Responses of *Acer saccharum* canopy trees and saplings to P, K and lime additions under high N deposition

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Received May 1, 2007; accepted August 1, 2007; published online December 3, 2007

Summary  Heavy atmospheric nitrogen (N) deposition has been associated with altered nutrient cycling, and even N saturation, in forest ecosystems previously thought to be N-limited. This observation has prompted application to such forests of non-N mineral nutrients as a mitigation measure. We examined leaf gas-exchange, leaf chemistry and leaf and shoot morphological responses of *Acer saccharum* Marsh. saplings and mature trees to experimental additions of non-nitrogenous mineral nutrients (dolomitic lime, phosphorus + potassium (P + K) and lime plus P + K) over 2 years in the Haliburton region of central Ontario, which receives some of the largest annual N inputs in North America. Nutrients were adsorbed in the mineral soil and taken up by *A. saccharum* trees within 1 year of fertilizer application; however, contrary to expectation, liming had no effect on soil P availability. Saplings and canopy trees showed significant responses to both P + K fertilization and liming, including increased foliar nutrient concentration, leaf size and shoot extension growth; however, no treatment effects on leaf gas-exchange parameters were detected. Increases in shoot extension preceded increases in diameter growth in saplings and canopy trees. Vector analysis of shoot extension growth and nutrient content was consistent with sufficiency of N but marked limitation of P, with co-limitation by calcium (Ca) in saplings and by Ca, Mg and K in canopy trees.

Keywords: canopy development, fertilization, liming, N saturation, photosynthesis, P limitation, resource allocation, sugar maple, vector analysis.

Introduction  Tree growth and primary productivity in temperate forests are typically limited by nitrogen (N) availability (Schlesinger 1997, Aber et al. 1998); however, in recent years many forests exposed to high rates of atmospheric N deposition have exhibited signs of N excess (Aber et al. 1989, Vitousek et al. 1997, Fenn et al. 1998). Observed reductions in the C:N ratio of soil and foliage, and increased nitrate leaching from the forest floor (Stoddard 1995, Aber et al. 1998), indicate changes in N cycling. High N inputs are associated with an increase in base cation leaching from soil (Aber et al. 1989, Houdijk and Roelofs 1993, Stoddard 1995, Fenn et al. 1998, Watmough and Dillon 2003, Watmough et al. 2004) and decreased soil phosphate availability (Mohren et al. 1986, Houdijk and Roelofs 1993, Compton and Cole 1998a, 1998b, Fluckiger and Braun 1998, Harrison et al. 1999). These changes in nutrient availability, as well as associated reductions in root mycorrhizal infection (Hutchinson et al. 1998, 1999), may have important impacts on tree physiological processes and growth in large areas of the temperate forest biome.

Liming and additions of non-nitrogenous mineral nutrients have been used in managed forests to avert nutritional deficiencies (Ljungstrom and Nihlgard 1995), including those induced by N deposition (Liu et al. 1994, Cote et al. 1995, Fenn et al. 1998). By raising soil pH, liming corrects topsoil acidification, temporarily increasing the availability of exchangeable cations, especially calcium (Ca) and magnesium (Mg), and reducing solubility of aluminum (Al) and iron (Fe) (Schierl and Kreutzer 1991, Mohamed et al. 1993, Belkacem and Nys 1995, Kreutzer 1995, Long et al. 1997). By increasing soil pH, liming may also increase phosphate (P) availability by lowering Al and Fe solubility and by increasing mineralization of organic matter. Additions of lime have been associated with increased foliar concentrations of P, N, Ca and Mg (Hendershot 1991, Long et al. 1997, Moore et al. 2000).

The success of targeted nutrient additions has usually been measured by visible assessments of crown health, soil and foliar nutrient analysis (Hendershot 1991, Fyles et al. 1994, Hendershot and Courchesne 1994, Ljungstrom and Nihlgard 1995) and changes in tree radial growth (Ouimet and Fortin 1992, Cote et al. 1993, 1995, Wilmot et al. 1996). Relatively little information is available on physiological responses of trees to additions of non-N nutrients, with studies to date mainly limited to seedlings and saplings (e.g., Brown 2002, Bungard et al. 2002, Brown and Courtin 2003, Carswell et al. 2005, Cheaib et al. 2005; but see Cordell et al. 2001, Lovelock et al. 2006). Large canopy trees show pronounced differences in physiological patterns from seedlings and saplings (Thomas and Winner 2002), and may be expected to differ in responses to nutrient additions because of differences in nutrient demand, root deployment and capacity to acclimate to changes in
the soil environment.

The Haliburton region in central Ontario receives some of the highest annual rates of N deposition in eastern North America (Ro and Vet 2003). In a recent study in this region we inferred deficiencies of P and basic cations from high correlations of A. saccharum Marsh. radial growth with local availability of P, and to a lesser extent Ca and Mg, in rooting-zone soil (Gradowski and Thomas 2006). In the present study, we applied major non-nitrogenous mineral nutrients and measured physiological and morphological responses of A. saccharum canopy trees and saplings over 2 years. Four hypotheses were tested: (1) liming increases availability of phosphate by decreasing soil acidity, thus having the same effect as direct addition of P; (2) A. saccharum shows increased physiological and growth performance (photosynthetic capacity, internode elongation and leaf proliferation) in response to liming and P + K fertilization; (3) physiological and growth effects of liming and P + K additions are more rapid and more pronounced in saplings than in mature canopy trees; and (4) canopy physiological and shoot extension responses precede radial growth responses in mature trees.

