



WILEY

Response of Sugar Maple to Calcium Addition to Northern Hardwood Forest

Author(s): Stephanie M. Juice, Timothy J. Fahey, Thomas G. Siccama, Charles T. Driscoll, Ellen G. Denny, Christopher Eagar, Natalie L. Cleavitt, Rakesh Minocha and Andrew D. Richardson

Source: *Ecology*, May, 2006, Vol. 87, No. 5 (May, 2006), pp. 1267-1280

Published by: Wiley on behalf of the Ecological Society of America

Stable URL: <https://www.jstor.org/stable/20069067>

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at <https://about.jstor.org/terms>



Ecological Society of America and *Wiley* are collaborating with JSTOR to digitize, preserve and extend access to *Ecology*

JSTOR

RESPONSE OF SUGAR MAPLE TO CALCIUM ADDITION TO NORTHERN HARDWOOD FOREST

STEPHANIE M. JUICE,¹ TIMOTHY J. FAHEY,^{1,6} THOMAS G. SICCAMA,² CHARLES T. DRISCOLL,³ ELLEN G. DENNY,²
CHRISTOPHER EAGAR,⁴ NATALIE L. CLEAVITT,¹ RAKESH MINOCHA,⁴ AND ANDREW D. RICHARDSON⁵

¹Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, New York 14853 USA

²Yale School of Forestry and Environmental Studies, Greeley Lab, 370 Prospect Street, New Haven, Connecticut 06511 USA

³Department of Civil and Environmental Engineering, 220 Hinds Hall, Syracuse University, Syracuse, New York 13244 USA

⁴USDA Forest Service, P.O. Box 640, Durham, New Hampshire 03824 USA

⁵Complex Systems Research Center, University of New Hampshire, Morse Hall, 39 College Road, Durham, New Hampshire 03824 USA

Abstract. Watershed budget studies at the Hubbard Brook Experimental Forest (HBEF), New Hampshire, USA, have demonstrated high calcium depletion of soil during the 20th century due, in part, to acid deposition. Over the past 25 years, tree growth (especially for sugar maple) has declined on the experimental watersheds at the HBEF. In October 1999, 0.85 Mg Ca/ha was added to Watershed 1 (W1) at the HBEF in the form of wollastonite (CaSiO₃), a treatment that, by summer 2002, had raised the pH in the O_{ie} horizon from 3.8 to 5.0 and, in the O_a horizon, from 3.9 to 4.2. We measured the response of sugar maple to the calcium fertilization treatment on W1.

Foliar calcium concentration of canopy sugar maples in W1 increased markedly beginning the second year after treatment, and foliar manganese declined in years four and five. By 2005, the crown condition of sugar maple was much healthier in the treated watershed as compared with the untreated reference watershed (W6). Following high seed production in 2000 and 2002, the density of sugar maple seedlings increased significantly on W1 in comparison with W6 in 2001 and 2003. Survivorship of the 2003 cohort through July 2005 was much higher on W1 (36.6%) than W6 (10.2%). In 2003, sugar maple germinants on W1 were ~50% larger than those in reference plots, and foliar chlorophyll concentrations were significantly greater (0.27 g/m² vs. 0.23 g/m² leaf area). Foliage and fine-root calcium concentrations were roughly twice as high, and manganese concentrations twice as low in the treated than the reference seedlings in 2003 and 2004. Mycorrhizal colonization of seedlings was also much greater in the treated (22.4% of root length) than the reference sites (4.4%). A similar, though less dramatic, difference was observed for mycorrhizal colonization of mature sugar maples (56% vs. 35%). These results reinforce and extend other regional observations that sugar maple decline in the northeastern United States and southern Canada is caused in part by anthropogenic effects on soil calcium status, but the causal interactions among inorganic nutrition, physiological stress, mycorrhizal colonization, and seedling growth and health remain to be established.

Key words: *Acer saccharum*; acid deposition; calcium; forest decline; Hubbard Brook; manganese; mycorrhizae; soil acidification; stress physiology; sugar maple.

INTRODUCTION

Elevated deposition of strong acids derived from atmospheric pollution has been widely documented, and the effects of acid deposition on terrestrial and aquatic ecosystems have been demonstrated conclusively (Driscoll et al. 2001). The mechanism underlying these effects depends primarily upon the leaching loss of basic cations from soils and consequent reductions in soil pH, soil base saturation, and the mobilization of aluminum in soils. In some base-poor watersheds, effects on a variety of acid-sensitive biotic populations may occur, including forest trees. For example, the decline of red spruce (*Picea rubens* Sarg.) in the northeastern U.S. mountains

has been tentatively tied to disruption of plant calcium nutrition by acid deposition (Shortle and Smith 1988, Minocha et al. 1997, DeHayes et al. 1999). Similarly, many studies suggest that acid deposition and soil base cation depletion may be involved as predisposing factors (Manion 1991) in the widespread, but patchy, decline of sugar maple (*Acer saccharum* Marsh.) in eastern North America (Mader and Thompson 1969, Bernier and Brazeau 1988, Adams and Hutchinson 1992, Kolb and McCormick 1993, Ouimet and Camiré 1995, Wilmot et al. 1995, Horsley et al. 2000, Wargo et al. 2002). Because sugar maple is among the most abundant and valuable forest trees in this region, its decline is a cause for serious concern, and the mechanisms and progression of the decline are of great interest.

Long-term studies of the biogeochemical budgets of forested watersheds at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, USA (Likens and

Manuscript received 20 September 2005; accepted 7 October 2005; final version received 23 November 2005. Corresponding Editor: J. Richards.

⁶ Corresponding author. E-mail: tjf5@cornell.edu

Bormann 1995), especially the biogeochemical reference watershed (W6), have provided the basis for quantifying the effects of acid deposition on calcium cycling and soil base status of northern hardwood forest ecosystems (Likens et al. 1996, 1998, Gbondo-Tugbawa and Driscoll 2003). These studies have demonstrated that soil base saturation on W6 was greatly reduced during the 20th century, partly as a result of the high rates of cation leaching by acid deposition. As a result, the acid neutralizing capacity of surface waters in the HBEF experimental watersheds is very low. Coincidentally, these studies reported that the biomass accumulation in the largely even-aged Hubbard Brook forest stopped increasing considerably earlier than had been predicted (Whittaker et al. 1974), but a causal linkage between these observations was not established (Likens et al. 1996). More recently, Fahey et al. (2005) documented that in the late 1990s, aboveground net primary production (ANPP) in the biogeochemical reference watershed (W6) at the HBEF was 23% lower than had been measured in 1956–1965 by Whittaker et al. (1974) and 12% lower than predicted for the late 1990s by the forest production model PnET (Aber et al. 1995). Most of this reduced ANPP on W6 was associated with slow growth rates of sugar maple. Also, unusually high mortality of canopy trees of this species was observed from 1985 to 2002, especially at higher elevations (above 650 m; 2.7% per year) (T. G. Siccama, *unpublished data*). Foliar calcium concentrations are consistently much lower for sugar maple trees from these higher elevation sites (4 g/kg) than for lower elevation stands (6–7 g/kg) where soil base status is generally higher (Likens et al. 1996, Johnson et al. 2000). The abundance of sugar maple in the sapling layer throughout W6 has declined markedly since the earliest measurements in the mid-1960s, and if current trends continue, the abundance of sugar maple on W6 will be greatly reduced in the future. Hence, there are strong indications that in some parts of the HBEF, sugar maple may be adversely affected by soil base cation depletion, owing in part to acid deposition.

Sugar maple normally forms symbiotic associations with arbuscular mycorrhizal (AM) fungi. The AM symbiosis is important to the inorganic nutrition of host plants (Smith and Read 1997), and AM colonization has been shown to enhance plant growth and survival at low soil pH (Heijne et al. 1996, Clark 1997). The development of AM may be restricted by low soil or plant tissue pH or base cation status (Hepper and O'Shea 1984, Jartsfer et al. 1998), and there is some evidence that AM formation in *Acer* species may be reduced at low soil pH (Frankland and Harrison 1985, Ouimet et al. 1995, Coughlan et al. 2000). However, the relationships among sugar maple decline, AM colonization, and acid rain depletion of soil bases remain equivocal (Ouimet et al. 1995, Coughlan et al. 2000).

In October 1999, calcium was added to one of the experimental watersheds (W1) at the HBEF in the form of wollastonite (CaSiO_3), with the long-term objective of

gradually returning the exchangeable calcium in the soil to preindustrial levels over a 10–20 year period (Peters et al. 2004). Exceptionally high seed production by sugar maple was observed throughout the HBEF in fall 2002, resulting in a large cohort of sugar maple germinants in 2003. The objective of the present study was to quantify the response of this cohort of sugar maple germinants and canopy maple trees to the calcium fertilization treatment. Based upon the documented calcium depletion at the HBEF and the sensitivity of sugar maple to low soil calcium availability, we hypothesized that the treatment would improve the inorganic nutrition, mycorrhizal colonization, and overall health of sugar maple on W1.

METHODS

Study area

The Hubbard Brook Experimental Forest (HBEF) is located in north-central New Hampshire, USA (43°56' N, 71°45' W). Detailed descriptions of climate, hydrology, topography, and vegetation of the HBEF are presented in Likens and Bormann (1995). The climate is humid continental with short, cool summers and long, cold winters. Mean air temperature in July is 19°C and –9°C in January. Annual precipitation averages 140 cm, and a continuous snowpack usually develops early each winter to a depth of ~1.5 m. Soils are well-drained, acid Spodosols (Haplorthods) of sandy-loam to loamy-sand texture formed from glacial till. Thick (mean 6.9 cm) organic horizons, with pH ranging from 3.4 to 3.8, overlie bouldery mineral soil. Overstory vegetation is dominated by northern hardwoods: sugar maple, American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.), which comprise >90% of the forest basal area. At the highest elevations, balsam fir (*Abies balsamea* (L) Mill.), red spruce (*Picea rubens* Sarg.), and paper birch (*Betula papyrifera* Marsh.) dominate the forest. The HBEF is mostly second-growth forest developed following logging in the late 19th and early 20th centuries. Some areas also were affected by the 1938 hurricane and subsequent salvage logging (Schwarz et al. 2003) and by an intense ice storm in 1998 (Rhoads et al. 2002, Houlton et al. 2003).

