N to shoots as amino acids (Smirnov and Stewart, 1985). This does have implications for growing Fraser fir in a horticultural setting where they are exposed to high irradiance, because reduction of NO_3^- in foliage can greatly reduce the C cost of N assimilation under light-saturated conditions (Smirnov and Stewart, 1985).

Because we are relying on an indirect estimate of initial plant biomass and nutrient pools to calculate uptake, it is important to evaluate the potential for errors in these estimates to confound our interpretations of the response of nutrient uptake to varying $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca ratios. Analysis of residuals from the regressions of biomass and nutrient pools against diameter and height indicates that errors in our initial estimates of biomass, N, P, K, Ca and Mg averaged 13, 11, 16, 13, 17 and 17%, respectively. While these errors are not ideal, they are unavoidable given the destructive nature of nutrient analysis. Furthermore, we argue that these errors do not significantly compromise our study for two reasons. First, errors in estimates of initial nutrient pools were small relative to variations in nutrient uptake among treatments. For example, note the 2–3-fold differences in uptake rates for P, K, Ca and Mg across $\text{NH}_4^+:\text{NO}_3^-$ ratio treatments. Second, errors in estimates of initial nutrient pools reflect random error and not systematic bias; there was no significant variation in initial plant size among treatments ($P = 0.914, 0.641$ for $\text{NH}_4^+:\text{NO}_3^-$ and Mg:Ca treatments, respectively). Therefore, any errors associated with estimates of initial nutrient pools would have reduced our power to detect differences among treatments but would not have introduced systematic bias into our study.

Differences in soil N availability and plant N acquisition strategies through ecological succession and among forest types have been of great interest to ecologists for decades. Nitrate is thought to dominate soil mineral N pools in recently disturbed sites (Vitousek et al., 1982) and in high pH soils (Pastor et al., 1984; Zak and Pregitzer, 1990), whereas NH_4^+ is thought to be dominant in late successional ecosystems (Rice and Panchoy, 1972) and on highly acidic soils (Pastor et al., 1984; Zak and Pregitzer, 1990; Ste-Marie and Pare, 1999). In general, plant N acquisition strategies are thought to mirror these patterns of NO_3^- and NH_4^+ availability, with species characteristic of late-successional habitats and/or strongly acidic soils

exhibiting strong preferences for NH_4^+ nutrition (Pate, 1983; Smirnoff et al., 1984; Rothstein et al., 1996; Lavoie et al., 1992; Kronzucker et al., 1997). As a highly shade-tolerant climax species characteristic of acidic forest soils, Fraser fir would appear to fit the profile of a strongly NH_4^+ adapted species. However, our results clearly demonstrate that Fraser fir seedlings are able to utilize NO_3^- , and plants grown on 75–100% NO_3^- outperformed those grown on high NH_4^+ concentrations in terms of growth, nutrition and photosynthesis. While these results are from a single, short-term study under highly controlled conditions, they nevertheless suggest that commercial production of Fraser fir in high nitrifying soils is not likely to be impacted by poor N nutrition as has been shown for other late-successional conifers (Jobidon et al., 1989; Kronzucker et al., 1997, 2003).

