

Nitrogen fertilization of trembling aspen seedlings grown on soils of different pH

Annie DesRochers, R. van den Driessche, and Barb R. Thomas

Abstract: Seedlings from three open-pollinated aspen (*Populus tremuloides* Michx.) families were grown in a greenhouse with four nitrogen (N) sources, each at two N levels and three pH levels. Nitrogen sources were ammonium sulfate ((NH₄)₂SO₄), calcium nitrate (Ca(NO₃)₂), ammonium nitrate (NH₄NO₃), and urea ((NH₂)₂CO); N levels were 50 and 200 mg·L⁻¹ (100-mL aliquots three times per week); and pH levels were 5, 6, and 7. Shoot dry mass and leaf area increased with pH in (NH₄)₂SO₄ and (NH₂)₂CO treatments, but were greatest at pH 6 in the NH₄NO₃ and Ca(NO₃)₂ treatments as a result of a pH × N source interaction. N level was directly related to growth, net assimilation, water-use efficiency, and leaf carbon isotope ratio, while N source had no effect on these parameters. Seedlings from families 2 and 3 grew larger than seedlings from family 1, which allocated relatively more dry matter to roots. Growth of the three families interacted with soil pH so that family 1 was largest at pH 7, but families 2 and 3 were largest at pH 6. Seedling boron (B) uptake was reduced by increasing pH and by the Ca(NO₃)₂ fertilizer. These results were interpreted to show that although the three families seemed well adapted to all the N and pH conditions to which they were exposed, growth could be increased by selection of a fertilizer most suitable to the pH and availability of other nutrients in the soil.

Résumé : Les semis de trois familles à pollinisation libre de peuplier faux-tremble (*Populus tremuloides* Michx.) ont été cultivés en serre avec quatre sources d'azote (N), chacune à deux niveaux de N et trois niveaux de pH. Les sources de N étaient le sulfate d'ammonium ((NH₄)₂SO₄), le nitrate de calcium (Ca(NO₃)₂), le nitrate d'ammonium (NH₄NO₃) et l'urée ((NH₂)₂CO). Les niveaux de N correspondaient à 50 et 200 mg·L⁻¹ (aliquotes de 100 mL trois fois par semaine) et les niveaux de pH étaient 5, 6, et 7. La masse sèche des pousses et la surface foliaire ont augmenté avec le pH dans les traitements avec (NH₄)₂SO₄ et (NH₂)₂CO mais étaient le plus élevés à pH 6 dans les traitements avec NH₄NO₃ et Ca(NO₃)₂ à cause d'une interaction entre la source de N et le pH. L'augmentation du niveau de N s'est traduite par une augmentation de la croissance, de l'assimilation, de l'efficacité d'utilisation de l'eau et du rapport du carbone isotopique dans les feuilles, alors que la source de N n'a pas eu d'effet sur ces paramètres. Les semis des familles 2 et 3 étaient plus gros que ceux de la famille 1 qui allouaient plus de matière sèche aux racines. Dans le cas de la croissance, il y avait une interaction entre les familles et le pH du sol de telle sorte que les semis de la famille 1 étaient plus gros à pH 7 alors que ceux des familles 2 et 3 étaient plus gros à pH 6. L'augmentation du pH et le fertilisant Ca(NO₃)₂ causaient une diminution du prélèvement du bore (B) par les semis. Bien que les trois familles semblaient bien adaptées à toutes les conditions de nutrition azotée et de pH auxquelles elles ont été exposées, l'interprétation des résultats montre que la croissance peut être augmentée par la sélection du fertilisant le mieux adapté aux conditions de pH et à la disponibilité des autres nutriments dans le sol.

[Traduit par la Rédaction]

Introduction

Nitrogen (N) is generally recognized as the most growth-limiting mineral nutrient in the boreal forest (Wollum and

Davey 1975). As a result, forest fertilization with N has been widely studied as a silvicultural tool to increase growth rates in conifer forests (Chappell et al. 1992; Tamm et al. 1999). Less attention has been paid to broadleaf trees (Ellis and von Althen 1973), although early research indicated the effectiveness of N fertilization on growth (Mitchell and Chandler 1939), and recent growing efforts have been directed to short rotation broadleaf crops, such as poplars and willows (Heilman 1992). The increasing importance of trembling aspen (*Populus tremuloides* Michx.) to forestry has heightened interest in stand management (Peterson and Peterson 1992), including the possible use of fertilization. Nitrogen, phosphorus (P), and potassium (K) fertilization of aspen root-sucker regeneration in Alaska substantially increased height and diameter growth (Coyne and van Cleve 1977), and further work in Alaska emphasized the importance of N, supplied as ammonium nitrate, in promoting aspen growth (Van Cleve and Oliver 1982). Urea fertilization of a 35-year-old aspen stand in Saskatchewan increased

Received 23 October 2001. Accepted 4 November 2002.

Published on the NRC Research Press Web site at <http://cjfr.nrc.ca> on 7 March 2003.

A. DesRochers.¹ Chaire industrielle CRSNG-UQAT-UQAM en aménagement forestier durable, Université du Québec en Abitibi-Témiscamingue, 445, boulevard de l'Université, Rouyn-Noranda, QC J9X 5E7, Canada.

R. van den Driessche. Biology Department, University of Victoria, P.O. Box 3020, Victoria, BC V8W 3N5, Canada, and New Dendrology Inc., 2361 Queenswood Drive, Victoria, BC V8N 1X4, Canada.

B.R. Thomas. Department of Renewable Resources, University of Alberta, GSB 751, Edmonton, AB T6G 2H1, Canada.

¹Corresponding author (e-mail: annie.desrochers@uqat.ca).

stem volume, particularly when P and K were also supplied (Yang 1991). Fertilization at planting could also be valuable in hastening establishment of aspen plantations, as found in establishment of hybrid poplar plantations (van den Driessche 1999).

The main available sources of N to plants are ammonium (NH_4^+) and nitrate (NO_3^-). Plants may show differential preferences for these two N sources depending on such factors as where assimilation takes place within the plant and the pH of the rooting medium (Raven and Smith 1976). Conifers are usually considered better adapted for uptake of $\text{NH}_4\text{-N}$ (Lavoie et al. 1992), as demonstrated in solution cultures (van den Driessche 1978; van Wijk and Prins 1993; Kronzucker et al. 1995, 1996). Some differences have also been reported in the growth response of conifer stands to sources of N, with ammonium nitrate being found more effective than urea (Dangerfield and Brix 1979; Nason et al. 1990).