Most of our research was conducted in and around two of the experimental small watersheds: the biogeochemical reference watershed (designated W6) and the calcium-treated watershed (W1). These watersheds are located on the south-facing slope (average slope angle 20–30%) of the Hubbard Brook valley. These gauged headwater catchments have been the subject of biogeochemical budget studies for many years (Likens and Bormann 1995). Field sampling for the present study was conducted in W6 and W1, as well as in study plots located adjacent to these experimental catchments. In particular, long-term sampling of sugar maple populations has been conducted in W6 since 1965 (*Methods: Demography and seedfall*). In addition, some process-level sampling (e.g., seedfall, litterfall) has been con-

ducted in permanent plots located immediately to the west of W6 (to avoid foot traffic in W6), designated hereafter as “west of W6 plots.” Detailed sampling of sugar maple seedlings for the present study was conducted within W1 and in reference (untreated) sites located immediately adjacent to W1 (hereafter, “reference plots”); these sites were chosen to minimize differences in site characteristics between treatment and reference sites. For most of these measurements, sampling was stratified by elevation zone. There were three elevation zones designated within the northern hardwood-dominated forest in and adjacent to W1: low (500–550 m), middle (550–600 m), and high (600–700 m).

Experimental treatment

In October 1999 (soon after leaf fall), 55 Mg of powdered and pelletized wollastonite (CaSiO_3) were applied by helicopter to W1. The delivery rate measured on the ground averaged 85 g Ca/m^2 and was highly uniform across the catchment (Peters et al. 2004). This delivery rate was chosen to roughly double soil base saturation and consequently increase soil pH to estimated preindustrial levels. By summer 2002, the treatment had increased pH in the O_{ie} horizon of W1 to 4.98 compared with 3.83 in W6 (P. M. Groffman, *personal communication*). A smaller but significant pH increase was also observed in the O_a horizon (4.22 vs. 3.88), while no significant response was detected in the upper mineral soil (4.36 vs. 4.26). In summer 2004, pH in the organic horizons was similar to 2002, and differences were beginning to appear in mineral soil (4.25 vs. 4.02; $P < 0.10$).

Demography and seedfall

Periodic counts of seedlings (stems <0.50 m tall) and small saplings (≥ 0.50 m tall and <2.0 cm dbh) were conducted in the low-elevation zone of the reference watershed (W6) in a random set of 24 quadrats of 1.0 m^2 beginning in 1965 and then at 5-year intervals from 1977 to 1997. Since 1998, these counts have been made annually in a set of 100 permanent 1.0- m^2 quadrats distributed in a stratified (by elevation) random design on both W1 and W6. The elevation at the base of W1 is 70 m lower than W6, and because seedling density varied markedly with elevation, all comparisons between W1 and W6 are confined to permanent plots in the same elevation range (550–740 m; 70 plots for W1 and 74 plots for W6). In 2004 and 2005, all seedlings measured in the plots were categorized as second-year (2004) or third-year (2005) germinants (i.e., originated in 2003) or as older seedlings. The vast majority of seedlings in 2003 were actually new germinants, but the exact proportion is not known because new germinants were not distinguished from older seedlings. In 1965, large saplings (stems 2.0–9.5 cm dbh) were measured in 208 10×10 m plots distributed on a grid across W6. In 1982 and 1987, large saplings were measured in 35 quadrats of 625 m^2 , and in 1992, 1997, and 2002, saplings were measured in 208 quadrats of 75 m^2 in W6.

Seedfall was measured in three 0.5-ha plots arranged along the elevation gradient in W1 and in the west of W6 plots using networks of 12 0.1- m^2 litter traps (Fahey et al. 2005). Sugar maple seeds were sorted from autumn litterfall collections and counted. A subsample of 30 seeds each from reference and treated sites was retained for measurement of average seed mass and element concentrations. Leaf fall of sugar maple was also measured for each trap so that seed production could be expressed on a unit leaf mass basis to correct for between-plot differences in sugar maple abundance.

Canopy sampling

Mid-canopy foliage of sugar maple was collected annually beginning in early August 1999 (pretreatment) through 2004 by sharpshooters. A stratified (three elevation zones) random sample of 10 canopy trees was sampled in each year. Samples were stored on ice during transport to the laboratory for chemical analysis (*Methods: Laboratory measurements*).

In late July and early August of 2005, visual evaluations of crown condition were conducted on 380 canopy trees of sugar maple encompassing the same ranges of elevation in W1 and W6 (600–700 m). The standard protocols of the North American Maple Project (Miller et al. 1991) were employed, and field staff received a day of training with Maple Project staff (Robert Cooke, Durham, New Hampshire, USA) immediately prior to beginning the survey. Three crown condition ratings were estimated visually by two-person teams viewing each tree from two directions: (1) crown vigor, an estimate of overall branch mortality; (2) foliage transparency, an estimate of skylight visible through foliated portions of branches; and (3) twig dieback, the percentage of crown silhouette exhibiting dieback originating from terminal portion of branches.

Seedling sampling

Sugar maple germinants were collected in early August 2003 (first-year germinants) and 2004 (second-year germinants) from randomly placed transects in the treated watershed (W1) and in the reference plots located adjacent to W1 (destructive sampling is prohibited in W6). In 2003, sampling was stratified by elevation, with 120 seedlings each collected from treated and reference areas in the low- and mid-elevation zones of the watersheds. Seedlings were not destructively sampled in the highest elevation zone because of very low densities there. Seedlings were carefully extracted from soil, with root systems intact, with the aid of a hand trowel. Samples were transported to the laboratory in plastic bags and processed the same day as collected. In 2004, 90 seedlings in total were collected randomly from treated and reference areas using the same approach.

In early August 2003 and in June 2004, field measurements of foliar “greenness” were conducted using a SPAD-502 (Minolta Camera, Osaka, Japan) as an estimate of chlorophyll content (Richardson et al.

2002). In 2003, 40 randomly chosen seedlings were measured in W1 and the reference plots in each of the three elevation zones (low, mid, and high) and similarly in the west of W6 plots (Bohlen et al. 2001). In 2004, 55 SPAD readings were made in both W1 and the reference plots. Seedling collections and SPAD readings were made in 2003 in additional sites: (1) 30 seedlings were collected from two paired control and fertilized plots in the HBEF, which received complete, balanced fertilizer for three years at the same rate and timing as described by Fahey et al. (1998); and (2) SPAD readings were taken on 50 seedlings in Sleeper's River watershed, a calcium-rich site in northeastern Vermont, USA (Shanley et al. 2002).

In July 2004, additional sampling was conducted in W1 and reference plots in the low-elevation zone. Photosynthesis was measured on randomly chosen, second-year germinants (12 seedlings from each site) using a portable photosynthesis system (LI-6400, LiCor, Lincoln, Nebraska, USA). Preliminary light response curves (photosynthetic photon flux density [PPFD] = 0–400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) indicated that A_{sat} , the light-saturated rate of photosynthesis, was achieved at PPFD $\sim 200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For the remainder of the measurements, illumination of the leaf was held constant at this level using a built-in red + blue LED light source. The CO_2 concentration in the reference analyzer was set to 400 $\mu\text{mol}/\text{mol}$, and relative humidity in the sample chamber was kept at $\sim 70\%$. At each site an additional eight seedlings were collected for subsequent biochemical analysis (for a total of 40 samples).

In July 2004, fine-root samples were collected from mature sugar maple trees in W1 and adjacent reference plots. Twenty samples of organic and upper mineral soil horizons were collected in each site at randomly chosen locations in the vicinity of overstory sugar maples in the low- and mid-elevation zones. Samples were returned to the laboratory, and, within 24 hours, sugar maple fine roots were sorted from the soil based on morphological criteria. Root clusters were washed in deionized water and preserved in 50% ethanol for analysis of mycorrhizal colonization.

Laboratory measurements

Seedlings from the treated watershed (W1) and adjacent reference plots were divided into root and shoot. Half of the root samples were gently washed with tap water and preserved in 50% ethanol for mycorrhizal analysis, while the others were rinsed with deionized water and dried to constant mass at 55°C . Stems were separated from leaves and dried at 55°C . In 2003, 10 discs (38.5 mm^2) were removed from each of 34 fresh leaves in each site for chlorophyll analysis after making SPAD measurements on each leaf. The rest of the leaves were dried at 55°C . The same procedures were used for seedlings from the paired reference and complete fertilizer plots, with 20 samples from each plot measured for chlorophyll and 10 preserved for mycorrhizal analysis.

Chlorophyll was extracted from leaf discs with dimethyl sulfoxide (DMSO) using the method of Richardson et al. (2002). Glass test tubes containing 7 mL DMSO were preheated to 65°C . Leaf discs were incubated for 30 min and then the volume was brought to 10 mL with DMSO. Absorbance was measured at 645 and 663 nm using a Genesys-20 spectrophotometer (Spectronic, Leeds, UK). Chlorophyll concentrations were then calculated according to the method of Richardson et al. (2002). The relationship between chlorophyll concentrations and SPAD readings was evaluated using a general linear model.