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References

- Adler, P.R., Wilcox, G.E., Markhardt, A.H., 1996. Ammonium decreases muskmelon root system hydraulic conductivity. *J. Plant Nut.* 19, 1395–1403.
- Al Gharbi, A., Hipkin, C.R., 1984. Studies on nitrate reductase in British angiosperms. I A comparison of nitrate reductase activity in ruderal, woodland-edge and woody species. *New Phytol.* 97, 629–639.
- Alm, A., Vogt, C., Werner, C., 1994. Growing Christmas trees, a guide for Minnesota and the North Central States. Univ. of Minnesota Ext. Bull. BU-02022, Univ. Minnesota Ext., St. Paul, MN.
- Beck, D.E., 1990. *Abies fraseri* (Pursh) Poir. Fraser fir. In: Silvics of North America, vol. 1, Conifers. Agric. Handb. 654. U.S. Dept. of Agric., Forest Service Washington, DC, pp. 47–51.
- Beevers, L., Hageman, R.H., 1969. Nitrate reduction in higher plants. *Annu. Rev. Plant. Physiol.* 20, 495–522.
- Boxman, A.W., Krabbendam, H., Bellemakers, M.J.S., Roelofs, G.M., 1991. Effects of ammonium and aluminum on the development and nutrition of *Pinus nigra* in hydroculture. *Environ. Pollut.* 73, 119–136.
- Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. *J. Plant Physiol.* 159, 567–584.
- Buchmann, N., Schulze, E.D., Gebhauer, G., 1995. ^{15}N -ammonium and ^{15}N -nitrate uptake of a 15-year-old *Picea abies* plantation. *Oecologia* 102, 361–370.
- Downs, M.R., Nadelhoffer, K.J., Melillo, J.M., Aber, J.D., 1993. Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. *Trees* 7, 233–236.
- Fisher, J.H., 1996. Adequate nutrient ranges for scotch pine, Douglas-fir and Fraser fir Christmas trees in Michigan. *Michigan Christmas Tree J.* 42, 25–27.
- Guo, S., Bruck, H., Sattelmacher, B., 2002. Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants - Nitrogen form and water uptake. *Plant Soil* 239, 267–275.
- Hanson, E.J., Throop, P.A., Serce, S., Ravenscroft, J., Paul, E.A., 2002. Comparison of nitrification rates in blueberry and forest soils. *J. Am. Soc. Hortic. Sci.* 127, 136–142.
- Jaworski, E.G., 1971. Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* 43, 1274–1279.
- Jeschke, W.D., Hartung, W., 2000. Root–shoot interactions in mineral nutrition. *Plant Soil* 226, 57–69.
- Jobidon, R., Thibault, J.R., Fortin, J.A., 1989. Phytotoxic effect of barley, oat, and wheat straw mulches in eastern Quebec forest plantations. 2. Effects on nitrification and black spruce (*Picea mariana*) seedling growth. *For. Ecol. Manag.* 29, 295–310.
- 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 solutions containing both NH_4^+ and NO_3^- . *Plant Soil* 151, 91–96.
- Knoepp, J.D., Turner, D.P., Tingey, D.T., 1993. Effects of ammonium and nitrate on nutrient uptake and activity of nitrogen assimilating enzymes in western hemlock. *For. Ecol. Manag.* 59, 179–191.
- Koelling, M.R., 2002. Fertilization recommendations for Fraser fir: Part II established plantings. *Michigan Christmas Tree J.* 48, 5–8.
- Koelling, M.R., Hart, J.B., Leefers, L., 1998. Christmas tree production in Michigan. Agric. Exp. Sta. Special Rep. SR619201, Mich. State Univ. Ext., East Lansing, MI.
- 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., Siddiqi, M.Y., Glass, A.D.M., Britto, D.T., 2003. Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis. *Physiol. Plantarum* 117, 164–170.
- Lavoie, N., Venzina, L.P., Margolis, H., 1992. Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. *Tree Physiol.* 11, 171–183.
- Lee, J.A., Stewart, G.R., 1978. Ecological aspects of nitrogen assimilation. *Adv. Bot. Res.* 6, 1–43.