Broadleaf trees such as *Populus* spp. may be better adapted to $\text{NO}_3\text{-N}$ uptake than conifers. *Populus xeuramericana* grown at a range of soil pH from 3 to 8 had greater dry matter production with $\text{NO}_3\text{-N}$ than with $\text{NH}_4\text{-N}$, although an interaction was evident between N sources and pH, with little difference among N sources at pH 8 (Evers 1964). However, urea has been used successfully for fertilizing young poplar plantations (Heilman 1990), and both ammonium nitrate (Coyne and Van Cleve 1977) and urea for fertilizing aspen stands (Yang 1991). Although aspen often grows in mixed stands with white spruce (*Picea glauca* (Moench) Voss) in northern Alberta, aspen seems well adapted to NO_3^- . The nitrate reductase enzyme is active in aspen roots without induction (Min et al. 1998), and the opposite is true for spruce, which preferentially absorbs NH_4^+ (Kronzucker et al. 1995, 1996).

Soil pH affects the preference with which $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ are taken up by trees (Evers 1964; van den Driessche 1978; Garnett and Smethurst 1999), but also affects the availability of other nutrients. In acid soils, P is adsorbed and less available to plants than at higher pH, whereas manganese (Mn), boron (B), copper (Cu), and zinc (Zn) are most available between about pH 5 and 6.5 (Mengel and Kirkby 1982).

In boreal aspen forest soils, the most abundant inorganic form of N may be in the NH_4^+ form. Study of an aspen forest soil in Saskatchewan showed that $\text{NH}_4\text{-N}$ was about 10 times as abundant as $\text{NO}_3\text{-N}$, despite a relatively high pH of about 6.7 (Huang and Schoenau 1996). Consequently, it is difficult to predict whether the source of N supplied in a fertilizer would affect the growth response obtained from aspen, particularly since the few reported forest experiments seem to have used only one source of N. Therefore, the objective of this study was to determine whether different N sources might influence growth responses of 1-year-old aspen seedlings grown in soil under greenhouse conditions. In addition, to determine the influence of pH on uptake response to N sources, the greenhouse soil was treated with calcium carbonate (CaCO_3) to achieve three pH levels. Instantaneous water-use efficiency and leaf carbon isotope ratios were also examined because research suggests that these characteristics are affected by N source (Raven and Farquhar 1990). Three aspen families were chosen from

north-central Alberta, making our results applicable to this area.

Materials and methods

Plant material

In June 1999, seed was collected from three female aspen trees located in north-central Alberta, Canada (family 1: 55°39'N, 111°17'W, elevation 619 m; family 2: 55°29'N, 113°24'W, elevation 676 m; family 3: 55°13'N, 113°27'W, elevation 637 m). This area is within the Boreal Mixedwood ecological region of Alberta (Beckingham and Archibald 1996) where aspen is the dominant tree species. Yearly precipitation averages 415 mm and dominant soil types are Grey Luvisols and Brunisols.

The seed lots were sent to a commercial nursery (K & C Silviculture, Red Deer, Alta.) and grown from February to June 2000 using standard growing techniques. In mid-June, 120 seedlings from each family were planted in 4.7-L pots containing an artificial soil mixture of two parts peat moss to one part vermiculite (v/v). The seedlings were grown for 6 weeks in a greenhouse with open sides and without additional lighting, heating, or humidity control, located in north-central Alberta (54°N, 112°W).

pH treatments

To create pH levels of 5, 6, and 7, CaCO_3 was added to the peat moss – vermiculite soil media in each pot, requiring a rate of 1.5, 14.3, and 25.0 g CaCO_3 per pot, respectively. One week after mixing the soil media and prior to N treatment applications, the actual pH levels averaged 5.65, 6.55, and 7.10.

Nitrogen treatments

Four different forms of nitrogen were applied (N sources): ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), ammonium nitrate (NH_4NO_3), and urea ($(\text{NH}_2)_2\text{CO}$). To control the possible confounding effects of sulphur (S) in the ammonium sulfate and the calcium (Ca) in the calcium nitrate, an adequate supply of S and Ca was included in all treatments. Nitrogen sources were applied at a low (50 $\text{mg}\cdot\text{kg}^{-1}$, or 3.57 mM, N) and high (200 $\text{mg}\cdot\text{kg}^{-1}$, or 14.29 mM, N) concentrations (N levels). The seedlings were supplied with 15 and 60 mg N weekly for the low and high N treatments, respectively, by watering with 100-mL aliquots of 50 and 200 $\text{mg}\cdot\text{L}^{-1}$ of N three times per week.

Nutrients other than N

Phosphorus was supplied as monobasic calcium orthophosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O}$), so that each pot contained approximately 2.5 g of P. Calcium requirements were satisfied by the CaCO_3 used to control pH, and by the calcium orthophosphate. Other macronutrients were supplied twice per week. Potassium (K), magnesium (Mg), and S requirements were supplied in macronutrient solutions of potassium sulfate (K_2SO_4) and magnesium sulfate ($\text{MgSO}_4\cdot 7\text{H}_2\text{O}$), giving 20 mg of K, 18.6 mg of S, and 8 mg of Mg, twice per week. Micronutrients were given at the same time as the macronutrients and included 0.4 mg of chelated iron (Fe), 0.004 mg of Mn and molybdenum (Mo), and 0.04 mg of Cu, Zn, and B.

Measurements

Height and basal diameter were measured immediately after planting and before destructive harvesting after 6 weeks of growth. After 5 weeks of growth, measurements of net assimilation (A), transpiration (E), and stomatal conductance (G_s) were made with a CIRAS-1 infrared gas analyzer (PP Systems, Haverhill, Mass.). Instantaneous water-use efficiency (WUE) was calculated as A/E .

At harvest, the leaves were collected and total leaf area was measured with a LI-COR 3100 leaf area meter (LI-COR, Lincoln, Nebr.). The seedlings were carefully washed free of all rooting medium, separated into leaves, stems, and roots, dried at 80°C for 2 days, and then weighed. All tissue samples were ground in a Cyclone Sample Mill with a 1-mm mesh size (UDY Corp., Fort Collins, Colo.). For each plant part, elemental analyses of P, K, Ca, sodium (Na), Mg, Fe, Mn, Cu, Zn, Mo, and B were carried out by dry-ashing the samples in a muffle furnace at 500°C and digesting with 30% hydrochloric acid, followed by atomic emission spectroscopy using an ARL 34000 RTB ICP spectrometer (Thermo Instruments Inc., Mississauga, Ont.). Nitrogen and S contents were determined by combustion elemental analysis using a CNS-2000 Elemental Analyzer (Leco Instruments Ltd., St-Josephs, Mich.). Whole-plant concentrations were calculated as the sum of the absolute amounts in each plant part, divided by the total dry mass of the plant. A subsample of the leaves was ground more finely with a Brinkmann MM2 ball grinder. Leaf carbon isotope ratios ($\delta^{13}\text{C}$) were then determined using a Carlo-Erba 1500 NCS directly interfaced to a V.G. Sira mass spectrometer (Middlewich, Cheshire, U.K.), which was tuned for continuous flow ^{13}C isotope ratio mass spectrometer (IRMS) measurements. The isotopic composition of the samples was calculated from

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where R_{sample} and R_{standard} are the ratios of $^{13}\text{C}/^{12}\text{C}$ in the sample and standard, respectively. Spring wheat grain was used as the working standard, with a carbon isotope composition of -23.47‰ relative to the Pee Dee Belemnite (PDB) standard.