The area of each leaf was measured using an LI-3100 Area Meter (LiCor, Lincoln, Nebraska, USA). Shoot length was measured to $\pm 1 \text{ mm}$. Root length and area were measured using a flatbed scanner and analyzed with Delta-T Scan software (Delta-T Devices, Cambridge, UK). After first removing laterals from the primary root axis, lateral roots were stained with methyl violet for 24 hours. Bouma et al. (2000) detail the procedure we used for computerized analysis of root systems. Finally, roots, stems, and leaves were weighed to $\pm 0.1 \text{ mg}$.

Fine roots were cleared and stained for mycorrhizal analysis using a modified version of the method of Brundrett et al. (1994). Lateral roots were cut into 2-cm segments and 10 segments were randomly selected for analysis. Roots were cleared using 10 mL of 10% KOH in a 90°C water bath, with KOH changed every 30 min until no pigment was visible in the solution. Roots were rinsed with deionized water and bleached (0.5% NH_4OH and 0.5% H_2O_2) in a 90°C water bath for 30 min, stained with 0.05% Parker Quink black ink (Parker Pen Products, Newhaven, UK) in 1:1 glycerol/vinegar for 9–12 h; de-stained in 1:1 glycerol/vinegar solution for 2–3 h; and wet-mounted and examined at $200\times$ magnification. For each sample, 100 observations were taken at 1-mm intervals along each root axis, with colonization scored on the basis of intersection of the ocular crosshairs with fungal structures: arbuscules, vesicles, coils, hyphae, and no structures. Mycorrhizal hyphae included only those fungal hyphae that were connected to other AM structures. Percentage colonization was calculated from the percentage of total observations of each structure relative to total observations.

The procedure for mycorrhizal analysis of highly pigmented, mature sugar maple roots was slightly different. Following staining and destaining, as before, the cortex of the roots was peeled from the vascular cylinder with jeweler's forceps under a dissecting microscope. The root cortex was flattened and wet-mounted uniformly onto a slide. For each slide, a cover slip with a square grid was placed over the root cortex, and intersections with the grid were scored as for seedling roots. This procedure allowed visualization of mycorrhizal structures without the strong clearing and bleaching procedures needed to clear vascular cylinders.

Dried samples of seedling roots, stems, and leaves were pooled randomly into three samples for chemical analysis. Samples were powdered in a ball mill and analyzed for nitrogen on a combustion analyzer (Costech Analytical, Valencia, California, USA). Analysis of all other elements was conducted following combustion of 0.1-g subsamples at 550°C for 4 h. After adding 0.5 mL of 50% H₂O₂, samples were reheated to 550°C for 2 h. Ash was dissolved in 5 mL of 50% ultrapure HNO₃ and brought to 10 mL final volume with deionized water. Solutions were analyzed by plasma spectroscopy (Spectro Analytical, Kleve, Germany). Overstory leaf samples were digested with H₂SO₄ and H₂SeO₃. Total calcium and manganese concentrations were analyzed by plasma spectroscopy (Varian, Palo Alto, California, USA).

Exchangeable ions, amino acids, and polyamines were extracted from freshly chopped tissues with 5% perchloric acid for the foliage and roots of seedlings used for photosynthesis measurements. Extracts were kept on ice during transport and stored at -20°C until they were processed. Exchangeable inorganic ion concentrations were determined by Varian Vista ICP (Varian, Palo Alto, California, USA). Amino acids and polyamines were determined by reverse-phase HPLC (Perkin-Elmer, Norwalk, Connecticut, USA) following procedures detailed by Minocha and Long (2004).

Results from the treated watershed, W1, and adjacent reference plots were analyzed using a fixed-effects two-way ANOVA with treatment and elevation zone as the main effects. Effects of elevation and year of collection on canopy foliage chemistry in W1 were evaluated using a two-way ANOVA. Student's *t* statistic was used to compare seedlings in the complete fertilization plots and to compare tissue chemistry between W1 and adjacent reference plots. Crown ratings of canopy trees were compared between W1 and W6 using the chi-square statistic. Seedling density in permanent plots was compared between the treated (W1) and the biogeochemical reference (W6) watersheds; these comparisons were confined to plots within the same elevation range (550–740 m). The difference in seedling density between pre- and posttreatment for each permanent plot was evaluated with a Wilcoxon test to detect the effects of treatment.

RESULTS

As expected, calcium fertilization of W1 caused a significant ($F_{4,8} = 16.36$, $P = 0.001$) increase in calcium concentrations of canopy sugar maple foliage (Fig. 1). The response was delayed until the second year after treatment, peaked in year three, and declined slightly thereafter. No significant elevational differences in foliar calcium were observed either before or after treatment. Foliar manganese remained constant for three years after treatment before declining significantly ($F_{4,8} = 3.13$; $P = 0.007$) in years 4 and 5 (Fig. 1). Unlike calcium, foliar manganese showed a very strong elevational pattern with much greater concentrations at high (2.14

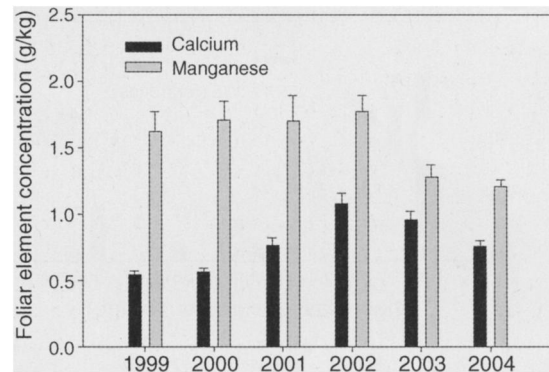


FIG. 1. Concentrations (mean \pm SE) of calcium and manganese in mid-canopy foliage of sugar maple before (1999) and after (2000–2004) addition of calcium (85 g/m²) in the form of wollastonite on Watershed 1 (W1), Hubbard Brook Experimental Forest, New Hampshire, USA.

± 0.14 g/kg; mean \pm SE) rather than mid (1.35 ± 0.06 g/kg) and low elevations (1.24 ± 0.06 g/kg).

In 2005, the crown condition of sugar maple trees on the treated watershed (W1) was generally healthier ($P < 0.01$) than for the reference watershed (W6), as illustrated by frequency histograms of crown vigor, branch dieback, and foliage transparency (Fig. 2). For example, 42% of surviving canopy sugar maples on W6 exhibited >15% branch dieback (a common threshold used to indicate ill health in sugar maple; [Allen et al. 1995]), whereas the corresponding figure for W1 was only 12%. In contrast, since being tagged in 1999, the percentage mortality of canopy sugar maples in W1 and W6 was not much different (16.2% vs. 15.6%, respectively). On an annual basis these rates (2.7% per year) are considerably elevated in comparison with healthy maple populations (1.25% per year; Buchman et al. 1983). However, it is important to note that some of this high mortality can be attributed to damage by the 1998 ice storm, which affected W1 and W6 to a very similar degree (Rhoads et al. 2002).

Density of sugar maple seedlings (<0.50 m tall) was consistently higher in the reference watershed (W6) than the treated watershed from 1998 (pretreatment) to 2000 (Fig. 3). Based upon permanent plots encompassing the same elevation range (550–740 m), seedling density in W6 during this period was much lower than had been observed in 1965, 1977, and 1982, but similar to 1987 and 1992 (Fig. 4). Similarly, the density of saplings declined markedly over the interval of long-term sampling on W6 (Fig. 4). Very high seedling density was observed in 1997 following the exceptionally large seed crop year of 1996 (e.g., 582 ± 69 seeds/m², all values mean \pm SE, in the mid-elevation plot west of W6). Seedling density declined markedly with increasing elevation in both W1 and W6, especially above ~600 m (Fig. 5).

A significant increase in sugar maple seedling density in the treated watershed, but not the reference water-

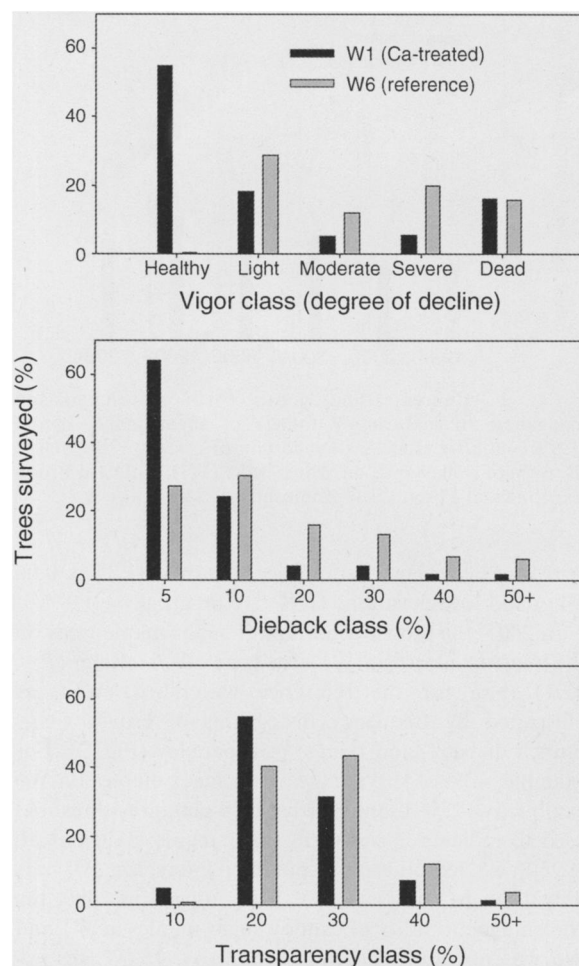


FIG. 2. Frequency histograms (percentage of stems surveyed) of crown ratings (vigor, dieback, and transparency) for canopy sugar maple trees on Ca-treated W1 and reference W6, Hubbard Brook Experimental Forest, New Hampshire, USA. See text for detailed descriptions of crown ratings.

shed, was observed in 2001 (Fig. 3) following a moderate seed crop in 2000 (89.3 ± 13.2 seeds/m² for low and mid-elevation in the treated watershed). Following the large sugar maple seed crop of 2002, seedling density in July 2003 was ~50% higher on the treated than the reference watershed (Fig. 3), a significant ($P < 0.001$) response that was clearly attributable mostly to the high density of first-year germinants on the treated watershed. Seedfall in 2002 was not significantly different between W1 (103 ± 11 seeds/m²) and the west of W6 permanent reference plots (131 ± 11 seeds/m²). Expressed per unit of sugar maple leaf dry mass (to correct for differences in sugar maple abundance among plots), seedfall averaged 0.77 ± 0.27 seeds/g leaf litter mass in W1 and 1.25 ± 0.48 seeds/g leaf litter mass in the west of W6 plots. Hence, the much higher density of new germinants in the treated watershed resulted either from higher seed germination or higher early survivorship, or both. Seedfall was much lower in the high-elevation

hardwood zone (600–700 m) on both watersheds (0.29 ± 0.09 seeds/g leaf litter mass) than at mid and low elevations (500–600 m; 1.37 ± 0.09 seeds/g leaf mass). This seedfall pattern was reflected in the decline of seedling density above 600 m elevation in both watersheds (Fig. 5).