Materials and methods

Site description

The study area is located in the Haliburton Forest, central Ontario, Canada (44°55′N, 78°45′W). The soils are of glacial origin (Sherborne till), dry to moderately fresh and rapidly to well drained. On upland sites, soils are shallow to moderately deep brunisols or juvenile podzols, with low (<10%) organic matter content, pH 4.2–5.1, cation exchange capacity (CEC) 3.55 ± 0.37 cmol kg⁻¹ and a base saturation of 38.5 ± 5.8 (Gradowski, personal observations). The predominantly silty sands are derived from granite or granite-gneiss deposits of the Precambrian Shield. The dominant humus form is fibrimor. Land morphology is heterogeneous with undulating topography. Mean annual precipitation is about 1050 mm and mean annual temperature is 5 °C; mean annual number of days with temperatures below 0 °C is 78.4 (Environment Canada 2004). The Haliburton region receives some of the highest annual rates of nitrate deposition in eastern North America (Ro and Vet 2003), with total N inputs of ~20 kg ha⁻¹ year⁻¹. Northern hardwood forests in this region are dominated by sugar maple (A. saccharum) with a significant component of American beech (Fagus grandifolia Ehrh.), yellow birch (Betula alleghaniensis Britton) and hemlock (Tsuga canadensis (L.) Carr.). The study was conducted in a sugar-maple-dominated stand (mean basal area 19.7 m² ha⁻¹) harvested under a selection management system 6 years before our initial measurements. Previous selection harvests have resulted in relatively uniform tree spacing in the study area.

Experimental design

Selected for study were 52 mature sugar maple trees (diameter at breast height (DBH) 37.6 ± 9.1 cm) of similar canopy position (dominant or co-dominant), with no visible trunk or crown defects. Trees were located near unpaved forest roads to facilitate crown access with a mobile aerial lift (Scanlift SL-240, Specialty Equipment, Indianapolis, IN). The minimum distance between trees was 25 m, which was similar to mean tree height. We also included 138 sugar maple saplings (0.5–3 m tall) growing in the shade of the experimental trees. Independently, 24 full-sun sapling plots (1 m² area each) were established in the middle of an oval treeless opening about 0.8 ha in area. Full-sun saplings were 0.5–0.7 m tall at the time of treatment applications.

Individual trees and sapling plots were treated with two fertilizers (triple superphosphate (TST; Ca(H₂PO₄)₂H₂O) and potassium chloride (KCl)) alone (P + K) or in combination with dolomitic lime (about 56% calcium oxide, 40% magnesium oxide and 4% of impurities such as iron oxide, aluminum oxide, silicon dioxide and sulfur). Fertilizers (200 kg of P and K ha⁻¹) and lime (4 Mg ha⁻¹) were applied uniformly in a 5 m radius around each tree (likely the area of highest fine root density) and throughout the entire 1 m² area of each sapling plot in the open area. In July 2002, treatments were applied in a two-level factorial, randomized design to mature sugar maple trees and to the shaded saplings (13 sites per each of the four treatment groups: control (C), P + K fertilization (F), liming (L) and liming plus P + K fertilization (LF)). In July 2003, the same treatments were applied to open area (full sun) sapling plots (six plots per treatment) and reapplied to the mature trees and shaded saplings, because of the small changes in soil pH and P concentration following initial applications.

Soil sampling and analysis

In July 2002 and July 2004, after a few rainless days, composite mineral soil samples were collected within the drip-line of each of the 52 study trees. A minimum of 10 samples from a depth of 0–10 cm (approximately corresponding to the depth of the Ah horizon) and another 10 samples from 10–30-cm depth (corresponding to the location of maximum root density) were randomly collected beneath each tree crown. Samples for each tree were bulked separately for each depth, dried for 48 h at 105 °C, stored in plastic bags and passed through a 2-mm sieve before analysis. Additionally, in July 2002–2004, the pH of three fresh soil samples from each site was determined in a saturated soil-distilled-water paste with a glass electrode (McLean et al. 1983). Samples were allowed to stand at room temperature for 1 hour before pH was measured. To reduce the time-related variation in soil data to a minimum, each soil sampling was conducted within a period of 1 or 2 days and included all treatment sites.

Concentrations of C and N in mineral soil were determined by C/H/N/S combustion analysis (Costech Analytical Technologies, Valencia, CA). Available P was determined colorimetrically by the ammonium molybdate method (Allen 1974) following extraction with 0.03 M ammonium fluoride in 0.025 M hydrochloric acid (Bray 1; Bray and Kurtz 1945, Olsen and Sommers 1982). Availability of K, Ca, Mg, Na, Fe, Al, Mn and Zn cations was determined by measuring their concentration in 1 N neutral ammonium acetate extracts by inductively coupled plasma (ICP) spectrometry (Page et al.
2003. Leaf, stem and root mass fractions were determined by estimated based on survival of 10 saplings randomly tagged in or per sapling plot. In August 2004, in each plot, mortality was determined by averaging 10 measurements per tree canopy layer (Li-Cor, Lincoln, NE) equipped with a red-blue light source leaves with a portable gas-exchange analyzer (LI-6400, measurements were made on three healthy, randomly chosen between 0900 and 1500 h EST in July 2004. At each location, leaves of mature trees and in upper-canopy leaves of saplings gases with a TCD detector.