- Lu, Y.X., Li, C.J., Zhang, F.S., 2005. Transpiration, potassium uptake and flow in tobacco as affected by nitrogen forms and nutrient levels. *Ann. Bot.* 95, 991–998.
- MehneJakobs, B., Gulpen, M., 1997. Influences of different nitrate to ammonium ratios on chlorosis, cation concentrations and the binding forms of Mg and Ca in needles of Mg-deficient Norway spruce. *Plant Soil* 188, 267–277.
- Mengel, K., Kirkby, E.A., 2001. *Principles of Plant Nutrition*, fifth ed. Kluwer Academic Publishers, Dordrecht, Boston.
- Mohammed, G.H., Binder, W.D., Gillies, S.L., 1995. Chlorophyll fluorescence—a review of its practical forestry applications and instrumentation. *Scand. J. For. Res.* 10, 383–410.
- Ohlund, J., Nasholm, T., 2001. Growth of conifer seedlings on organic and inorganic nitrogen sources. *Tree Physiol.* 21, 1319–1326.
- Olsthoorn, A.F.M., Keltjens, W.G., Van Baren, B., Hopman, M.C.G., 1991. Influence of ammonium on fine root development and rhizosphere pH of Douglas-fir seedlings in sand. *Plant Soil* 133, 75–81.
- Pastor, J., Aber, J.D., McLaugherty, C.A., Melillo, J.M., 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. *Ecology* 65, 256–268.
- Pate, J.S., 1983. Patterns of nitrogen metabolism in higher plants and their ecological significance. In: Lee, J.A., McNeill, S., Rorison, I.H. (Eds.), *Nitrogen as an Ecological Factor*. Blackwell Scientific Publications, Oxford, UK, pp. 225–255.
- Peuke, A.D., Jeschke, W.D., Hartung, W., 1994. The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long distance transport of abscisic acid depending on nitrogen nutrition and salt stress. *J. Exp. Bot.* 45, 741–747.
- Pill, W.G., Lambeth, V.N., 1977. Effects of NH_4^+ and NO_3^- nutrition with and without pH adjustment on tomato growth, ion composition, and water relations. *J. Am. Soc. Hort. Sci.* 102, 78–81.
- Raven, J.A., Wollenweber, B., Handley, L.L., 1992. A comparison of ammonium and nitrate as nitrogen sources for photolithotrophs. *New Phytol.* 121, 19–32.
- Rice, E.L., Pancholy, S.K., 1972. Inhibition of nitrification by climax ecosystems. *Am. J. Bot.* 59, 1033–1040.
- Rothstein, D.E., Lisuzzo, N.J. Optimal nutrition and diagnosis for *Abies fraseri* Christmas trees in Michigan. *Northern J. Appl. For.*, in press.
- Rothstein, D.E., Zak, D.R., Pregitzer, K.S., 1996. Nitrate deposition in northern hardwood forests and the nitrogen metabolism of *Acer saccharum* Marsh. *Oecologia* 108, 338–344.
- Rygiiewicz, P.T., Bledsoe, C.S., Zasoski, R.J., 1984a. Effects of ectomycorrhizae and solution pH on $[\text{N-15}]$ ammonium uptake by coniferous seedlings. *Can. J. For. Res.* 14, 885–892.
- Rygiiewicz, P.T., Bledsoe, C.S., Zasoski, R.J., 1984b. Effects of ectomycorrhizae and solution pH on $[\text{N-15}]$ nitrate uptake by coniferous seedlings. *Can. J. For. Res.* 14, 893–899.
- Smirnov, N., Stewart, G.R., 1985. Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. *Physiol. Plantarum* 64, 133–140.
- Smirnov, N., Todd, P., Stewart, G.R., 1984. The occurrence of nitrate reduction in the leaves of woody plants. *Ann. Bot. London* 54, 364–374.
- Ste-Marie, C., Pare, D., 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biol. Biochem.* 31, 1579–1589.
- Stewart, G.R., Lee, J.A., Orebamjo, T.O., Havill, D.C., 1974. Ecological aspects of nitrogen metabolism. In: Bielski, R.L., Ferguson, A.R., Cresswell, M.M. (Eds.), *Mechanisms of Regulation of Plant Growth*. Bulletin 12, The Royal Society of New Zealand, Wellington, NZ, pp. 41–47.
- Truax, B., Gagnon, D., Cevrier, N., 1994. Nitrate reductase activity in relation to growth and soil N forms in red oak and red ash planted in three different environments: forest, clear-cut and field. *For. Ecol. Manage.* 64, 71–82.
- Van Dijk, H.F.G., Louw, M.H.J., Roelofs, J.G.M., Verburgh, J.J., 1990. Impact of artificial ammonium-enriched rainwater on soils and young coniferous trees in a greenhouse. Part II Effects on the trees. *Environ. Pollut.* 63, 41–59.
- Vitousek, P.M., Gosz, J.R., Grier, C.C., Melillo, J.M., Reiners, W.A., 1982. A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. *Ecol. Monogr.* 52, 155–177.
- Zak, D.R., Pregitzer, K.S., 1990. Spatial and temporal variability of nitrogen cycling in northern Lower Michigan. *For. Sci.* 36, 367–380.