In 2004, we distinguished second-year germinants from older seedlings, and the difference in density of second-year germinants between W1 and W6 was even greater than observed in 2003 (1.73 germinants/m² on W1 vs. 0.45 germinants/m² on W6). Hence, late-summer and overwinter mortality was much lower in the treated than the reference watershed (Fig. 3). Above ~600 m elevation there were almost no surviving germinants on the reference watershed, whereas moderate survivorship was observed on W1 (Fig. 5). This pattern of higher survivorship for the 2003 cohort of sugar maple seedlings on the treated watershed continued in 2005, as the difference in density between W1 (1.11 stems/m²) and W6 (0.20 stems/m²) became even more pronounced (Fig. 3).

Sugar maple germinants on W1 had significantly greater leaf, stem, and root biomass than those in adjacent reference plots in both 2003 and 2004 (Table 1). The proportional difference in germinant biomass between the sites remained about the same in both years, as all tissues were about one-third greater in W1 than reference plots in both years. There was no effect of the treatment on the root : shoot mass ratio of sugar maple germinants. Although maple germinants on the treated watershed were taller in both years, this pattern was significant ($P < 0.01$) only in 2004. No treatment effects were observed for two indices of plant morphology: leaf mass per unit area and specific root length (Table 1). Finally, average seed mass was similar between W1 (38 ± 6 mg) and reference plots (43 ± 4 mg) adjacent to W6.

Total chlorophyll concentration in leaves of sugar maple germinants in 2003 was significantly higher ($P < 0.001$) in W1 than for adjacent reference plots (Table 2), a pattern that was also observed for both chlorophyll *a* and chlorophyll *b*. Surprisingly, no elevation effect (low vs. mid) on total chlorophyll was observed. Application of complete fertilizer to two experimental plots elsewhere in the HBEF also resulted in significant increases ($P < 0.001$) in chlorophyll concentrations relative to control plots (data not shown). Chlorophyll concentrations were linearly correlated with SPAD “greenness” reflectance readings ($r = 0.74$, $n = 120$) so that the latter can be regarded as an effective surrogate for leaf chlorophyll concentration (Richardson et al. 2002).

Greenness measurements of sugar maple germinants in 2003 from W1 and adjacent reference plots were parallel to chlorophyll measurements, with the additional observation that seedlings from the highest elevation zone (600–700 m) were comparable to those from lower and mid-elevations, i.e., treatment effect ($P < 0.001$) but no elevation effect (Table 2). In June 2004, the differences in greenness between W1 and

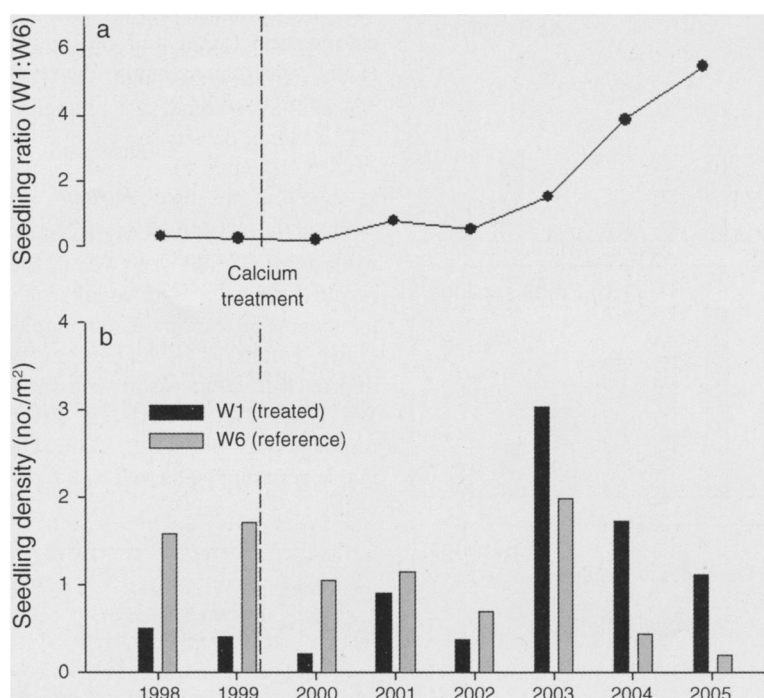


FIG. 3. Comparison of sugar maple seedling density over the same elevation range (550–740 m) on Ca-treated watershed (W1) and reference watershed (W6) at Hubbard Brook Experimental Forest, New Hampshire. The dashed vertical line indicates the calcium application that occurred at the end of the 1999 growing season.

adjacent reference plots, though still significant ($P < 0.05$), were smaller than for 2003, possibly reflecting late summer and overwinter mortality of the least healthy germinants in reference plots.

Greenness measurements of sugar maple germinants from other untreated plots in the HBEF and elsewhere illustrated broadscale spatial variation in seedling health as reflected in chlorophyll content. For example, the highest greenness values that we measured for sugar maple germinants in 2003 (36.2 ± 0.5 SPAD units) were for a plot at Sleeper's River watershed, a base-cation-rich forest located 80 km northwest of the HBEF (Shanley et al. 2002) where surface soil pH averages 6.3 (J. Shanley and S. Bailey, *unpublished data*). Within the HBEF, greenness of sugar maple germinants was especially high (31.9 ± 0.7 SPAD units) in the west of W6 permanent reference plot at mid-elevation. This plot also exhibited very high seedling density in July 2003 (61.3 ± 5.9 seedlings/m²) and had notably higher forest floor pH (4.2; Fisk et al., *in press*) than W1 and the adjacent reference plots (pH = 3.4–3.8).

Plant tissue chemistry differed in a variety of ways between sugar maple germinants on W1 and adjacent reference plots. As expected, concentrations of calcium were much greater ($P < 0.01$) in fine roots, foliage, and stem tissue from the treated watershed (Table 3); roughly twofold greater calcium concentrations were observed in W1 both years. Conversely, concentrations of manganese were much lower in treated seedlings than reference seedlings. Surprisingly, in 2003, nitrogen

concentrations in foliage and fine roots of new germinants were significantly lower in the treated watershed (Table 3), despite the higher chlorophyll and greenness values there (Table 2). Although no other significant differences in tissue chemistry were observed, concentrations of magnesium, phosphorus, and aluminum tended to be lower in seedlings from the treated watershed than the adjacent reference plots (Table 3). In the treated plots, calcium and magnesium concentration declined in concert from the first to second year, so that the Ca:Mg ratio remained constant. All element concentrations tended to be higher in seeds from the reference watershed than from the treated watershed (e.g., 14.4 g Ca/kg in W6 vs. 9.7 g Ca/kg in W1). Patterns of exchangeable ion concentrations were similar to those for total concentrations, with significantly higher calcium and lower manganese in the treated than reference sites (data not shown).

In 2004, photosynthesis rates (at PPFD = 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of second-year germinants were nearly identical between W1 (2.67 ± 0.20 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and adjacent reference plots (2.66 ± 0.26 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Concentration of the polyamine putrescine in foliage and roots was not significantly affected by the calcium treatment. In contrast, significantly higher concentrations of the amino acid proline were observed in seedlings of the reference plots than in W1 for both foliage (ref = 37.2 ± 5.5 $\mu\text{mol/kg}$ vs. W1 = 24.2 ± 1.8 $\mu\text{mol/kg}$ fresh mass) and roots (ref = 28.0 ± 5.7 $\mu\text{mol/kg}$ vs. W1 = 15.6 ± 6.0 $\mu\text{mol/kg}$ fresh mass).