determined by C/H/N/S analysis (Costech Analytical Tech-
nologies), and chromatographic separation and detection of gases as a 2-ml vial containing N,N-dimethylformamide (DMF) to extract the chlorophylls and stored in the dark at 4 °C for up to 2 weeks. Chlorophyll extracts in DMF maintain stability for about 20 days (Moran and Porath 1980). Absorbance of the extracts was measured at 664, 647 and 480 nm with an S2000 spectroradiometer (Ocean Optics, Dunedin, FL). Concentrations of chlorophyll (a+b) were calculated as described by Wellburn (1994). For morphological chemical analyses, leaves were collected by hand from top and bottom portions of each tree canopy and stored in plastic bags for a few hours in a dark cooler until their fresh mass and leaf area could be determined. Subsequently, the leaves were scanned, dried for 72 h at 70 °C and stored in paper envelopes. Dry leaf samples were weighed, ground in a mortar with liquid nitrogen and combined for each tree crown layer. Powdered leaf subsamples were digested in a solution of concentrated sulfuric acid and hydrogen peroxide (Lowther 1980). The digests were analyzed for P concentration by the colorimetric molybdenum blue method (Allen 1974) and for concentrations of Ca, Mg, K, Fe, Al, Mn and Na by ICP analysis (Page et al. 1982). Total leaf nitrogen content (N\text{t}) of powdered leaf subsamples was determined by C/H/N/S analysis (Costech Analytical Technologies), and chromatographic separation and detection of gases with a TCD detector.

Gas exchange was measured in upper- and lower-canopy leaves of mature trees and in upper-canopy leaves of saplings between 0900 and 1500 h EST in July 2004. At each location, measurements were made on three healthy, randomly chosen leaves with a portable gas-exchange analyzer (LI-6400, Li-Cor, Lincoln, NE) equipped with a red-blue light source mounted above the leaf chamber. Photosynthetic capacity (A\text{max}) was determined at a photosynthetic photon flux of 1000 μmol m\textsuperscript{-2} s\textsuperscript{-1}, as were stomatal conductance (g\textsubscript{s}), transpiration rate (E), water-use efficiency (WUE = A\text{max}/E) and intercellular CO\textsubscript{2} concentration (C\textsubscript{i}). Photosynthetic nitrogen-use efficiency (PNUE) was computed on a leaf area basis as A\text{max}/N\text{t}. For each leaf measured, the mean of three values was recorded at 20-s intervals, after 5–15 minutes of induction. A CO\textsubscript{2} concentration of 350 μmol mol\textsuperscript{-1}, relative humidity of 60–80% and leaf temperature of 20–25 °C were maintained during A\text{max} measurements.

Current-year shoot extension (± 1 mm), root collar diameter (± 0.1 mm for saplings) number of leaves per shoot and single leaf area (Li-Cor LI-3000 leaf area meter, ± 1 mm\textsuperscript{2}) were determined by averaging 10 measurements per tree canopy layer or per sapling plot. In August 2004, in each plot, mortality was estimated based on survival of 10 saplings randomly tagged in 2003. Leaf, stem and root mass fractions were determined by averaging values for five saplings randomly collected in each plot in August 2004. Saplings were dried for 72 h at 70 °C and their leaves, stems and roots weighed.

**Tree core sampling and analysis**

To minimize damage to tree boles, tree cores were collected at a height of 35 cm above ground. Tree cores were collected in August 2004, mounted on plywood boards and sanded. Annual ring widths were measured to the nearest 0.001 mm with a tree ring increment measurement system (WinDendro v6.1D, 1996; Regent Instruments, Université du Québec a Chicoutimi, Québec).

Five-year mean growth before the nutrient additions (1998–2002), and mean 2-year growth following nutrient additions (2003–2004) were evaluated statistically to examine nutrient addition effects on diameter increment. The most recent tree increments likely best reflect current growing conditions, although 5-year means have been widely used (e.g., Lorimer and Frelich 1989, Payette et al. 1990, Latham and Tappeiner 2002, Vizcayno-Soto and Cote 2004) to average out year-to-year variation in diameter growth resulting from climatic variation. To ensure correct measurement of annual increments, two cores collected from each tree were compared and results averaged. In case of pattern discrepancies, the cores were remeasured.

**Vector nutrient analysis**

Foliar data were subjected to graphical vector nutrient analysis (Timmer and Stone 1978); whereby mean concentrations of foliar nutrients are plotted against their mean content in the entire plant (or plant part). The magnitude of the vector joining the point describing the above relationship in the control with that in a given treatment indicates the effect of that treatment in terms of changes in nutrient content (mg per shoot), concentration (mg g\textsuperscript{-1} dry mass) and foliar biomass (g per shoot). Vector direction represents the underlying process associated with these changes. For example, an increase in total nutrient content accompanied by no increase in nutrient concentration is expected under steady-state conditions and is indicative of nutrient sufficiency; an increase in nutrient content accompanied by an increase in nutrient concentration (and biomass) is indicative of nutrient limitation. Detailed descriptions of vector diagnosis can be found in Timmer and Morrow (1984), Haase and Rose (1995), Koricheva (1999), Timmer and Teng (1999) and Salifu and Timmer (2003). When only a portion of tree biomass was sampled, it was assumed that nutrient concentration (mg g\textsuperscript{-1} dry mass) and content (mg per current-year shoot) in sampled foliage adequately reflected relative changes in the sampled trees (Timmer and Morrow 1984, Timmer and Teng 1999).