Two replicates of soil media for each treatment combination were collected at the end of the experiment, dried at 50°C, and milled to 1 mm. The pH was determined in a 1:1 soil-water mixture. Available P was extracted in a weak acetic acid solution (0.5 M) mixed with 0.015 M of ammonium fluoride and 1.0 N ammonium acetate, and determined by colorimetry (Technicon Industrial Syst., Tarrytown, N.Y.). Exchangeable cations (Ca, Mg, Na, and K) were extracted with ammonium acetate and determined by atomic emission spectroscopy using a TJA IRIS Advantage ICP (Thermo Jarrell Ash, Mass.). Hot-water soluble B was also determined by atomic emission spectroscopy (Thermo Jarrell Ash, Mass.). Ammonium, NO_3^- , and available S were simultaneously extracted in a weak solution of calcium chloride (0.001 M) mixed with KCl (100 ppm K) (Kowalenko 1993; Maynard and Kalra 1993) and determined by flow injection analysis (Tecator AB, Hoganas, Sweden).

Table 1. Percent differences in mean plant measurement between the highest and lowest response to each treatment main effect after 6 weeks of growth.

Plant measurement	Treatment		
	N source ^a	N level ^b	pH ^c
Height	2.8 (0.71)	0.0 (0.99)	3.1 (0.31)
Basal diameter	2.2 (0.63)	2.3 (0.12)	5.1 (0.01)
Leaf area	5.4 (0.42)	6.3 (0.04)	9.4 (0.04)
Leaf dry mass	5.8 (0.44)	9.6 (<0.001)	12.1 (<0.001)
Shoot dry mass	4.4 (0.46)	5.5 (0.03)	9.6 (0.003)
Root dry mass	4.7 (0.54)	5.5 (0.02)	13.9 (<0.001)
A	5.7 (0.13)	9.5 (<0.001)	4.2 (0.18)
WUE	2.7 (0.18)	6.3 (0.05)	3.7 (0.93)
$\delta^{13}\text{C}$	0.4 (0.83)	1.0 (0.02)	0.5 (0.55)
G_s	3.4 (0.77)	2.0 (0.41)	2.2 (0.86)

Note: P values of main effects are shown in parentheses. A , net assimilation rate; WUE, water-use efficiency; $\delta^{13}\text{C}$, carbon isotope ratio; G_s , stomatal conductance.

^aAmmonium sulphate, calcium nitrate, ammonium nitrate, and urea.

^b50 and 200 mg·kg⁻¹ N.

^cpH 5, 6, and 7.

Experimental design

The experiment was a completely randomized block design with four N sources \times two N levels \times three pH levels \times three aspen families replicated in five blocks to compensate for possible environmental gradients in the greenhouse. Height and basal diameter were used as covariates in an analysis of variance for the growth and nutrient measurements. The data were statistically analyzed with the SAS statistical package (version 8.0, SAS Institute Inc., Cary, N.C.). A significance level of $P < 0.05$ was chosen.

Results

Growth

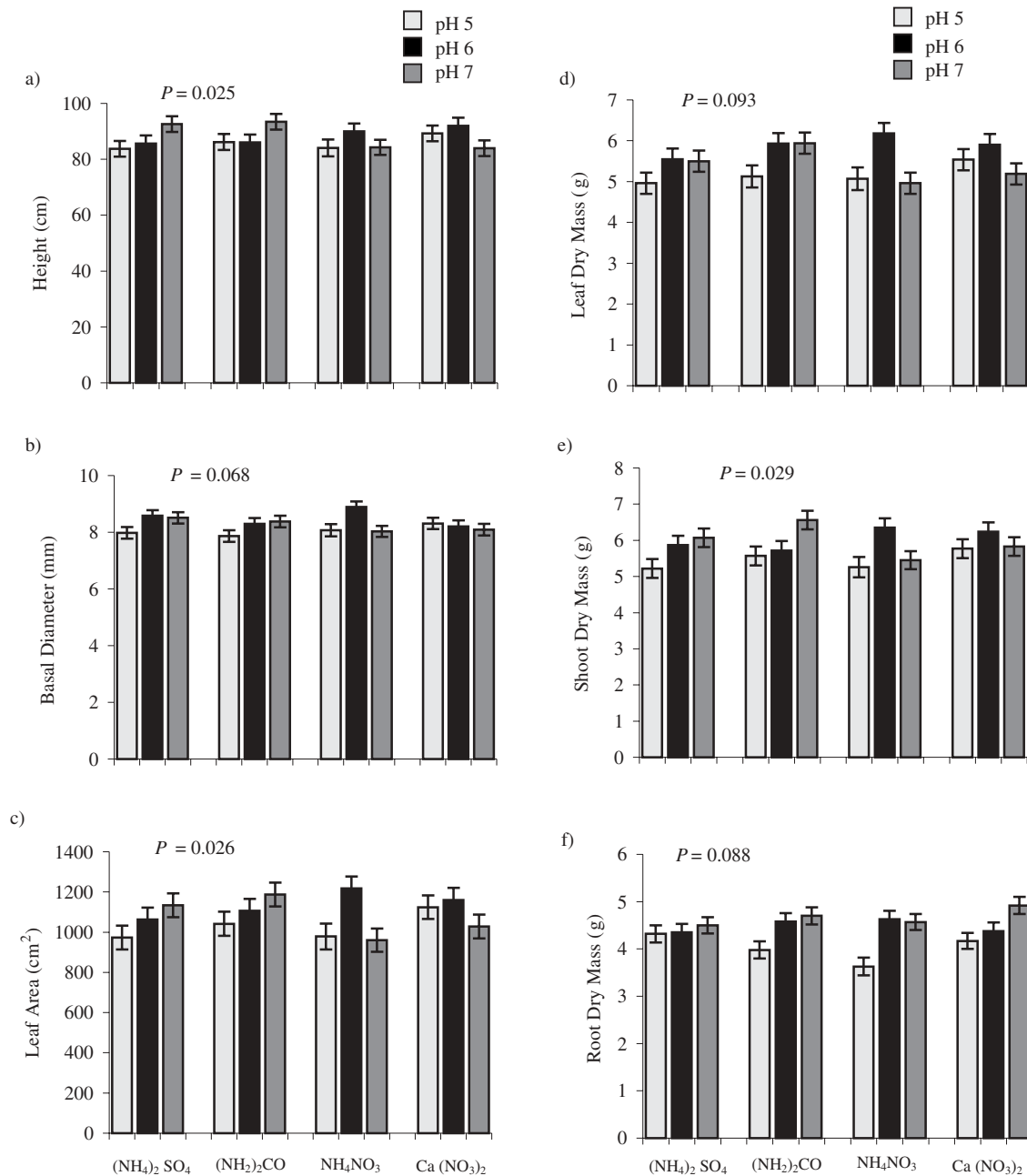
The treatment effects on growth were in order of pH $>$ N level $>$ N source (Table 1). Significant interactions between N source and pH showed that height, shoot dry mass, and leaf area increased with pH in the $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_2)_2\text{CO}$ treatments and were greatest at pH 6 in the NH_4NO_3 and $\text{Ca}(\text{NO}_3)_2$ treatments (Fig. 1).

