

Influence of experimental snow removal on root and canopy physiology of sugar maple trees in a northern hardwood forest

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Abstract Due to projected increases in winter air temperatures in the northeastern USA over the next 100 years, the snowpack is expected to decrease in depth and duration, thereby increasing soil exposure to freezing air temperatures. To evaluate the potential physiological responses of sugar maple (*Acer saccharum* Marsh.) to a reduced snowpack, we measured root injury, foliar cation and carbohydrate concentrations, woody shoot carbohydrate levels, and terminal woody shoot lengths of trees in a snow manipulation experiment in New Hampshire, USA. Snow was removed from treatment plots for the first 6 weeks of winter for two consecutive years, resulting in lower soil temperatures to a depth of 50 cm for both winters compared to reference plots with an undisturbed snowpack. Visibly uninjured roots from trees in the snow removal plots had significantly higher (but sub-lethal) levels of

relative electrolyte leakage than trees in the reference plots. Foliar calcium: aluminum (Al) molar ratios were significantly lower, and Al concentrations were significantly higher, in trees from snow removal plots than trees from reference plots. Snow removal also reduced terminal shoot growth and increased foliar starch concentrations. Our results are consistent with previous research implicating soil freezing as a cause of soil acidification that leads to soil cation imbalances, but are the first to show that this translates into altered foliar cation pools, and changes in soluble and structural carbon pools in trees. Increased soil freezing due to a reduced snowpack could exacerbate soil cation imbalances already caused by acidic deposition, and have widespread implications for forest health in the northeastern USA.

Keywords Soil freezing · Root injury · Woody shoot growth · Carbohydrate and cation concentrations · *Acer saccharum*

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Introduction

Among the numerous changes climate models have projected for the northeastern United States (USA) are an increase in mean annual air temperature and amount of precipitation. It is projected that global temperatures may rise between 1.1 and 6.4 °C in the twenty-first century with a 20–30 % increase in precipitation projected to accumulate—primarily as rain rather than snow (IPCC 2007). The combination of these changes in climate is expected to delay the onset of snowpack, as well as decrease snowpack depth and duration throughout winter. Over the next 100 years in the northeastern USA, the number of days each year with snow on the ground is projected to decrease

(IPCC 2007). Hydrologic models estimate a reduction in snowpack by as much as 53 %, with the greatest change projected for areas that currently experience air temperatures just below freezing (Federer 2001). Snow insulates the soil from severe freezing and minimizes the frequency and extent of freeze/thaw events (Stadler et al. 1996; Shanley and Chalmers 1999). Thus, soil temperatures below 0 °C are currently rare in this region when a snow layer is established by December (Decker et al. 2003). At the Hubbard Brook Experimental Forest (HBEF), New Hampshire, USA, between 1956 and 2009, soil frost events have been common (e.g., 62–76 % of years at two measurement stations, respectively), though the great majority of these frosts are shallow (<10 cm deep) (Campbell et al. 2010). It is generally projected that, with a reduction and a later onset of the snowpack, soil freezing may occur more frequently (Brown and DeGaetano 2011). However, specific projections for HBEF are more variable, with there likely to be no major changes in annual frost depth, but an increase in freeze–thaw events (Campbell et al. 2010).

Past snow removal studies have documented increased losses of nitrate (NO_3^-) in leachate (Groffman et al. 2001), which results in soil acidification that leaches cations such as calcium (Ca) from soils (Fitzhugh et al. 2003). At the HBEF, soil freezing is an additional cause of soil acidification in a region that has already experienced significant inputs of atmospheric acid deposition. Anthropogenic acid deposition beginning in the 1950s has led to depleted base cation reserves in the soil (Likens et al. 1996). At the HBEF, Ca pools in the soil have been depleted by up to 50 %, and due to slow rates of weathering and reduced cation deposition, Ca pools will not be replenished in the foreseeable future (Likens et al. 1998). In addition to being an essential plant nutrient, the acid neutralizing capacity of soil Ca can help to buffer further acidic inputs (Driscoll et al. 2001). Additional acidification caused by soil freezing could exacerbate the influence of acid deposition on cation nutrition, including the increased availability of aluminum (Al)—a potentially phytotoxic element (Cronan and Grigal 1995). The combined effects of soil freezing and atmospheric deposition may lead to reduced Ca uptake and increased Al availability—factors that have been associated with reduced growth and increased crown decline in sugar maple trees (Schaberg et al. 2006b; Huggett et al. 2007).

The physiological response of trees to soil freezing has been less studied than biogeochemical alterations within soils. However, it is known that tree roots are sensitive to freezing stress (Sakai and Larcher 1987) and that soil freezing can injure tree roots in the field (Tierney et al. 2001). Roots are damaged through the extra- and intracellular freezing of cells and tissues that occur at low temperature thresholds that differ somewhat among species

(Schaberg et al. 2011). Root injury may also occur through mechanical action as the surrounding soil heaves and subsides with the freezing and thawing of soil water (Sutinen et al. 1998), although the relative importance of mechanical damage to injury in northern forests has been questioned (Cleavitt et al. 2008).

Increases in root damage from freezing may have numerous implications for tree health and function. At a fundamental level, root damage and loss would likely limit plant uptake of water and nutrients (Waisel et al. 1996). Initial belowground perturbations could then translate into distal changes in crown physiology if transpirational recharge was diminished. In particular, alterations in foliar cations (supplied via the transpiration stream) and carbohydrates (produced in leaves if stomata are open and leaves take up and assimilate CO_2) are two likely downstream consequences of freeze-induced root damage. Because cation (especially Ca) nutrition is critical to tree stress response systems (McLaughlin 1999; Schaberg et al. 2001), and carbohydrates fuel both growth and the production of biochemical defense compounds (Koricheva et al. 1998), changes in these parameters may be especially consequential to tree health and productivity.