**Statistical analysis**

Data were subjected to analysis of variance (ANOVA), and Tukey’s Studentized Range post-hoc test was used to establish treatment differences. The mean change in annual diameter increment was calculated as the difference between mean annual diameter increments within the last 2 years (post-treatment ra-
dial increment) and the mean annual diameter increment within the previous 5 years (pretreatment radial increment). The mean change in yearly post- and pretreatment shoot elongation was calculated similarly. Relationships among variables were analyzed by Pearson’s correlation and multiple linear regression.

Results

Soil

Soil pH averaged 4.61 ± 0.20 before treatment, and no significant differences in soil pH were found the first year after treatment. After the second application of dolomite, soil pH increased to 4.80 ± 0.20 (P = 0.059), whereas in sites fertilized with P + K, it decreased to 4.37 ± 0.29 (P = 0.011; Table 1). Initially, total soil N content in the study area averaged 0.30 ± 0.07% with a C:N ratio of 15.95 ± 1.52, and these values did not differ significantly from values measured 2 years after the initial treatments. Availability of soil phosphate before treatment was generally low (14.7 ± 12.7 mg kg⁻¹) and was similar across plots. Two years after the first addition of P + K, soil P concentration in fertilized sites was significantly higher than in control sites (70.3 ± 46.5 mg kg⁻¹ at 10–30 cm depth, P < 0.0001). Similarly, soil K concentrations were significantly higher (P = 0.0032) in fertilized sites (22.9 ± 1.3 mg kg⁻¹) than in non-fertilized sites (21.8 ± 1.1 mg kg⁻¹). Addition of dolomite did not significantly change soil P or K availability within the first 2 years of the study. Soil concentrations of soluble Ca and Mg (pretreatment Ca, 218.6 ± 57.6 mg kg⁻¹; Mg, 27.9 ± 11.9 mg kg⁻¹) were significantly higher (P < 0.001) in limed sites (with post-treatment values of 326 ± 177 mg Ca kg⁻¹ and 36.9 ± 11.4 mg Mg kg⁻¹). Addition of P + K fertilizers did not change the concentration of soluble Ca and slightly lowered the concentration of Mg in soil.

Foliar nutrient concentrations

One year after treatment, foliar P concentrations in saplings growing in the open area was significantly higher (P = 0.001, Table 2) in fertilized (F and LF) plots (mean 0.18 ± 0.05%) than in non-fertilized (C and L) plots (mean 0.10 ± 0.01%). Similarly, open-area saplings in fertilized plots had significantly higher (P = 0.0035) foliar K concentrations (1.14 ± 0.09%) than saplings in non-fertilized plots (0.99 ± 0.12%). Foliar Ca concentrations were higher in L plots (1.25 ± 0.13%) than in C plots (1.04 ± 0.18%); however, overall differences in foliar Ca concentrations between treatments were not significant. There were no significant differences in foliar C, N, Mg or Al concentrations among saplings 1 year after treatment.

Pretreatment foliar P concentrations in canopy trees were near the minimum value recommended for healthy A. saccharum growth by Kolb and McCormick (1993) and Vizcayno-Soto and Cote (2004). Foliar K concentrations of untreated trees were about in the middle of the recommended range. In 2004, after 2 years of P + K additions, foliar P concentration in fertilized canopy trees was significantly (P < 0.0001) higher than in non-fertilized trees (Figure 1). The P + K treatment increased tree mean foliar K concentration by over 15% relative to the control concentration (P > 0.05). Liming did not significantly change foliar P, K, Ca or Mg concentrations. Foliar concentrations of Mg and Ca were initially near the lower limit of the recommended range and increased only slightly and non-significantly (P > 0.05) in response to P + K fertilization (Figure 1).

Vector analysis of tree foliar data showed that additions of P, K, Ca and Mg caused simultaneous increases in mean leaf dry mass, relative P, K, Ca, Mg concentration (% of leaf dry mass) and relative P, K, Ca, Mg content (mean total amount of nutrient content per shoot; Figure 2). This set of simultaneous changes is consistent with a tree response to addition of limit-

Table 1. Soil variables for the mature tree fertilization trial 2 years after application of the initial treatment (2004). Shown are the mean values and standard deviation (SD). Abbreviations: top = samples collected within the top 10 cm of mineral soil; bottom = samples collected at a depth of 10–30 cm of mineral soil; and CEC = cation exchange capacity. Letters indicate Tukey groupings for post-hoc comparisons (P < 0.05).