Height, basal diameter, and shoot, leaf, and root dry mass showed significant differences among families (Fig. 2). This was primarily caused by the greater growth responses of seedlings from families 2 and 3, compared with family 1 ($P < 0.001$). Leaf area and shoot and leaf dry mass also showed significant interactions between families and pH in which the growth response of family 1 was greatest at pH 7, but families 2 and 3 had greater growth at pH 6 (Fig. 2).

Nutrients

Increase from pH 5 to 6 with $\text{NH}_4\text{-N}$ nutrition resulted in increased leaf mass and indicated that N and Ca were limiting (Fig. 3a, vector 1). The concentrations of P, K, and B decreased (Fig. 3a, vector 2), diluted by increased leaf mass. Further increase to pH 7 showed similar increase in leaf mass, but possible deficiency of K as well as N and Ca

Fig. 1. Mean height, basal diameter, leaf area, and leaf, root, and shoot dry mass of aspen seedlings for each N source \times pH combination, across all three aspen families. The P values correspond to the N source \times pH interaction. Error bars are SEs.



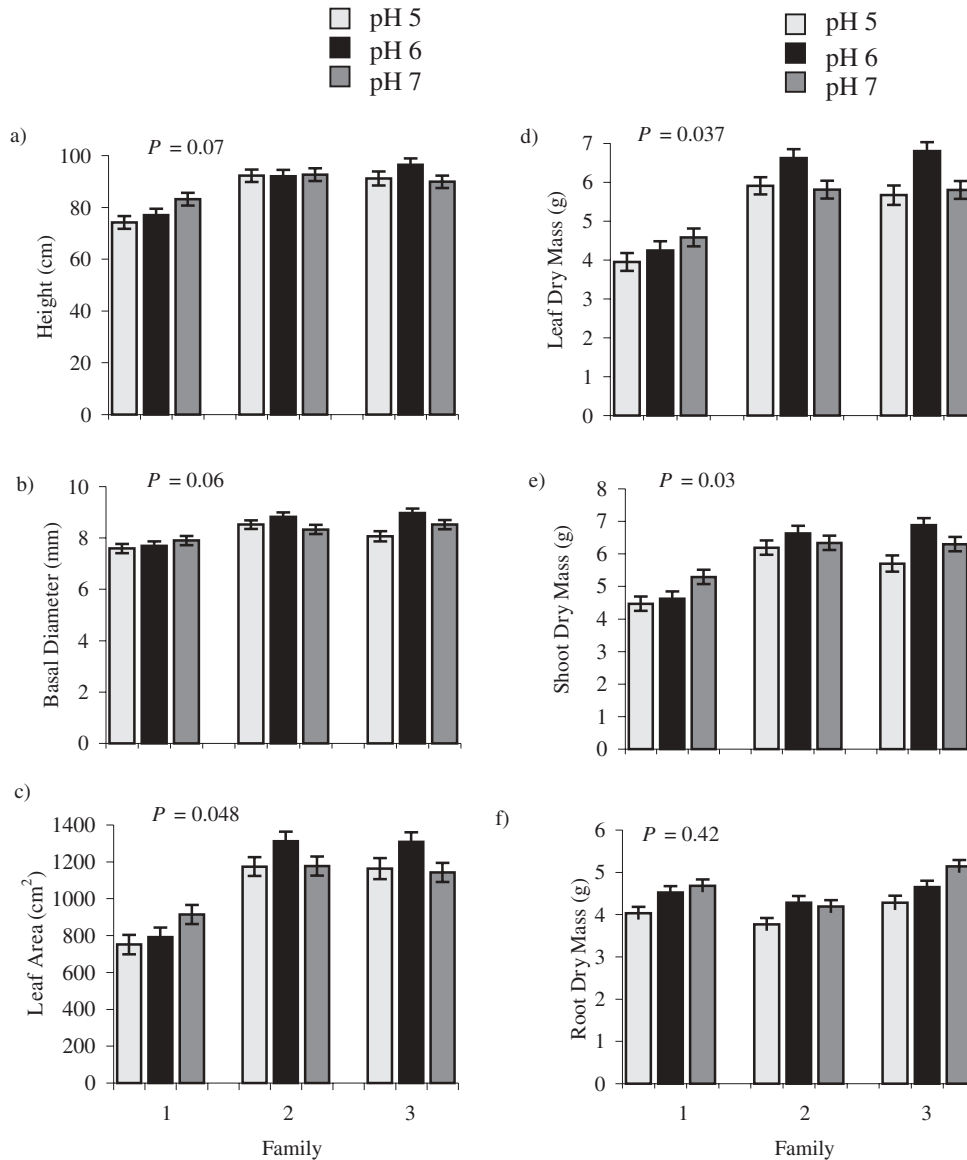
(Fig. 3b, vector 1). Concentrations and content of P and B were substantially reduced.

Increase in pH from 5 to 6 under the $\text{NO}_3\text{-N}$ treatments caused very similar responses in leaf mass, nutrient concentrations, and content as in the corresponding $\text{NH}_4\text{-N}$ treatments (Figs. 3a and 4a). However, P appeared more diluted in the $\text{NO}_3\text{-N}$ treatment than in the $\text{NH}_4\text{-N}$ treatment at pH 6. There were several differences among the N sources at pH 7, where leaf mass decreased below the pH 5 value in the $\text{NO}_3\text{-N}$ treatments (Fig. 4b). At pH 7, $\text{NO}_3\text{-N}$ appeared to cause an increase in nutrient concentration (“luxury consumption”) of K and Ca (Fig. 4b, vector 4) and a reduction in concentrations and content of N, B, and P (Fig. 4b, vector 5).

Leaf B concentrations showed significant interactions for N source \times N level and for N source \times pH: an increase in N level resulted in a decrease of leaf B concentration except for the NH_4NO_3 source, where an increase in N level resulted in an increase in leaf B concentration (Fig. 5a). Boron concentration also decreased with increasing pH, and the reductions in B concentration between pH 6 and 7 were proportionally greater in the $\text{NH}_4\text{-N}$ compared with the $\text{NO}_3\text{-N}$ treatments (Fig. 5b). There were no significant effects of N source on N or P concentrations in whole plants or in individual plant parts.

Increase in N level increased mean leaf N concentration from 30.2 to 34.1 $\text{g}\cdot\text{kg}^{-1}$ ($P < 0.001$), leaf P concentration

Fig. 2. Height, basal diameter, leaf area, and leaf, root, and shoot dry mass of aspen seedlings for each family × pH combination. The *P* values correspond to the family × pH interaction. Error bars are SEs.



from 5.25 to 5.45 g·kg⁻¹ (*P* = 0.045), and decreased leaf K concentration from 14.2 to 13.3 g·kg⁻¹ (*P* < 0.001).