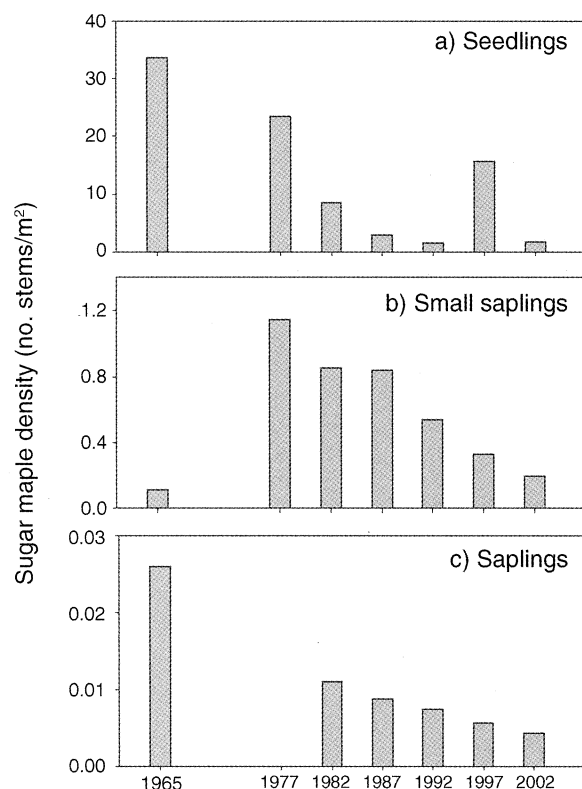


FIG. 4. Long-term trends in density of sugar maple regeneration on reference watershed (W6) at Hubbard Brook Experimental Forest, New Hampshire, USA. (a) Seedlings are defined as stems <50 cm tall. (b) Small saplings are stems ≥ 50 cm tall and <2.0 cm dbh. (c) Saplings are stems 2.0 to <9.5 cm dbh. Note the different y-axis scales.

Mycorrhizal colonization of sugar maple germinants was significantly greater ($P < 0.001$) in W1 than the reference plots in 2003 (Fig. 6), and no differences between elevation zones were observed. The abundance of all mycorrhizal structures, arbuscules, coils, and vesicles was much greater in the treated sites as well. Mycorrhizal colonization increased in 2004, but the large difference ($P < 0.001$) between treated and reference sites was maintained (Fig. 6). Hence, calcium fertilization resulted in a striking and sustained increase in mycorrhizal colonization of sugar maple germinants on the treated watershed at the HBEF. In contrast, the addition of complete fertilizer to two plots elsewhere in the HBEF resulted in increased growth, but significantly reduced mycorrhizal colonization of first-year sugar maple germinants in comparison with reference plots ($P < 0.05$). The levels of colonization in the reference plots of the latter study were significantly greater than in the reference plots adjacent to W1 (e.g., total hyphal colonization 12.3% vs. 4.4%, $P < 0.001$), apparently reflecting the high, broadscale spatial variation in sugar maple seedling health and abundance noted earlier. Finally, mycorrhizal infection of mature sugar maple trees was also significantly different ($P < 0.001$) between

W1 and adjacent reference plots. On W1, total colonization (56%) and the abundance of arbuscules (13%) were greater than in the reference plots (35% and 4%, respectively).

DISCUSSION

General

The addition of 0.85 Mg/ha of calcium in the form of wollastonite (CaSiO_3) to W1 at the HBEF in fall 1999 resulted in increased foliar calcium and decreased manganese in canopy sugar maples (Fig. 1). By 2005, crown condition of maples on W1 was significantly improved in comparison with the reference watershed, W6 (Fig. 2). Similarly, a striking and prolonged response was observed in the 2003 cohort of sugar maple germinants, as well as a significant, but transient,

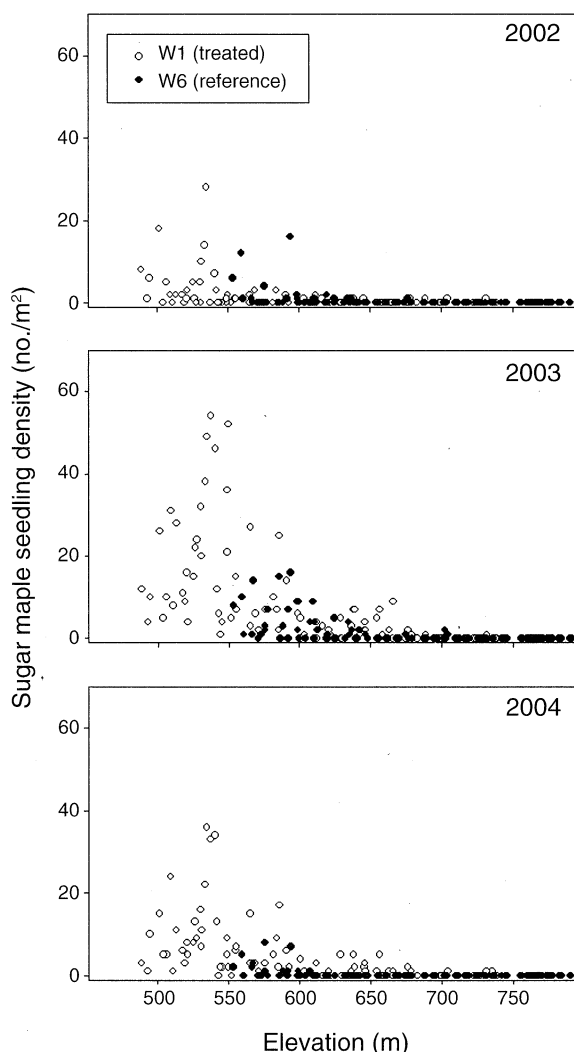


FIG. 5. Elevational patterns in sugar maple seedling density (2002–2004) on Ca-treated watershed (W1) and reference watershed (W6) at Hubbard Brook Experimental Forest, New Hampshire, USA.

TABLE 1. Individual seedling biomass components, root:shoot biomass ratio, plant height, leaf mass per unit area (LMA), and specific root length (SRL) for sugar maple seedlings from reference and Ca-treated (W1) sites at Hubbard Brook Experimental Forest, New Hampshire, USA.

Collection year and site	Seedling biomass (mg dry mass per plant)				Root:shoot ratio	Height (mm)	LMA (g/m ²)	SRL (m/g)
	Leaf	Stem	Root	Total				
2003								
Reference	41.4 (3.1)	22.9 (0.6)	27.9 (3.8)	92.2 (7.5)	0.43	81.0 (2.0)	18.4 (0.1)	44.9
Treated	62.0 (1.9)	33.3 (1.0)	40.4 (3.6)	135.7 (6.5)	0.42	87.8 (1.8)	19.5 (0.3)	47.2
2004								
Reference	66.1 (3.0)	65.0 (2.9)	69.3 (4.8)	200.4 (10.7)	0.53	85.9 (2.0)	29.0 (1.0)	nd
Treated	109.2 (4.0)	93.4 (3.0)	103.0 (6.2)	305.6 (13.2)	0.51	97.5 (1.8)	30.5 (0.8)	nd

Notes: All means set in boldface type differed significantly ($P < 0.01$) between reference and treated sites for within-year comparisons. No data are available (nd) for SRL in 2004.

increase in density of the 2001 cohort (Fig. 3). For the 2003 cohort, density, growth, survivorship, and foliar chlorophyll were observed to increase greatly in W1 compared with adjacent reference plots. Most intriguing, these responses coincided with differences in arbuscular mycorrhizal colonization, as seedlings in the reference plots exhibited very low infection rates (<5% of root length), whereas those in the treated, high pH soils had high infection (>20%; Fig. 6). A similar, albeit less dramatic, response was observed for canopy sugar maples. Although the mechanistic connections between these observed responses and the responses of tree inorganic nutrition (Fig. 1, Table 3) are not conclusively demonstrated by this study, the fact is clear that in some parts of the HBEF (especially the experimental watersheds W1 and W6), sugar maple suffers from a deficiency of soil calcium or consequent low soil pH, or both. Sugar maple decline in the study area is most severe at higher elevations (above 600 m), where soil base cation pools are smallest (Johnson et al. 2000) and have been most severely depleted by acid deposition (Likens et al. 1996, 1998). This provides further support for the likely role of acid deposition in regional maple decline (Moore et al. 2000, Bailey et al. 2004). Moreover, reduced growth (Fahey et al. 2005) and regeneration of maple (Fig. 4) are evident throughout these experimental watersheds, and the striking seedling response to the calcium treatment in W1 suggests that

soil acidification effects on regeneration may accompany or precede canopy decline.

Mycorrhizae

The complexity of potential interactions among AM colonization, soil base cation depletion, plant nutrition, and sugar maple decline limits the conclusiveness of mechanistic connections based on current literature and the present study. Although we observed much higher AM infection and arbuscule formation for mature sugar maple trees on Ca-treated W1, the sampled trees in our reference sites did not exhibit severe decline symptoms, and the connections between calcium availability, AM colonization, and decline of mature sugar maple at our site remains to be established. In general, low soil pH seems to be associated with foliar cation deficiency and sugar maple decline (Horsley et al. 2000), and with lower AM colonization (Klironomos et al. 1993, 1995, Hutchinson et al. 1999), but comparisons of AM colonization between declining and healthy trees and stands have produced conflicting results (Ouimet et al. 1995, Zahka et al. 1995). Although the striking response to calcium addition of AM colonization of sugar maple seedlings in our field sites (Fig. 6) confirms previous greenhouse experiments (Coughlan et al. 2000, St. Clair and Lynch 2004; but see Cooke et al. 1993), the question of whether the stimulation of AM colonization was a cause or a consequence of their enhanced health, growth, and survivorship (Fig. 3, Table 1) will require additional experimental study. It is notable that complete fertiliza-

TABLE 2. Chlorophyll concentrations in foliage of first-year germinants from Ca-treated and adjacent reference plots, and greenness (SPAD units) for first-year germinants from three elevation zones on the Ca-treated and adjacent reference areas at Hubbard Brook Experimental Forest, New Hampshire, USA.

Site	N	Chlorophyll concentration (g/m ² leaf area)			Greenness (SPAD units)†		
		Total chlorophyll	Chlorophyll a	Chlorophyll b	Low elevation	Middle elevation	High elevation
Reference	67	0.227 (0.009)	0.160	0.067	28.2 (0.7)	28.5 (0.8)	28.4 (0.7)
Treated	70	0.268 (0.008)	0.191	0.077	32.7 (0.7)	31.8 (0.7)	32.1 (0.6)

† See Richardson et al. (2002).