| Top       | Bottom      | C (%) | N (%) | C:N | P (ppm) | C (%) | N (%) | C:N | P (ppm) | K (ppm) | Ca (ppm) | Mg (ppm) | Al (ppm) | Fe (ppm) | CEC (cmol kg⁻¹) | pH |
|-----------|-------------|-------|-------|-----|---------|-------|-------|-----|---------|---------|----------|----------|----------|----------|----------|------------------|-----|
| Control   |             | 7.48  | 0.48  | 15.8| 18.7 a  | 4.11  | 0.26  | 15.93| 22.4 a  | 22.1 ab  | 174.6 a  | 20.3 a   | 43.2     | 1.63     | 4.19     | 4.62               |     |
| SD        |             | 1.85  | 0.15  | 1.44| 12.96   | 0.91  | 0.05  | 1.37 | 10.95   | 1.26     | 56.83     | 8.81     | 11.26    | 1.68     | 0.69     | 0.34               |     |
| Fertilizer|             | 6.55  | 0.4   | 16.4| 184.3 b | 4.5   | 0.27  | 16.94| 70.3 b  | 22.9 ab  | 187.1 a  | 18.7 a   | 39.6     | 1.56     | 4.28     | 4.37               |     |
| SD        |             | 1.56  | 0.1   | 0.66| 142.49  | 1.05  | 0.07  | 0.83 | 46.48   | 1.33     | 62.65     | 6.8      | 3.89     | 1.57     | 0.78     | 0.29               |     |
| Lime      |             | 7.09  | 0.42  | 17.1| 19.1 a  | 4.21  | 0.26  | 16.11| 20.2 a  | 21.5 a   | 326.0 b  | 36.9 b   | 39.9     | 1.57     | 4.84     | 4.8                |     |
| SD        |             | 1.31  | 0.07  | 0.76| 10.03   | 0.65  | 0.04  | 0.9  | 9.67    | 0.57     | 176.81    | 11.37    | 16.25    | 1.25     | 0.95     | 0.2                |     |
| Lime + fertilizer |     | 7.22  | 0.43  | 17.1| 158.7 b | 4.01  | 0.25  | 16.27| 78.8 b  | 23.0 b   | 322.9 b  | 34.6 b   | 38.6     | 1.5      | 4.73     | 4.56               |     |
| SD        |             | 1.84  | 0.13  | 1.51| 85.5    | 0.88  | 0.07  | 1.64 | 56.48   | 1.25     | 160.4     | 13.33    | 15.56    | 2.24     | 0.86     | 0.34               |     |

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ing nutrients. The magnitude of tree response to additions of P and Mg was particularly conspicuous, whereas responses to additions of Ca and K were lower, suggesting that trees were less deficient in Ca and K than in P and Mg. Foliar N concentration remained constant as its total content increased, a pattern consistent with N sufficiency (Figure 2). Among saplings, foliar biomass remained nearly constant when only P + K was added. The uptake of both nutrients was thus in excess of that required for maximum growth. Additions of lime and lime + P + K were associated with a greater increase in leaf dry mass compared with values for control and fertilized saplings (Table 2). Foliar concentrations of P, K and Ca and total foliar content of P, K and Ca increased in fertilized saplings. This pattern is consistent with deficiency of P, K and Ca. Although P deficiency was especially pronounced, P uptake was associated with sapling foliar biomass increase only when Ca was available. Additions of P + K were associated with depletion of foliar Mg in saplings. As in mature trees, foliar N concentration remained nearly constant as total N content increased, a pattern consistent with N sufficiency in saplings (Figure 2).

**Leaf area and dry mass**

Mean number of leaves per shoot in 2004 was significantly higher in upper canopies than in lower canopies of mature trees (P = 0.05) and saplings (P = 0.0009), but there were no significant differences among treatments (P = 0.67; Figure 3C). Sapling leaves were significantly larger than lower-canopy leaves of mature trees (P = 0.003; Figure 3A), and lower-canopy leaves were significantly larger than upper-canopy leaves of mature trees (P < 0.0001). Leaf area (Figure 3A) and the total leaf area per current-year shoot (Figure 3D) were significantly larger (P = 0.008 and P = 0.022, respectively) among A. saccharum treated with lime than among controls. Leaf dry mass was 23.9–37.4% higher in treated trees than in controls (Figure 3B). The effect of liming on leaf mass per area (LMA) was statistically significant at α = 0.05 and that of fertilization at α = 0.1 (Table 2). Leaf mass per area (LMA) was significantly higher (P = 0.0001) in the upper canopy than in the lower canopy of mature trees and saplings, but no treatment effect on LMA was discernible (P = 0.54).

**Photosynthetic gas exchange and chlorophyll**

Overall, canopy trees showed no significant treatment effects on $A_{\text{max}}$, $g_s$, $C_i$, PNUE, WUE, P, and $C_n$. Chlorophyll concentrations were lower in leaves of mature trees treated with lime than in controls (Figure 4A). Chlorophyll concentrations were lower in the combined treatment indicating a significant (P = 0.024)
negative interaction of liming and P + K fertilization (Table 2). This trend was especially pronounced in the lower tree crown. The concentration of chlorophyll was positively correlated with foliar N concentrations ($r^2 = 0.42$) and Ca ($r^2 = 0.26$), but showed no significant correlation with foliar P, K, Mg or Al. Photosynthetic capacity was positively correlated with chlorophyll concentration ($r^2 = 0.31$). We did not collect chlorophyll data for saplings.