Gas exchange

Net assimilation (*A*), water-use efficiency (WUE), and leaf carbon isotope ratios ($\delta^{13}\text{C}$) increased with N level (Table 2). Neither N source nor pH had a significant effect on *A*, WUE, or $\delta^{13}\text{C}$. Stomatal conductance (G_s) was not significantly influenced by any of the treatments. A positive correlation existed between leaf dry mass and $\delta^{13}\text{C}$ ($r^2 = 0.26$, *P* < 0.001) and between leaf dry mass and plant N ($r^2 = 0.76$, *P* < 0.001). All three families significantly differed in their $\delta^{13}\text{C}$, with -27.83, -27.19, and -27.58‰ for families 1, 2, and 3, respectively.

Soil

By the end of the experiment, mean soil pH had increased by 0.37 units for the pH 5 treatment and decreased by 0.26

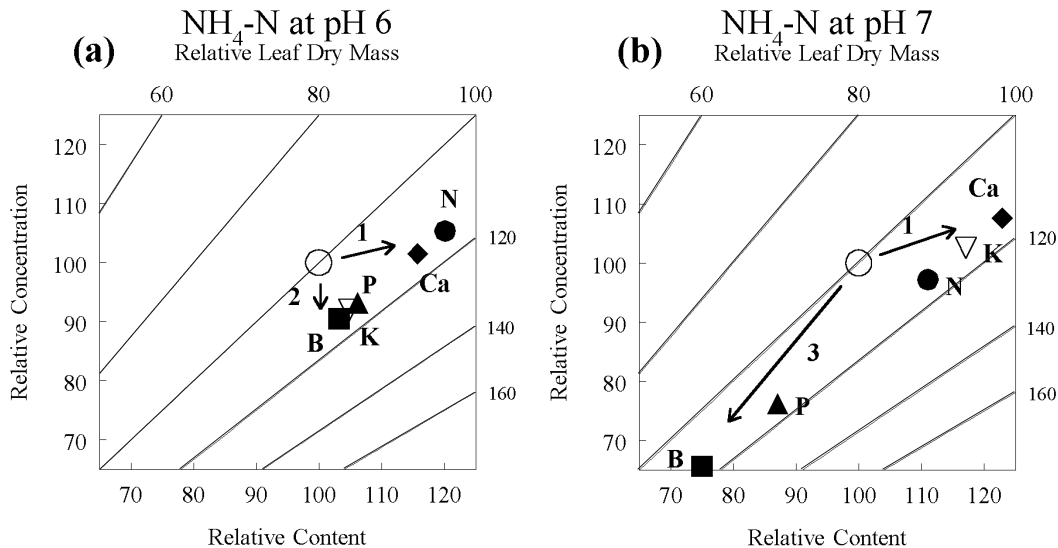
Table 2. Mean growth measurements, net assimilation rate (*A*), water-use efficiency (WUE), stomatal conductance (G_s), and leaf carbon isotope ratio ($\delta^{13}\text{C}$) for each N level and treatment *P* value across the three aspen families.

Measurement	N level (mg·kg ⁻¹) ^a		<i>P</i>
	50	200	
Shoot dry mass (g)	5.53 (0.14)	6.09 (0.14)	0.03
Root dry mass (g)	4.24 (0.08)	4.55 (0.08)	0.02
Leaf area (cm ²)	1035 (26)	1125 (26)	0.04
Leaf dry mass (g)	5.15 (0.12)	5.81 (0.12)	<0.001
<i>A</i> (μmol·m ⁻² ·s ⁻¹)	15.18 (0.22)	16.85 (0.22)	<0.001
WUE (<i>A</i> / <i>E</i>)	5.66 (0.13)	6.04 (0.13)	0.05
G_s (mmol·m ⁻² ·s ⁻¹)	298.42 (6.29)	305.73 (6.20)	0.41
$\delta^{13}\text{C}$ (‰)	-27.7 (0.07)	-27.4 (0.07)	0.02

Note: Standard errors are shown in parentheses.

^aDegrees of freedom = 359.

Fig. 3. Vector diagrams showing changes of leaf mass and nutrients (N, P, K, Ca, and B) in $\text{NH}_4\text{-N}$ treatments ($(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_2)_2\text{CO}$) at (a) pH 6 and (b) pH 7, relative to pH 5: vector 1 represents increase in leaf mass, nutrient concentration, and content; vector 2 represents increase in leaf mass and nutrient dilution; and vector 3 represents increase in leaf mass, but decrease in nutrient concentration and content. Values are scaled to pH 5 set to 100 and represented by the large open circle (Timmer 1991).



units for the pH 6 treatment ($P < 0.05$), while pH 7 remained unchanged. There was no significant effect of N source on soil pH, except for $\text{Ca}(\text{NO}_3)_2$ under the pH 7 treatment, which significantly increased pH above the other N sources, to pH 7.3 ($P < 0.05$). The high N level significantly decreased soil pH by 0.25 units, on average, compared with the low N level ($P < 0.001$), except for the pots that received $(\text{NH}_2)_2\text{CO}$, for which no difference was detected between the two N levels (N level \times N source interaction, $P = 0.03$).

Soil B concentration was negatively correlated with soil pH ($r^2 = 0.30$, $P < 0.001$) (Fig. 6) without being correlated with soil Ca ($r^2 = 0.003$, $P = 0.50$). Soil available P increased with N level ($P < 0.001$) and decreased with increasing soil pH ($P < 0.001$), but was not affected by N source ($P = 0.44$).

Discussion

This study suggests that aspen seedlings are well adapted to utilize both NH_4 and NO_3 N sources, which may partly explain the success of this species over a wide ecological and geographical range (Perala 1990). However, slight differences existed in growth response to N sources (depending on soil pH), which could be exploited in a fertilization strategy to maximize growth. The $\text{NH}_4\text{-N}$ sources, $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_2)_2\text{CO}$, produced an increased growth response with an increase in pH (Fig. 1), which was expected, since $\text{NH}_4\text{-N}$ uptake is favored when the H^+ ion concentration of the rooting medium is reduced (Raven and Smith 1976). Conversely, growth response to the two $\text{NO}_3\text{-N}$ sources, $\text{Ca}(\text{NO}_3)_2$ and NH_4NO_3 , was greatest at pH 6, suggesting that $\text{NO}_3\text{-N}$ fertilizers would result in the greatest growth response at pH 6 and below, and that $\text{NH}_4\text{-N}$ fertilizers would be beneficial in soils of pH 7 or higher.