TABLE 3. Element concentrations in leaf, root, and (in 2004) stem tissues of new sugar maple seedlings growing on Ca-treated and adjacent reference sites at Hubbard Brook Experimental Forest, New Hampshire, USA.

Tissue by site	Concentration (g/kg)					
	N	P	Ca	Mg	Mn	Al
2003, new germinants						
Leaf						
Reference	22.3 (0.8)	1.95 (0.18)	6.90 (0.64)	1.99 (0.14)	1.79 (0.28)	0.079 (0.02)
Treated	19.3 (0.6)	1.41 (0.18)	12.60 (0.55)	1.58 (0.16)	0.83 (0.06)	0.042 (0.01)
Root						
Reference	24.4 (1.4)	3.65 (0.93)	2.52 (0.89)	1.49 (0.36)	0.68 (0.32)	1.32 (0.84)
Treated	18.3 (0.4)	2.52 (0.21)	6.46 (0.64)	2.06 (0.51)	0.30 (0.01)	0.94 (0.47)
2004, 1-yr-old seedlings						
Leaf						
Reference	16.5 (0.3)	1.06 (0.13)	5.74 (0.35)	1.50 (0.08)	1.60 (0.19)	0.025 (0.007)
Treated	16.7 (0.4)	0.91 (0.07)	10.59 (0.55)	1.32 (0.14)	0.81 (0.11)	0.025 (0.011)
Root						
Reference	10.6 (0.3)	1.38 (0.20)	5.13 (0.36)	1.42 (0.13)	0.35 (0.02)	0.83 (0.19)
Treated	10.5 (1.2)	0.99 (0.08)	10.21 (2.12)	1.54 (0.11)	0.24 (0.04)	0.53 (0.06)
Stem						
Reference	7.0 (0.3)	1.05 (0.15)	3.07 (0.40)	0.80 (0.12)	0.67 (0.15)	0.018 (0.008)
Treated	7.0 (0.5)	0.80 (0.06)	6.17 (0.68)	0.78 (0.04)	0.33 (0.02)	0.019 (0.006)

Notes: All means set in boldface type differed significantly ($P < 0.01$) between reference and treated sites for within-year comparisons. Standard errors are in parentheses.

tion in two plots at HBEF elicited a typical response of increased maple seedling growth and reduced mycorrhizal colonization. Conversely, Ouimet et al. (1996) observed no effect of AM inoculation on sugar maple germinant growth at a variety of soil nutrient levels, whereas St. Clair and Lynch (2004) measured higher photosynthesis rates under base cation enrichment that also stimulated mycorrhizal colonization. The potential role of changes in AM fungi species composition for sugar maple response to soil acidification also deserves further study (Coughlan et al. 2000).

Tree nutrition

The principal responses of inorganic nutrition of sugar maple to the calcium treatment at HBEF were

greatly increased foliar and fine-root calcium concentration as well as greatly decreased manganese (Fig. 1, Table 3). Reductions in root uptake of calcium owing to excessive manganese availability have been reported widely (Horst and Marschner 1990), and it seems plausible that manganese-induced calcium deficiency has affected sugar maple seedlings on acidified soils at the HBEF. Concentrations of manganese were significantly lower in stream water in the treated watershed ($25.3 \pm 4.9 \mu\text{g/L}$) than the reference watershed ($40.9 \pm 4.9 \mu\text{g/L}$) in 2004 (C. T. Driscoll, *unpublished data*), and previous studies have shown oxidation and hydrolysis of manganese in soil following base cation addition to northern hardwood forests (Driscoll et al. 1989).

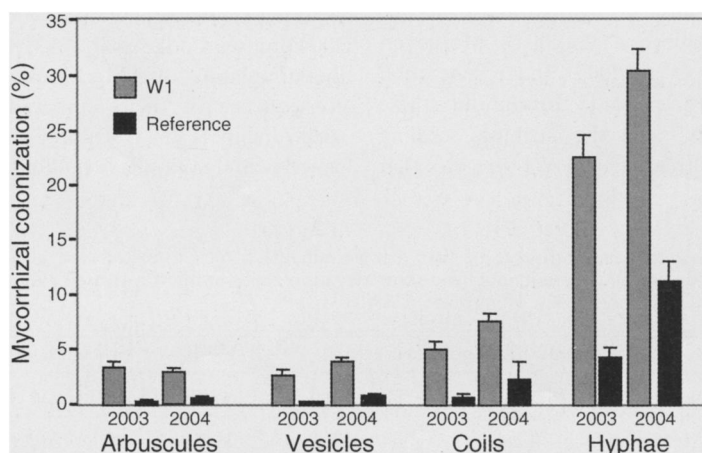


FIG. 6. Percentage of mycorrhizal colonization (mean \pm SE) of roots of sugar maple seedlings (germinated in spring 2003) growing in Ca-treated Watershed 1 (W1) and in adjacent reference plots. Error bars indicate standard error.

The concentrations of manganese observed in foliage of sugar maple seedlings from the reference plots adjacent to W1 (1.6–1.8 g/kg) were higher than those causing symptoms of manganese toxicity in 12-week-old seedlings grown under laboratory conditions (0.9 g/kg; McQuattie and Schier 2000). However, we observed a general chlorosis across leaf blades in our study rather than the localized chlorosis along leaf veins and margins that characterize manganese toxicity (McQuattie and Schier 2000). Others have noted a similar marked reduction in foliar manganese of sugar maple seedlings in response to base cation additions in the laboratory. The range of values of seedling foliar manganese in our field study (1.3–2.0 g/kg; Table 3) was higher than observed by Ouimet et al. (1996; 0.5–1.0 g/kg) but lower than for St. Clair and Lynch (2004; 2.8–6.3 g/kg) in greenhouse studies. Decline of canopy sugar maple has been associated previously with foliar manganese in the range of ~2.5 g/kg (Kolb and McCormick 1993, Long et al. 1997, Horsley et al. 2000), similar to the levels observed prior to treatment in the high-elevation zone on W1 (2.54 ± 0.26 g/kg) where decline is most severe. In summary, it seems plausible that correction of either manganese toxicity or manganese-induced calcium deficiency accounted, in part, for the response of sugar maple to calcium treatment on W1 at the HBEF. The concentrations of calcium in canopy foliage of trees and seedlings prior to treatment and in reference sites (5.7–6.9 g/kg) fall near the deficiency range for sugar maple as summarized in various field surveys and fertilization experiments (Kolb and McCormick 1993, Wilmot et al. 1995, 1996, Long et al. 1997). As noted earlier, mature sugar maple trees in areas of W6 with severe decline symptoms exhibit exceptionally low foliar calcium concentrations (4.0 g/kg; Likens et al. 1998; T. J. Fahey, *unpublished data*).

A less likely explanation for the sugar maple response to calcium fertilization is amelioration of aluminum toxicity. Although marginally significant ($P < 0.1$) decreases in foliar aluminum were observed in new germinants, and fine-root aluminum tended to be lower at the treated site (Table 3), molar Ca:Al ratios were well above the diagnostic thresholds that have been cited as indicative of aluminum toxicity (Thornton et al. 1986, Cronan and Grigal 1995). For example, fine-root molar Ca:Al increased from 1.3 to 4.6 the first year after treatment, whereas the values suggestive of impacts in field studies range from 0.1 to 0.2 (Cronan and Grigal 1995).

The interactions among soil calcium, plant mineral nutrition, and mycorrhizal colonization in sugar maple are not entirely clear and could be quite complex. Although AM colonization can enhance plant calcium status (Schultz and Kormanik 1982), not all studies indicate such a response (Ouimet et al. 1996). As noted earlier, calcium may promote mycorrhizal development and mycorrhizae may improve tree health. Our observations suggest that calcium addition promoted improved maple growth, health, mineral nutrition, and

mycorrhizal status, but the causal web of these responses cannot be conclusively established from our results.

Despite significantly higher foliar chlorophyll concentrations in the sugar maple germinants on the treated watershed, foliar nitrogen concentrations in the first year were significantly lower than for reference seedlings (Table 3). In contrast, the one-year-old seedlings exhibited no treatment effect on tissue nitrogen concentration. In general, foliar nitrogen concentration and photosynthetic capacity are strongly correlated in broadleaf trees (Ellsworth and Reich 1992), and so it is not surprising that there were no treatment-related differences in seedling photosynthetic rates. Decreased chlorophyll content is a characteristic response to a wide variety of stress factors (Carter 1993), and the chlorophyll content of the new germinants in the reference plots may have been sufficiently low to impair photosynthesis (Kull and Niinemets 1998). However, in the one-year-old seedlings on which photosynthesis was measured, there was no evidence of such an effect. Moreover, despite clear evidence of very low seedling survival in the high-elevation zone (Fig. 3), foliar “greenness” did not differ significantly with elevation in either treated or reference plots (Table 2). It is possible that late summer and overwinter mortality of the least healthy germinants selected for seedlings with less chlorosis. St. Clair and Lynch (2004) observed both higher photosynthesis and foliar nitrogen in seedlings on base-enriched than reference soils; however, the greenhouse conditions in that study would be very different from the forest understory environment at the HBEF.

Previous studies have demonstrated a strong inverse relationship between foliar free-putrescine levels and soil calcium for several hardwood and conifer species (Minocha et al. 1997, 2000), and addition of lime to calcium-depleted soils elicited a decrease in foliar free-putrescine (Wargo et al. 2002). However, neither putrescine nor its precursor amino acid, arginine, showed such a response in the present study. Interestingly, among all the amino acids and polyamines analyzed (Minocha and Long 2004), only proline exhibited a treatment response in the sugar maple seedlings, with higher concentrations in foliage from the reference than the treated site. High growing season precipitation and recent rainfall at the time of seedling sampling would discount the likelihood of drought stress in explaining the proline response. Further study of this intriguing result is warranted.