**Growth**

One year after treatment initiation, no significant differences in leaf area, leaf dry or fresh mass, root collar diameter, leaf, stem or root mass fraction, or mortality attributable to treatment were recorded among saplings growing in the open areas. However, sapling shoot extension increased in all treatments, and in the plots treated with P + K combined with dolomite (LF) shoot extension increase was significantly higher ($P < 0.05$) than in the control treatment (Figure 5). The increase in shoot extension was significantly ($P = 0.0124$; Table 2) higher in all fertilized plots (F and LF pooled) than in plots where no P + K was added (in plots treated with P + K and lime it was 224% of that recorded in control plots). There was no effect of soil amendment on growth of shaded saplings ($P = 0.2$).

Two years after treatment, shoot extension in crowns of treated trees averaged 48.8–65.8% more than in control trees (Figure 5). Post-treatment shoot extension relative to mean shoot growth before treatment was significantly higher ($P = 0.0002$) in all treated trees compared with controls. However,
there was a significant negative interaction \((P = 0.005)\) between P + K fertilization and liming on shoot elongation growth: shoot elongation in the LF treatment was marginally lower than in either L or F (Figure 5, Table 2). No significant difference in tree radial growth was recorded among treatments within the first 2 years of nutrient additions: the difference between the mean yearly basal area increment before and after treatment decreased slightly, but not significantly \((P > 0.05)\), in P + K fertilized plots (Figure 5).

**Discussion**

Although lime additions increased soil pH, there was no detectable increase in available P, contrary to our first hypothesis. Nevertheless, additions of either dolomitic lime or P + K fertilizer resulted in rapid changes in foliar nutrient concentrations and shoot extension growth of both saplings and mature canopy trees, consistent with our second hypothesis. The positive effects of liming on shoot extension and related variables thus likely reflect direct effects of Ca and Mg. Although the observed responses generally indicate a predominant limitation of P, co-limitation by Ca and Mg (and to a lesser extent K) is supported by vector analysis. In contrast to the positive effects on shoot extension growth, we detected no effects of treatments on leaf-level gas exchange. A plausible explanation is that non-N nutrient additions enhanced shoot extension mainly by altered allocation patterns, rather than enhanced leaf-level carbon uptake. Contrary to our third hypothesis, treatment effects on shoot extension were similar in saplings and mature canopy trees; in both cases, shoot extension re-
We hypothesized that liming would mimic effects of P fertilization by enhancing P availability: phosphate availability in the soil solution is controlled by precipitation with Fe and Al, which is highly pH-dependent (Simard et al. 1994, Schlesinger 1997, McDowell et al. 2003). In addition, increases in pH in response to liming may increase the decomposition rate of forest litter (Condron et al. 1993, Geissen and Brummer 1999, Bauhus et al. 2004), which provides the majority of phosphate for plant uptake in hardwood forests (Yanai 1992). Nevertheless, P availability in mineral soil did not increase significantly within 2 years following dolomitic lime application (Table 1). We observed only a small (6.1%) statistically non-significant increase in foliar P concentration in the upper crowns of limed trees, and increases in foliar P concentration in the lower crowns of limed trees and in limed saplings were negligible. These findings suggest that liming did not substantially increase the availability of P, despite a marginally significant increase in soil pH. An 8-week-long incubation of 20 × 20 × 20 cm blocks of forest floor removed from the study area and treated with dolomitic lime also failed to increase P availability (Gradowski and Thomas, unpublished data).

Local soils at the study site are derived from granite parent material naturally depleted in phosphate-carrying apatite (Lightfoot et al. 1997, Nedachi et al. 2005). When enrichment of the soil P pool from mineral weathering is minimal, most P is either in organic form in the upper soil profile or bound by Fe and Al in deeper horizons (Yanai 1992, Crews et al. 1995, Cross and Schlesinger 1995). The low amounts of available inorganic P in the mineral soil at the control sites (Table 1) appear to support this scenario. Two years after the application of dolomitic lime, soil pH had increased from 4.61 ± 0.20 to 4.80 ± 0.20, which would result in only a small increase in solubility of phosphate precipitated by Al and Fe (Schlesinger 1997) with correspondingly small effects on P availability. Also, a naturally low availability of mineral phosphate is often associated with conservative, efficient P cycling between soil and vegetation, so P loss due to leaching is minimal (Fisher and Binkley 2000). We speculate that P released from iron and aluminum oxalates by liming was minimal and was efficiently and quickly immobilized by soil microorganisms (Fisher and Binkley 2000). Liming decreases P availability in forest litter (Condron et al. 1993, Chepkwony et al. 2001), which is commonly the main source of plant-available P in temperate forests (Yanai 1992). Thus, we conclude that the minimal increase in P availability at the limed sites was likely related to a combination of factors: naturally low amounts of mineral phosphate in the study area; liming-mediated inhibition of P release from organic sources; and efficient immobilization of P by soil microorganisms.