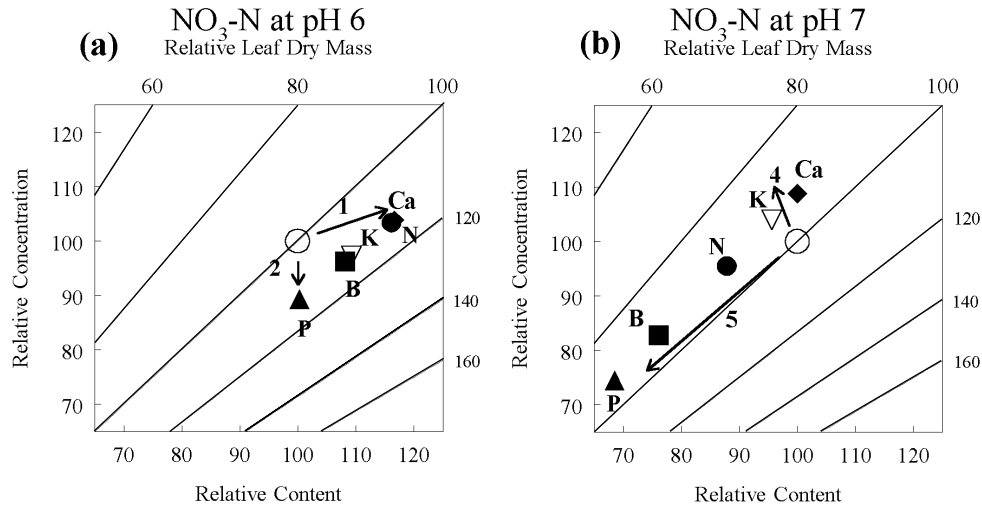
It appears that the effects of N source on plant nutrient levels, noted mostly in agricultural crops (Tisdale et al. 1985), are indirectly due to changes caused by NH_4 or NO_3

fertilization on soil pH. For example, N sources causing a decrease in soil pH will usually increase P uptake by changing its molecular form, which makes it more available to plants (Soon and Miller 1977). In our study, N source did not significantly affect soil pH, which probably explains why uptake of most nutrients was not strongly affected by the different N sources. Whole plant and leaf B concentrations were, however, particularly sensitive to N source (Fig. 5), suggesting a more direct effect of N source or a greater influence on B uptake to slight changes in soil pH. Uptake of NH_4^+ is compensated by the release of H^+ ions in the soil medium, increasing its acidity. This could explain the higher leaf B concentrations obtained under the $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_2)_2\text{CO}$ treatments (Fig. 5b), since available B increased with acidity (Fig. 6). Calcium is often said to have an antagonistic effect on B uptake (Olsen 1972), but it was later shown to be indirect, again reflecting the effect of Ca on soil pH (Gupta and MacLeod 1981). The effects of N source on B uptake were reduced at pH 6 and absent at pH 7 (Fig. 5b), stressing the importance of pH on B concentration. Boron was probably never deficient in any treatment combination; it remained in concentrations above $30 \text{ mg}\cdot\text{kg}^{-1}$, and B deficiency symptoms usually occur at concentrations under $15 \text{ mg}\cdot\text{kg}^{-1}$ for most plants (Gupta et al. 1985).

Interestingly, the growth response to NH_4NO_3 was similar to that of $\text{Ca}(\text{NO}_3)_2$ (Fig. 1), although half of its N is in the NH_4 form. It could be argued that this is because aspen preferentially takes up the NO_3 component of NH_4NO_3 . Closer examination of leaf B concentrations in response to N level and N source (Fig. 5a) might rather suggest that under the high level of NH_4NO_3 , $\text{NH}_4\text{-N}$ was preferentially absorbed and rendered B more available. However, the concentrations of NH_4^+ and NO_3^- in the soil at the end of the experiment provided no evidence about this (data not shown).

Growth responses of these aspen seedlings to N-level treatments were relatively small. The low level of N supply (3.6 mM) may have largely satisfied their N requirements

Fig. 4. Vector diagrams showing changes of leaf mass and nutrients (N, P, K, Ca, and B) in $\text{NO}_3\text{-N}$ treatments ($\text{Ca}(\text{NO}_3)_2$ and NH_4NO_3) at (a) pH 6 and (b) pH 7, relative to pH 5: vector 1 represents increase in leaf mass, nutrient concentration, and content; vector 2 represents increase in leaf mass and nutrient dilution; vector 4 represents decrease in leaf mass with increase in nutrient concentration (“luxury consumption”); and vector 5 represents decrease in leaf mass with decreases in nutrient concentration and content. Values are scaled to pH 5 set to 100 and represented by the large open circle (Timmer 1991).



because only a small increase (0–9.6%) in growth was evident with an increase in N supply to 14.3 mM. This is consistent with an experiment in which aspen was grown at N levels of 0, 0.25, and 0.5 M (Hemming and Lindroth 1999), where no differences in growth were detected between the two higher treatment levels. This would be expected if the lower N level was already satisfying N requirements in the present experiment. Czapowskyj and Safford (1979) also found little growth response of trembling aspen to N fertilizer treatments in Maine.

The vector diagrams indicated relatively similar response of leaf mass as well as nutrient concentrations and contents when pH increased from 5 to 6 for both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ (Figs. 3 and 4). Further increase to pH 7 showed different responses according to N source. The $\text{NH}_4\text{-N}$ treatment still resulted in an increase in leaf mass, while it was decreased with the $\text{NO}_3\text{-N}$ treatments. For both N sources, the increase to pH 7 caused much greater changes in internal nutrient relationships than the increase to pH 6. However, these changes were not detrimental to leaf growth in the $\text{NH}_4\text{-N}$ treatment, as they were in the $\text{NO}_3\text{-N}$ treatment. The differences that might have been responsible were the decrease of N and the strong decrease of P in the $\text{NO}_3\text{-N}$ treatment, which did not occur in the $\text{NH}_4\text{-N}$ treatment. Reduction in P uptake at pH 7 probably resulted from formation of insoluble calcium phosphate as a result of liming (Mengel and Kirkby 1982). Reduced uptake of N at pH 7 may have been related to reduced uptake of other nutrients and the need to maintain an internal balance of N with these nutrients.

Theoretically, stomatal closure should be reflected in higher $\delta^{13}\text{C}$, because intercellular spaces beneath closed stomata become increasingly enriched in $^{13}\text{CO}_2$, resulting in greater levels of its fixation. Hence, WUE would be expected to be higher in plants exhibiting higher $\delta^{13}\text{C}$ (Farquhar et al. 1982). In our study, seedlings receiving the high N treatment effectively showed higher instantaneous WUE and higher $\delta^{13}\text{C}$ (Table 2). This suggests that the seedlings which received the high N level and which grew more

aboveground biomass were more water stressed than the plants receiving the low N level. This is supported by the positive relationship between $\delta^{13}\text{C}$ and leaf dry mass. In addition, family 2, which had similar aboveground growth but smaller root dry mass than family 3, had significantly higher $\delta^{13}\text{C}$ values. A simple explanation for this may be that the larger plants depleted the water supply from the pots more quickly than smaller plants.