Demography

The calcium fertilization of W1 in 1999 caused a marked increase of maple seedling density in comparison with the reference watershed. This response first appeared in July 2001, followed by a more marked response in July 2003, which followed a heavy seed crop in 2002 (Fig. 1). This result indicates that either seed germination or early survival of sugar maple was greatly increased because seed deposition was not significantly

affected by the calcium treatment. Because both individual seed mass and seed chemistry were not much different between treated and reference sites, it seems likely that low early survivorship of germinants in the reference watershed was the principal cause of the low density there, although the possible role of maternal effects deserves further study. Gardescu (2003) observed very low survivorship of sugar maple germinants through June (0–1%) in most years in one forest stand in central New York, USA, the exception being the year following a heavy seed year, when a high-density (>50 stems/m²) cohort showed 44% survival through spring. In two other stands, she observed 27% and 54% spring survival. The principal causes of spring mortality were damage by pear thrips larvae (*Taeniothrips inconsequens*), caterpillar larvae (especially *Clepsis metaleucana*), and slugs. None of these damage agents was abundant at our sites (N. Cleavitt, *personal observation*), and obvious evidence of leaf herbivory differences was not observed.

Survivorship of sugar maple germinants remained much higher on the treated than the reference watershed for two years following the initial survey of the 2003 cohort. From July 2003 to July 2004, survivorship averaged 56.5% on treated W1 and 19.0% on reference W6; from July 2004 to July 2005, survivorship values were 64.5% for W1 and 45.4% for W6. This continued enhancement of seedling survivorship on W1 reinforces the apparent role of the calcium treatment in mitigating soil acidification effects on the health of sugar maple seedlings. Previous studies have reported wide variations in survivorship of older sugar maple seedlings (Hett and Loucks 1971, Forcier 1973, Boerner and Brinkman 1996). Gardescu (2003) ascribed mortality of 2–5 year old seedlings to a variety of causes including insects, disease, physical damage, and drought.

The long-term effects on forest composition and structure of decreasing sugar maple regeneration on the reference watershed at Hubbard Brook deserve further study, as do the possible consequences and implications of increased seedling abundance on the treated watershed. Sugar maple is characterized as a “seedling bank” species (Marks and Gardescu 1998) in which a large pool of small seedlings of different ages accumulates over prolonged periods and is replenished by occasional years of high seed regeneration; canopy gaps eventually release some of these seedlings to be recruited into larger size classes. On the reference watershed, the seedling bank appears to be depleted: total seedling density in 2002 and 2004 (0.3–0.5 seedlings/m²) was much lower than was observed prior to the 1990s (3–30 seedlings/m²), and the frequency of 1-m² plots that contained sugar maple seedlings was low (e.g., 9.5% in 2005); hence, a relatively small percentage of canopy gaps would release sugar maple at the present time. By comparison, in 2005, 38.6% of 1-m² plots on the treated watershed contained maple seedlings, and if future large seed crops result in additional sugar maple regeneration, the seedling bank on W1 should be replenished gradually, as noted by

Marks and Gardescu (1998) for central New York. Maple sapling abundance has declined steadily on the reference watershed since 1965 (Fig. 4), and the future outlook for the species on this watershed appears bleak in the absence of mitigative measures.

In conclusion, recent declines in the health and recruitment of sugar maple in the experimental watersheds at the HBEF appear to be linked in part to the depletion of calcium in the soil and consequent reductions in soil pH. The addition of calcium to W1 increased maple health, growth, and survivorship. The mechanism of maple response to calcium addition was associated with improved mineral nutrition, with either higher calcium or lower manganese, or both, in foliage and fine roots. In seedlings, chlorosis was also eliminated. Furthermore, mycorrhizal colonization was greatly stimulated by the calcium fertilization treatment, as both seedlings and mature trees in reference sites adjacent to W1 had low levels of mycorrhizal hyphae and structures. However, the causal relationships among inorganic nutrition, mycorrhizal colonization, and maple growth and health remain to be established. Because previous work at the HBEF has demonstrated that soil calcium was depleted by acid deposition, these results strongly support the contention that the growth and health of sugar maple on acid soils in the northeastern United States has been affected by pollution and atmospheric deposition. Mechanistic studies of seedling, sapling, and overstory responses to calcium addition on W1 and elsewhere should contribute to clarifying the causal web of interactions among pH, calcium and manganese in soils, mycorrhizal symbiosis, and sugar maple growth and nutrition.

ACKNOWLEDGMENTS

The authors thank the following individuals for assistance with this research: Robert Cooke, Becky Fahey, Madeleine Fairbairn, Melany Fisk, Jane Hislop, Marian Hovencamp, Suzanne Wapner, and Cindy Wood. This research was supported by grants from the National Science Foundation (DEB, LTER Program, Ecosystems Program, and REU Program), and is a contribution of the Hubbard Brook Ecosystem Study.

LITERATURE CITED

- Aber, J. D., S. V. Ollinger, C. A. Federer, P. B. Reich, M. L. Goulden, D. W. Kicklighter, J. M. Melillo, and R. G. Lathrop. 1995. Predicting the effects of climate change on water yield and forest production in the northeastern U.S. *Climate Research* 5:207–222.
- Adams, C. M., and T. C. Hutchinson. 1992. Fine-root growth and chemical composition in declining Central Ontario sugar maple stands. *Canadian Journal of Forest Research* 22:1489–1503.
- Allen, D. C., A. W. Molloy, R. R. Cooke, D. Lachance, and C. Barnett. 1995. North American Maple Project: seven year report. 1997–505–401. USDA Forest Service, Northeastern Area State and Private Forestry, Washington, D.C., USA.
- Bailey, S. W., S. B. Horsley, R. P. Long, and R. A. Hallett. 2004. Influence of edaphic factors in sugar maple nutrition and health on the Allegheny Plateau. *Soil Science Society of America Journal* 68:243–252.