**Nutrient limitation patterns**

Based on the optimum range approach (Kolb and McCormick 1993, Vizcayno-Soto and Cote 2004), P + K fertilization satisfied or exceeded the foliar requirements of mature *A. saccharum* trees, whereas liming resulted in only small improvements toward putative optimum concentrations. Foliar N concentrations were originally within the optimum range and did not change significantly in response to either treatment. The applicability of comparisons with the optimum range, however, is limited to mature trees, and provides only a general guide when more than one nutrient is limiting tree growth (Lozano and Huynh 1989, Timmer 1991). Vector nutrient analysis (Timmer and Stone 1978) is based on interrelationships among foliar nutrient concentration, total nutrient uptake by the growing plant and biomass increase. Initially developed with a focus on conifer saplings, vector analysis has recently been used to examine nutrient limitation patterns of mature conifers (Luyssaert et al. 2004) and broad-leaved trees (Timmer and Teng 1999, Moore et al. 2004) and understory vegetation (Moran and Moran 1998). Vector analysis of *A. saccharum* foliar data suggested that all added nutrients (P, K, Ca, Mg) were deficient in the mature trees, particularly P and Mg, whereas saplings showed pronounced deficiency in P and Ca and sufficiency in Mg supply. The concentration of foliar N remained roughly constant with increases in leaf biomass in both trees and saplings, indicating N sufficiency (Figure 2). Only mature trees exhibited a clear response to Mg addition (i.e., simultaneous increases in foliar nutrient concentration, content, foliar biomass and shoot extension), perhaps indicating a stronger physiological demand for Mg in mature trees than in saplings. However, foliar Mg concentrations in untreated saplings were
approximately twice as high as in canopy trees, suggesting that site differences may account for this pattern.

Thus, both vector analysis and interpretations based on optimum ranges suggest that the availability of P, Ca, Mg, and to a lesser degree K, play an important role in limiting growth of mature *A. saccharum* in the study area, but that N availability was sufficient. Growth of *A. saccharum* saplings was most strongly limited by P availability. These findings are consistent with prior evidence suggesting deficiencies in Ca and Mg (Bernier and Brazeau 1988a, 1988b, Long et al. 1997, Moore et al. 2000, Horsley et al. 2002, Timmer et al. 2003, but also see Yanai et al. 2005), P (Pare and Bernier 1989a, 1989b, Kinch 1989, Eggyed 1990, Yanai 1998, Timmer et al. 2003, St. Clair and Lynch 2005) and K (Eggyed 1990, Pare et al. 1993, Burke and Raynal 1998, Horsley et al. 2002) in *A. saccharum* stands at various locations in eastern North America, and confirm inferences based on relationships between diameter increment and soil nutrient availability at the same site (Gradowski and Thomas 2006). Our analyses indicate that soil N content in the study area was high and easily accessible to saplings and mature trees alike, consistent with N excess (Vitousek et al. 1997, Aber et al. 1998, Fenn et al. 1998).

A third potential line of evidence for nutrient limitation patterns is provided by the ratios of foliar nutrients, or ecological stoichiometry (Sterner and Elser 2002, Ågren 2004, Gusewell 2004). The high foliar N:P ratios that we observed before addition of nutrients (mean 17.95) are indicative of N excess and P-limitation in forest ecosystems generally (Tessier and Raymond 2003). There is less comparative data on diagnostic ratios of other nutrients in forest systems, but optimum nutrient ratios for deciduous tree seedlings grown under conditions of free access of N:P:K:Ca:Mg = 100:15:65:7:9 have been described (Knecht and Göransson 2004). We observed ratios in control plots of about 100:6:56:57:11 (see Figure 1), suggesting a primary limitation of P, a slight deficiency of K, a slight excess of Mg and a large excess of Ca. However, prior studies have noted unusually high demands for Ca in *A. saccharum* (e.g., Knecht and Göransson 2004). Patterns of Ca limitation in *A. saccharum* thus appear to be a case in which general stoichiometric trends are not relevant.

Together, these findings indicate multiple nutrient limitations with a pronounced role of Ca, Mg and especially P. Based on the presumption that N was historically limiting (Aber et al. 1998, Schlesinger 1997), our findings indicate a shift from a principle limitation by N to a principle limitation by P. Although such a shift may be widespread, preexisting site conditions, in particular an underlying bedrock naturally depleted of P-rich apatite, may have predisposed the study area to P limitation.

### Physiological and growth responses

We detected no effects of soil amendments on any of the leaf-level gas-exchange parameters examined (*A* max, *g* s, *C* i, WUE and PNUE), despite pronounced effects of liming and P + K additions on shoot extension growth in both saplings and mature trees. The most likely explanation for this disparity is that the primary response to treatments was a change in allocation: additions of limiting mineral nutrients resulted in increased resource allocation to canopy growth and decreased allocation to root growth and metabolic function. Prior studies on root dynamics in forest ecosystems have frequently reported reduced fine root production in response to additions of limiting nutrients (e.g., Haynes and Gower 1995, Maier and Kress 2000). A companion study based on the experiments reported here similarly documented large decreases in fine root production (48–81% of controls) and soil CO 2 efflux (49–70% of controls) in response to liming and P + K additions (Peng 2007).