Increases in $\delta^{13}\text{C}$ resulting from N fertilization have been explained by others such that CO_2 assimilation (A) proportionally increases more than stomatal conductance (G_s) with a given increase in N (Livingston et al. 1998; Siegwolf et al. 2001). This would limit the supply of CO_2 inside the leaf and therefore induce more ^{13}C fixation. Accordingly, we found that plant N concentration was positively correlated with $\delta^{13}\text{C}$, and that the high N level significantly increased A while changes in G_s were not detected (Table 2). In our study, however, plant N was well correlated with leaf dry mass and did not significantly increase the amount of variation in $\delta^{13}\text{C}$ explained by leaf dry mass alone. This suggests that although stomatal conductance on these aspen seedlings did not significantly increase with N level, it was sufficient to compensate for the increased demand in CO_2 created by the high N level. This could constitute a competitive advantage for the species in areas subjected to periodic droughts, since increased stomatal conductance could also increase water vapor loss.

Plants receiving $\text{NH}_4\text{-N}$ are said to have a lower WUE than those receiving $\text{NO}_3\text{-N}$, and it has been suggested that the N source may influence the fractionation of plant C that is fixed by carboxylases other than Rubisco (Raven and Farquhar 1990). However, certain factors such as the site of C fixation within the plant and its C/N ratio result in variation from species to species, and there is certainly no evidence of N source effect on WUE or $\delta^{13}\text{C}$ in this experiment with aspen.

In conclusion, although aspen showed no marked differences in response to the four types of N fertilizer, anticipated

Fig. 5. Mean leaf B concentration of aspen seedlings for each (a) N source and N level, and (b) N source and pH. Bars with the same letters are not significantly different at $P < 0.05$.

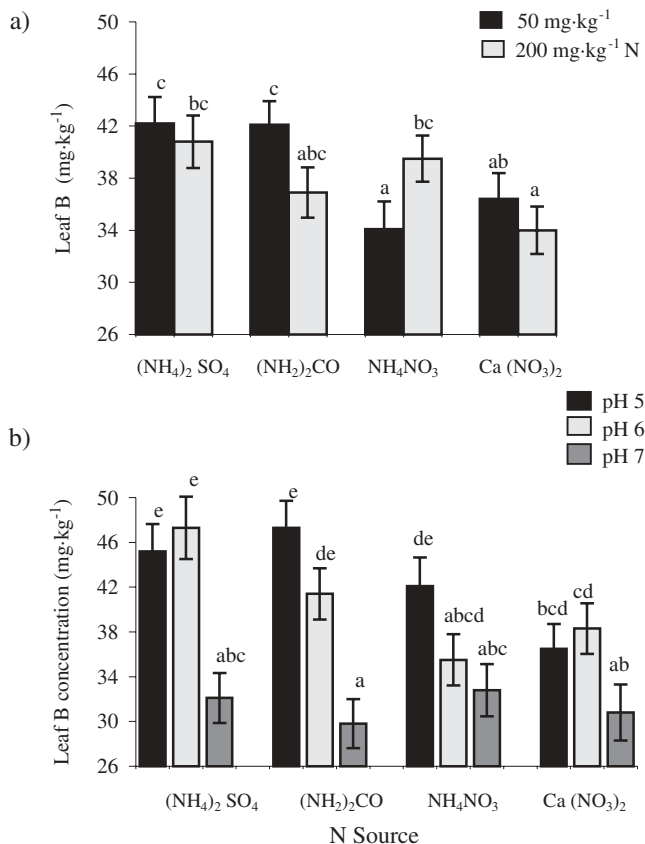
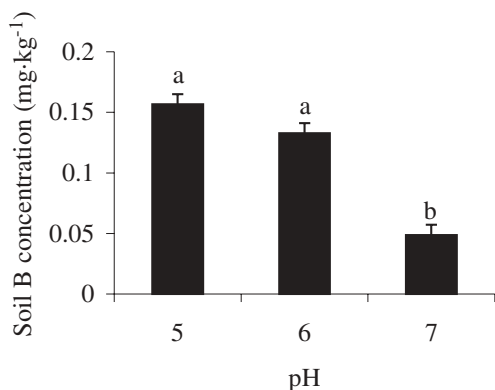


Fig. 6. Mean soil B concentration at pH 5, 6, and 7. Bars with the same letter are not significantly different at $P < 0.05$.



interactions with pH were observed. That is, the NH₄-N sources increased growth more at higher pH and the NO₃-N sources increased growth more at lower pH. Although the three families seemed well adapted to all the N and pH conditions to which they were exposed, growth could be increased by selection of a fertilizer most suitable to the pH and availability of various other nutrients at a particular site. These results were obtained in an artificial soil under greenhouse conditions, which may have induced more rapid growth and therefore better defined responses to treatments than would be obtained in the field. The extent to which

these responses would be detected in the field would depend on site conditions; however, our results should provide a basis for interpreting responses of aspen to N source and pH in the field. Differences in growth among families seem to have been associated with differences in dry matter distribution, and perhaps WUE, suggesting that selection and breeding could have important effects on productivity. Nitrogen source had no detectable effect on carbon discrimination, although WUE and $\delta^{13}\text{C}$ were positively related to plant N concentration.

Acknowledgements

This research was funded by Alberta-Pacific Forest Industries Inc., in collaboration with Forest Resource Improvement Association of Alberta, and the Natural Sciences and Engineering Research Council of Canada through an Industrial Research Fellowship to A.D. We also thank the University of Alberta, Norwest Labs, and Kerriane Koehler and Alan Bradfield for greenhouse and laboratory work.

References

- Beckingham, J.D., and Archibald, J.H. 1996. Field guide to ecosites of northern Alberta. Can. For. Serv. North. For. Cent. Spec. Rep. 5.
- Chappell, H.N., Weetman, G.F., and Miller, R.E. 1992. Forest fertilization: sustaining and improving nutrition and growth of western forests. Univ. Wash. Inst. For. Resour. Contrib. 73.
- Coyne, P.I., and van Cleve, K. 1977. Fertilizer induced morphological and chemical responses of a quaking aspen stand in Interior Alaska. For. Sci. **23**: 92–102.
- Czapowskyj, M.M., and Safford, L.O. 1979. Growth response to fertilizer in a young aspen-birch stand. USDA For. Serv. Res. Note NE-274.
- Dangerfield, J., and Brix, H. 1979. Comparative effects of ammonium nitrate and urea fertilizers on tree growth and soil processes. In Proceedings of Forest Fertilization Conference. Edited by S.P. Gessel, R.M. Kenady, and W.A. Atkinson. College of Forest Resources, University of Washington, Seattle, Wash. Contrib. 40. pp. 133–139.
- Ellis, R.C., and von Althen, F.W. 1973. Fertilization of adult and juvenile hardwoods in Ontario. Forest fertilization: symposium proceedings. USDA For. Serv. Gen. Tech. Rep. NE-3. pp. 174–187.
- Evers, F.H. 1964. Die Bedeutung der Stickstoffform für Wachstum und Ernährung der Pflanzen, insbesondere der Waldbäume. Mitt. Ver. Forstl. Standortsk. Forstpfl. Zucht, **14**: 19–37.
- Farquhar, G.D., O'Leary, M.H., and Berry, J.A. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. **9**: 121–137.
- Garnett, T.P., and Smethurst, P.J. 1999. Ammonium and nitrate uptake by *Eucalyptus nitens*: effects of pH and temperature. Plant Soil, **214**: 133–140.
- Gupta, U.C., and MacLeod, J.A. 1981. Plant and soil boron as influenced by soil pH and calcium sources on Podzol soils. Soil Sci. **131**: 20–25.
- Gupta, U.C., Jame, Y.W., Campbell, C.A., Leyshon, A.J., and Nicholaichuck, W. 1985. Boron toxicity and deficiency: a review. Can. J. Soil Sci. **65**: 381–409.
- Heilman, P.E. 1990. Growth and N status of *Populus* in mixture with red alder on recent volcanic mudflow from Mount Saint Helens. Can. J. For. Res. **20**: 84–90.