- Bernier, B., and M. Brazeau. 1988. Foliar nutrient status in relation to sugar maple dieback in the Quebec Appalachians. *Canadian Journal of Forest Research* **18**:754–761.
- Boerner, R. E. J., and J. A. Brinkman. 1996. Ten years of tree seedling establishment and mortality in an Ohio deciduous forest complex. *Bulletin of the Torrey Botanical Club* **123**:309–317.
- Bohlen, P. J., P. M. Groffman, C. T. Driscoll, T. J. Fahey, and T. G. Siccama. 2001. Plant–soil–microbial interactions in a northern montane hardwood forest. *Ecology* **82**:965–978.
- Bouma, T. J., K. L. Nielsen, and B. Koutstall. 2000. Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant and Soil* **218**:185–196.
- Brundrett, M. C., L. Melville, and L. Peterson. 1994. Practical methods in mycorrhizal research. Ninth North American Conference on Mycorrhizae, Guelph, Ontario, Canada. Mycologue Publications 161, Waterloo, Ontario, Canada.
- Buchman, R. G., S. P. Pederson, and N. R. Walters. 1983. A tree survival model with application to the species of the Great Lakes region. *Canadian Journal of Forest Research* **13**:601–608.
- Carter, G. A. 1993. Responses of leaf spectral reflectance to plant stress. *American Journal of Botany* **80**:239–243.
- Clark, R. B. 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant and Soil* **192**:15–22.
- Cooke, M. A., P. Widden, and I. O'Halloran. 1993. Development of vesicular-arbuscular mycorrhizae in sugar maple (*Acer saccharum*) and effects of base-cation amendment on vesicle and arbuscule formation. *Canadian Journal of Botany* **71**:1421–1426.
- Coughlan, A. P., Y. Dalpé, L. Lapointe, and Y. Piché. 2000. Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research* **30**:1543–1554.
- Cronan, C. S., and D. F. Grigal. 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality* **24**:209–226.
- DeHayes, D. H., P. G. Schaberg, G. J. Hawley, and G. R. Strimbeck. 1999. Acid rain impacts on calcium nutrition and forest health. *BioScience* **49**:789–800.
- Driscoll, C. T., G. F. Fordham, W. A. Ayling, and L. M. Oliver. 1989. Short-term changes in the chemistry of trace metals following calcium carbonate treatment. *Canadian Journal of Fishery and Aquatic Science* **46**:249–257.
- Driscoll, C. T., G. B. Lawrence, A. J. Bulger, T. J. Butler, C. S. Cronan, C. Eagar, K. F. Lambert, G. E. Likens, J. L. Stoddard, and K. C. Weathers. 2001. Acidic deposition in the Northeastern United States: sources and inputs, ecosystem effects, and management strategies. *BioScience* **51**:180–198.
- Ellsworth, D. S., and P. B. Reich. 1992. Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. *Functional Ecology* **6**:423–435.
- Fahey, T. J., J. J. Battles, and G. F. Wilson. 1998. Response of early successional northern hardwood forests to changes in nutrient availability. *Ecological Monographs* **68**:183–212.
- Fahey, T. J., et al. 2005. The biogeochemistry of carbon at Hubbard Brook. *Biogeochemistry* **75**:109–176.
- Fisk, M. C., W. Kessler, A. Goodale, T. J. Fahey, P. M. Groffman, and C. T. Driscoll. 2006. Microarthropod responses to calcium addition in a northern hardwood forest, Hubbard Brook Experimental Forest, USA. *Pedobiologia*, in press.
- Forcier, L. K. 1973. Seedling pattern and population dynamics, and the reproductive strategies of sugar maple, beech and yellow birch at Hubbard Brook. Dissertation. Yale University, New Haven, Connecticut, USA.
- Frankland, J. D., and A. F. Harrison. 1985. Mycorrhizal infection of *Betula pendula* and *Acer pseudoplatanus*: relationships with seedling growth and soil factors. *New Phytologist* **101**:133–151.
- Gardescu, S. 2003. Herbivory, disease, and mortality of sugar maple seedlings. *Northeastern Naturalist* **10**:253–268.
- Gbondo-Tugbawa, S. S., and C. T. Driscoll. 2003. Factors controlling long-term changes in soil pools of exchangeable basic cations and stream acid neutralizing capacity in a northern hardwood forest ecosystem. *Biogeochemistry* **63**:161–185.
- Heijne, B., D. van Dam, C. W. Heil, and R. Bobbink. 1996. Acidification effects on mycorrhizal vesicular-arbuscular (VAM) infection, growth and nutrient uptake of established heathland herb species. *Plant and Soil* **179**:197–206.
- Hepper, C. M., and J. O'Shea. 1984. Vesicular arbuscular mycorrhizal infection in lettuce (*Lactuca sativa*) in relation to calcium supply. *Plant and Soil* **82**:61–68.
- Hett, J. M., and O. L. Loucks. 1971. Sugar maple (*Acer saccharum* Marsh.) seedling mortality. *Journal of Ecology* **59**:507–520.
- Horsley, S. B., R. P. Long, S. W. Bailey, R. A. Hallett, and T. J. Hall. 2000. Factors associated with the decline disease of sugar maple on the Allegheny plateau. *Canadian Journal of Forest Research* **30**:1365–1378.
- Horst, W. J., and H. Marschner. 1990. Mineral nutrition of higher plants. Academic Press, Boston, Massachusetts, USA.
- Houlton, B. Z., C. T. Driscoll, T. J. Fahey, G. E. Likens, P. M. Groffman, E. S. Bernhardt, and D. C. Buso. 2003. Nitrogen dynamics in ice storm damaged forest ecosystems: implications for nitrogen limitation theory. *Ecosystems* **6**:431–443.
- Hutchinson, T. C., S. A. Watmough, E. P. S. Sager, and J. D. Karagatzides. 1999. The impact of simulated acid rain and fertilizer application on a mature sugar maple (*Acer saccharum* Marsh.) forest in central Ontario, Canada. *Water, Air, and Soil Pollution* **109**:17–39.
- Jarstfer, A. G., P. Farmer-Koppenol, and D. M. Sylvia. 1998. Tissue magnesium and calcium affect arbuscular mycorrhizal development and fungal reproduction. *Mycorrhiza* **7**:237–242.
- Johnson, C. E., C. T. Driscoll, T. G. Siccama, and G. E. Likens. 2000. Element fluxes and landscape position in a northern hardwood forest watershed-ecosystem. *Ecosystems* **3**:159–184.
- Klironomos, J. H. 1995. Arbuscular mycorrhizae of *Acer saccharum* in different soil types. *Canadian Journal of Botany* **73**:1824–1830.
- Klironomos, J. N., P. Moutoglou, B. Kendrick, and P. Widden. 1993. A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple forest soils. *Canadian Journal of Botany* **71**:1472–1480.
- Kolb, T. E., and L. H. McCormick. 1993. Etiology of sugar maple decline in four Pennsylvania stands. *Canadian Journal of Forest Research* **23**:2395–2402.
- Kull, O., and Ü. Niinemets. 1998. Distribution of leaf photosynthetic properties in tree canopies: comparison of species with different shade tolerance. *Functional Ecology* **12**:472–479.
- Likens, G. E., and F. H. Bormann. 1995. *Biogeochemistry of a forested ecosystem*. Second edition. Springer-Verlag, New York, New York, USA.
- Likens, G. E., C. T. Driscoll, and D. C. Buso. 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* **272**:244–246.
- Likens, G. E., C. T. Driscoll, D. C. Buso, T. G. Siccama, C. E. Johnson, G. M. Lovett, T. J. Fahey, W. A. Reinert, D. F. Ryan, C. W. Martin, and S. W. Bailey. 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* **41**:89–173.
- Long, R. P., S. B. Horsley, and P. R. Lilja. 1997. Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Canadian Journal of Forest Research* **27**:1560–1573.

- Mader, D. L., and B. W. Thompson. 1969. Foliar and soil nutrients in relation to sugar maple decline. *Soil Science Society of America Proceedings* **33**:794–800.
- Manion, P. D. 1991. Tree disease concepts. Prentice Hall, Englewood Cliffs, New Jersey, USA.
- Marks, P. L., and S. Gardescu. 1998. A case study of sugar maple (*Acer saccharum*) as a forest seedling bank species. *Journal of the Torrey Botanical Society* **125**:287–296.
- McQuattie, C. J., and G. A. Schier. 2000. Response of sugar maple (*Acer saccharum*) seedlings to manganese. *Canadian Journal of Forest Research* **30**:456–467.
- Miller, I., B. Lachance, W. G. Burkman, and D. C. Allen. 1991. North American Sugar Maple Decline Project: organization and field methods. General Technical Report NE-154, USDA Forest Service, Radnor, Pennsylvania, USA.
- Minocha, R., and S. Long. 2004. Simultaneous separation and quantitation of amino acids and polyamines of forest tree tissues and cell cultures within a single high-performance liquid chromatography run using dansyl derivatization. *Journal of Chromatography A* **1035**:63–73.
- Minocha, R., S. Long, A. H. Magill, J. D. Aber, and W. McDowell. 2000. Foliar free polyamine and inorganic ion content in relation to soil and soil solution chemistry in two fertilized forest stands at the Harvard Forest, Massachusetts. *Plant and Soil* **222**:119–137.
- Minocha, R., W. C. Shortle, G. B. Lawrence, M. B. David, and S. C. Minocha. 1997. Relationships among foliar chemistry, foliar polyamines, and soil chemistry in red spruce trees growing across the northeastern United States. *Plant and Soil* **191**:109–122.
- Moore, J. D., C. Camiré, and R. Ouimet. 2000. Effects of liming on the nutrition, vigor, and growth of sugar maple at Lake Clair Watershed, Quebec, Canada. *Canadian Journal of Forest Research* **30**:725–732.
- Ouimet, R., and C. Camiré. 1995. Foliar deficiencies of sugar maple stands associated with soil cation imbalances in the Quebec Appalachians. *Canadian Journal of Soil Science* **75**:169–175.
- Ouimet, R., C. Camiré, and V. Furlan. 1995. Endomycorrhizal status of sugar maple in relation to tree decline and foliar, fine-roots, and soil chemistry in the Beauce region, Quebec. *Canadian Journal of Forest Research* **73**:1168–1175.
- Ouimet, R., C. Camiré, and V. Furlan. 1996. Effect of soil K, Ca, and Mg saturation and endomycorrhization on growth and nutrient uptake of sugar maple seedlings. *Plant and Soil* **179**:207–216.
- Peters, S. C., J. D. Blum, C. T. Driscoll, and G. E. Likens. 2004. Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. *Biogeochemistry* **67**:309–329.
- Rhoads, A. G., S. P. Hamburg, T. J. Fahey, T. G. Siccama, E. N. Hane, J. Battles, C. Cogbill, J. Randall, and G. Wilson. 2002. Effects of a large ice storm on the structure of a northern hardwood forest. *Canadian Journal of Forest Research* **32**:1763–1775.
- Richardson, A. D., S. P. Duigan, and G. P. Berlyn. 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist* **153**:185–194.
- Schultz, R. C., and P. P. Kormanik. 1982. Vesicular-arbuscular mycorrhiza and soil fertility influence mineral concentrations in seedlings of eight hardwood species. *Canadian Journal of Forest Research* **12**:829–834.
- Schwarz, P. A., T. J. Fahey, and C. M. McCulloch. 2003. Factors controlling spatial variation of tree species abundance in a forested landscape. *Ecology* **84**:1862–1878.
- Shanley, J. C., C. Kendall, T. E. Smith, D. M. Wolock, and J. J. McDonnell. 2002. Controls on old and new water contributions to stream flow at some nested catchments in Vermont, USA. *Hydrological Processes* **16**(3):589–609.
- Shortle, W. C., and K. T. Smith. 1988. Aluminum-induced calcium deficiency syndrome in declining red spruce. *Science* **240**:1017–1018.
- Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis. Second edition. Academic Press, San Diego, California, USA.
- St. Clair, S. B., and J. P. Lynch. 2004. Base cation stimulation of mycorrhization and photosynthesis of sugar maple on acid soils are coupled by foliar nutrient dynamics. *New Phytologist* **165**:581–590.
- Thornton, F. C., M. Schaedle, and D. J. Raynal. 1986. Effect of aluminum on the growth of sugar maple in solution culture. *Canadian Journal of Forest Research* **16**:892–896.
- Wargo, P. M., R. Minocha, B. L. Wong, R. P. Long, S. B. Horsley, and T. J. Hall. 2002. Measuring changes in stress and vitality indicators in limed sugar maple on the Allegheny Plateau in north-central Pennsylvania. *Canadian Journal of Forest Research* **32**:629–641.
- Whittaker, R. H., F. H. Bormann, G. E. Likens, and T. G. Siccama. 1974. The Hubbard Brook Ecosystem Study: forest biomass and production. *Ecological Monographs* **44**:233–252.
- Wilmot, T. R., D. S. Ellsworth, and M. T. Tyree. 1995. Relationships among crown condition and stand nutrition in seven northern Vermont sugarbushes. *Canadian Journal of Forest Research* **25**:386–397.
- Wilmot, T. R., D. S. Ellsworth, and M. T. Tyree. 1996. Base cation fertilization and liming effects on nutrition and growth of Vermont sugar maple stands. *Forest Ecology and Management* **84**:1123–1134.
- Zahka, G. A., K. L. Baggett, and B. L. Wong. 1995. Inoculum potential and other VAM fungi parameters in four sugar maple forests with different levels of stand dieback. *Forest Ecology and Management* **75**:123–134.