Although allocation changes seem the most likely explanation for the shoot extension responses observed, we cannot discount changes in net carbon gain, which seem likely to play a role in driving long-term growth responses. We observed a significant increase in chlorophyll concentration in response to liming and a positive correlation between foliar Ca and Mg and the chlorophyll concentration in canopy tree leaves. Low foliar Ca and Mg concentrations have previously been linked with leaf chlorosis and low chlorophyll concentrations in declining *A. saccharum* stands (Kinch 1989, Liu et al. 1997). An increased chlorophyll concentration generally results in increased absorbance of photosynthetically active radiation by leaves (e.g., Baltzer and Thomas 2005), which is expected to result in increased carbon gain under low-light conditions. Our photosynthetic measurements under saturating irradiance would not have detected such an effect. Perhaps more importantly, increasing leaf production in response to nutrient additions will result in an increased total leaf area within tree crowns, which is expected to increase total carbon fixation. Feedbacks within individual tree crowns, in particular intra-crown shading, may partially offset such gains, but a shade-tolerant species such as *A. saccharum* should be well adapted to benefit from increasing intra-crown LAI because leaf-level light compensation points are low. We speculate that increased foliar chlorophyll concentrations in limed trees, which were most pronounced in the lower canopy, may represent in part a response to increased intra-crown LAI and shading.

We are aware of only two previous studies that examined growth and physiological responses of canopy tree foliage to additions of non-N nutrients. Cordell et al. (2001) found large increases in *A* max and leaf chlorphyll but not in stand leaf area index in response to P fertilization in *Metrosideros polymorpha* Gaudich. on highly weathered, P-deficient soils in Hawaii. On younger, less P-deficient sites, *M. polymorpha* responded to P additions with increases in leaf size and LAI, but no change in *A* max. Lovelock et al. (2006) examined responses of the mangrove species *Rhizophora mangle* L. to P additions, and found large increases in CO 2 assimilation, stem hydraulic conductivity and canopy development in dwarf populations of *R. mangle* in which growth was strongly P-limited. Both *M. polymorpha* and *R. mangle*, although not typical tropical pioneer species, commonly colonize open habitats, and may accordingly have high plasticity in photosynthetic traits (Strauss-Debenedetti and Bazzaz 1996). In contrast, *A. sacch-
arum is among the most shade-tolerant trees in North America, and prior studies of saplings have shown only limited responses of leaf traits to altered environmental conditions (e.g., Baltzer and Thomas 2005). The large rapid increases in canopy foliage development observed in response to soil amendments are perhaps more surprising than the lack of leaf-level gas-exchange responses in this species.

Increased diameter increment of A. saccharum in response to additions of non-nitrogenous nutrients have been documented in many areas of north-eastern USA and Canada (Stone 1977, 1980, Hendershot and Jones 1989, Leech and Kim 1990, Hendershot 1991, Ouimet and Fortin 1992, Côté et al. 1993, 1995, Côté and Ouimet 1996, Wilmot et al. 1996). We detected no significant treatment effects on radial growth in either mature trees or saplings within the first 2 years of liming or P + K fertilization. This is consistent with studies indicating that fertilization effects on tree diameter growth, basal area and volume generally require several years to become evident (Lea et al. 1979, Stone 1980, 1986, Stanturf et al. 1989, Leech and Kim 1990). Mature A. saccharum growing in a 1-ha plot in Haliburton Forest showed a ~2-fold increase in radial increment but with a time-lag of no less than 9 years following application of dolomitic lime (Gradowski and Thomas, unpublished data). The lag in diameter increment response in mature trees is not surprising; however, large increases in stem elongation growth in the absence of any stem diameter response in saplings was unexpected, and must correspond to a pronounced change in shoot allometry, favoring height at the expense of diameter growth. Surprisingly, shoot elongation responses of mature canopy trees were similar in magnitude to those of sapling less than 1 m tall. Our results thus indicate mature trees respond rapidly to changes in the soil environment with changes in crown morphology, allocation and allometry that precede any positive response in tree radial growth.

In conclusion, both mature canopy trees and saplings of A. saccharum showed marked changes in leaf chemistry and canopy morphology in response to liming and P + K fertilization (e.g., leaf size, nutrient content and shoot extension), but showed no response in leaf-level gas exchange. The observed canopy responses preceded any diameter growth increases in both mature trees and saplings. We suggest that changes in shoot extension in tree crowns generally provide a more rapid and precise indication of tree responses to variation in soil resource availability than diameter growth. Strong limitation of tree performance by P and other non-nitrogenous nutrients in northern hardwood forests have important ecological and management implications. Given high heterogeneity in patterns of nutrient availability (Gradowski and Thomas 2006), one can infer that spatial patterns of tree performance and forest productivity are being altered: e.g., N-rich areas that may have been especially good for A. saccharum growth 50–100 years ago, may now be unfavorable, whereas P-, Ca- and Mg-rich areas may now have become the hotspots for tree growth. From an applied standpoint, additions of P, K or lime may be a viable strategy for enhancing forest productivity in areas affected by high N deposition, but should be preceded by detailed analysis of soil and foliar nutrient status.

Acknowledgments

We thank Peter Schleifenbaum and the staff of Haliburton Forest and Wildlife Reserve for logistical support and permission to conduct this work on private property, and Trevor Jones, Grant Domke, Rachel Mayberry, Sheelah Griffith, Hilary Thorpe, Tyler Peet, Liora Zimmerman and Rudy Gradowski for assistance with field work and laboratory analyses, Vic Timmer and Yuanxin Teng for critical input on soil and plant tissue analysis, and John Caspersen, Bob Jeffries, Myrna Simpson and Tom Hutchinson for advice on aspects of the study. This project was supported financially by NSERC Canada, the Ontario Premier’s Research Excellence Award program and by grant-in-aid awards from the University of Toronto, Faculty of Forestry.

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TREEmOLOGY VOLUME 28, 2008