- Heilman, P.E. 1992. Sustaining production: nutrient dynamics and soils. *In* Ecophysiology of short rotation crops. Edited by C.P. Mitchell, J.R. Ford-Robertson, T. Hinkley, and L. Senneryby-Forse. Elsevier, Amsterdam. pp. 216–230.
- Hemming, J.D.C., and Lindroth, R.L. 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. *J. Chem. Ecol.* **25**: 1687–1714.
- Huang, W.Z., and Schoenau, J.J. 1996. Forms, amounts and distribution of carbon, nitrogen, phosphorus and sulfur in a boreal aspen forest soil. *Can. J. Soil Sci.* **76**: 373–385.
- Kowalenko, C.G. 1993. Extraction of available sulfur. *In* Soil sampling and methods of analysis. Edited by M.R. Carter. CRC Press, Boca Raton, Fla. pp. 65–67.
- Kronzucker, H.J., Siddiqi, M.Y., and Glass, D.M. 1995. Kinetics of NO_3^- influx in spruce. *Plant Physiol.* **109**: 319–326.
- Kronzucker, H.J., Siddiqi, M.Y., and Glass, D.M. 1996. Kinetics of NH_4^+ influx in spruce. *Plant Physiol.* **110**: 773–779.
- Lavoie, N., Vezina, L.-P., and Margolis, H.A. 1992. Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. *Tree Physiol.* **11**: 178–183.
- Livingston, N.J., Whitehead, D., Kelliher, F.M., Wang, Y.-P., Grace, J.C., Walcroft, A.S., Byers, J.N., McSeveny, T.M., and Millard, P. 1998. Nitrogen allocation and carbon isotope fractionation in relation to intercepted radiation and position in a young *Pinus radiata* D. Don tree. *Plant Cell Environ.* **21**: 795–803.
- Maynard, D.G., and Kalra, Y.P. 1993. Nitrate and exchangeable ammonium nitrate. *In* Soil sampling and methods of analysis. Edited by M.R. Carter. CRC Press, Boca Raton, Fla. pp. 25–42.
- Mengel, K., and Kirkby, E.A. 1982. Principles of plant nutrition. International Potash Institute, P.O. Box, CH-3048 Worblaufen-Bern, Switzerland.
- Min, X., Siddiqi, M.Y., Guy, R., Glass, A.D.M., and Kronzucker, H.J. 1998. Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine. *Plant Cell Environ.* **21**: 1039–1046.
- Mitchell, H.L., and Chandler, R.F. 1939. The nitrogen nutrition and growth of certain deciduous trees of northeastern United States. With a discussion of the principles and practices of leaf analysis as applied to forest trees. The Black Rock Forest Bull. Vol. 11.
- Nason, G.E., Pluth, D.J., Hardin, R.T., and McGill, W.B. 1990. Dynamics of foliar N in Douglas-fir after spring and fall application of ammonium nitrate and urea. *Can. J. For. Res.* **20**: 1515–1523.
- Olsen, S.R. 1972. Micronutrient interactions. *In* Micronutrients in agriculture. Edited by J.J. Mortvedt, P.M. Giordano, and W.L. Lindsay. Soil Science Society of America, Inc., Madison, Wis. pp. 243–264.
- Perala, D.A. 1990. *Populus tremuloides* Michx. Quaking aspen. *In* Silvics of North America. Vol. 2. Hardwoods. U.S. Dep. Agric. Agric. Handb. 654.
- Peterson, E.B., and Peterson, N.M. 1992. Ecology, management, and use of aspen and balsam poplar in the prairie provinces, Canada. *For. Can. North. For. Cent. Spec. Rep.* 1.
- Raven, J.A., and Farquhar, G.D. 1990. The influence of N metabolism and organic acid synthesis on the natural abundance of isotopes of carbon in plants. *New Phytol.* **116**: 505–529.
- Raven, J.A., and Smith, F.A. 1976. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytol.* **76**: 415–431.
- Siegwolf, R.T.W., Matyssek, R., Saurer, M., Maurer, S., Gunthardt-Goerg, M.S., Schmutz, P., and Bucher, J.B. 2001. Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves. *New Phytol.* **149**: 233–246.
- Soon, Y.K., and Miller, M.H. 1977. Changes in the rhizosphere due to NH_4 and NO_3 fertilization and phosphorus uptake by corn seedlings. *Soil Sci. Soc. Am. J.* **41**: 77–80.
- Tamm, C.O., Aronsson, A., Popovic, B., and Flower-Ellis, J. 1999. Optimum nutrition and nitrogen saturation in Scots pine stands. *Studia Forest. Suecica*, **206**: 1–126.
- Timmer, V.R. 1991. Interpretation of seedling analysis and visual symptoms. *In* Mineral nutrition of conifer seedlings. Edited by R. van den Driessche. CRC Press, Boca Raton, Fla. pp. 113–134.
- Tisdale, S.L., Nelson, W.L., and Beaton, J.D. 1985. Soil fertility and fertilizers. 4th ed. Macmillan Publishing Company, New York.
- Van Cleve, K., and Oliver, L.K. 1982. Growth response of postfire quaking aspen (*Populus tremuloides* Michx.) to N, P, and K fertilization. *Can. J. For. Res.* **12**: 160–165.
- van den Driessche, R. 1978. Response of Douglas-fir seedlings to nitrate and ammonium nitrogen sources at different levels of pH and iron supply. *Plant Soil*, **49**: 607–623.
- van den Driessche, R. 1999. First year growth response of four *Populus trichocarpa* × *Populus deltoides* clones to fertilizer placement and level. *Can. J. For. Res.* **29**: 554–562.
- van Wijk, C.K., and 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 Soil*, **151**: 91–96.
- Wollum, A.G., and Davey, C.B. 1975. Nitrogen accumulation, transformation and transport in forest soils. *In* Forest soils and forest land management. Edited by B. Bernier and C.H. Winget. Presses de l'Université Laval, Sainte-Foy, Que. pp. 67–106.
- Yang, R.C. 1991. Fertilization: a tool for managing aspen in western Canada. *For. Can., Northwest Region, For. Manage. Note* 54.