We hypothesized that reduced snowpack accumulation and increased soil freezing results in sub-lethal root injury that then influences the physiology of the crowns of associated trees. We tested this hypothesis by removing snow for the first 6 weeks of winter for two consecutive years at the HBEF and measuring (1) soil temperatures, (2) root electrolyte leakage (a measure of incipient injury), (3) foliar cation and carbohydrate concentrations, (4) woody shoot carbohydrates, and (5) terminal woody shoot growth of sugar maple (*Acer saccharum* Marsh.) trees. Compared to co-occurring American beech and yellow birch, sugar maples have a higher proportion of fine roots in shallow organic and mineral soil horizons (Fahey and Hughes 1994), which should place them at an elevated risk of root damage following soil freezing.

Materials and methods

Study site and treatment

Our study was located at the HBEF, a National Science Foundation Long Term Ecological Research (LTER) site in the White Mountain National Forest, NH, USA (43°56'N, 71°45'W). Dominant tree species include an overstory of sugar maple and yellow birch (*Betula alleghaniensis* Britton). Understory plant species include American beech (*Fagus grandifolia* Ehrh.), striped maple (*Acer pennsylvanicum* L.), and hobblebush (*Viburnum alnifolium* L.). The climate consists of cool summers and long, cold

winters. Average winter air temperatures from 1957–2000 were $-4.7\text{ }^{\circ}\text{C}$ with January air temperatures averaging $-9\text{ }^{\circ}\text{C}$. Average yearly precipitation is 1,400 mm, with about 30 % of this historically falling as snow (Bailey et al. 2003). Typically, snowpack is present from mid-December to mid-April. Soils consist of base-poor spodosols developed on glacial till with shallow bedrock averaging 1 m in depth (Johnson et al. 2000), with an average pH of 3.9 (Fitzhugh et al. 2001).

Four snow removal plots and four reference plots (each $13 \times 13\text{ m}$) were established in 2007 (Templer et al. 2012). There were three or more dominant or codominant sugar maple trees within each plot, and the three largest of these were sampled for tree-based measurements (see below). Beginning in December 2008 and 2009, snow was removed for 6 weeks during early winter from the four treatment plots. Prior to the first snowfall, low-lying shrubs consisting mainly of hobblebush were pruned with hand clippers from both treatment and reference plots, which facilitated snow removal on treatment plots. Snow was removed from treatment plots with shovels within 24 h of a snowfall exceeding 5 cm, taking care to pack down and leave a 3-cm layer of snow to both protect the forest floor from disturbance and to maintain albedo comparable to reference plots. This base layer was maintained throughout the treatment period. Snow was removed for the first 6 weeks of snowfall and then allowed to accumulate throughout the remainder of winter. Snow removal did not result in compaction of soils as measured by a lack of difference between the soil bulk density in the reference and snow-removal plots (Templer et al. 2012).

Environmental data

Soil temperature was measured in each plot with copper-constantan thermocouples (Campbell Scientific, Logan, UT, USA) inserted at six depths below the forest floor (1, 3, 7, 15, 30, and 50 cm deep) from November 15, 2007 to November 2, 2010. Temperature measurements were made at 10-s intervals and hourly averages were logged on Campbell Scientific CR10x and CR10 dataloggers with AM416 and AM16/32 multiplexers. A T-107 temperature probe was used as a reference for the thermocouples.

Root injury

In April 2010, three samples of sugar maple fine roots ($<2\text{ mm}$ in diameter) were carefully collected using hand trowels from soils adjacent to each of the three trees per plot used for shoot and foliar analyses (described below). Samples were collected in groups of adjacent treatment pairs—one replication of snow removal and reference plots at a time. Root samples were identified to species using

morphological features (Yanai et al. 2008). The sampling period was chosen to coincide with the initial thaw of the snowpack to allow for the extraction of roots while preceding root growth and elongation for the season. Root tissues were placed in water-moistened paper towels in sealed plastic bags and immediately transported in a cooler to the Forest Service Laboratory in South Burlington, VT, USA, for further processing and analysis.

We measured the relative electrolyte leakage (REL: the leakage of electrolytes from roots directly transported from the field relative to electrolyte leakage from the same roots after heat-induced mortality) as an indicator of freezing injury (Schaberg et al. 2008, 2011). Measurements of REL from plant tissues are routinely used as a measure of membrane stability (i.e., the ability of cells to control the flow of electrolytes, especially potassium ions) following damage from a variety of stresses including dehydration (Kuhns et al. 1993), osmotic stress (Zwiazek and Blake 1991), high temperatures (Ruter 1996), and freezing (McKay 1998; Schaberg et al. 2008). Approximately 0.5 g of each sample was washed with cool tap water to remove soil particles, washed in a dilute detergent solution (0.1 % v/v Triton X-100—deionized water) to reduce surface tension on root exteriors, and then processed through three deionized water (pH ~ 6) rinses to remove superficial ions. Washed samples were then chopped into 5-mm sections. Six subsamples of chopped root for each of the three samples per plot were measured volumetrically to obtain 0.1–0.2 g of fine root tissue. Each sample was placed into 64-cell styrene trays to measure the initial electrical conductivities of the samples (electrolyte leakage soon after samples were excavated in the field) and final conductivities (electrolyte leakage after samples were dried for 48 h at $50\text{ }^{\circ}\text{C}$ to kill tissues). Prior to conductivity measurements, 3 mL of 0.1 % Triton X-100 were added to each cell and the trays were agitated overnight (for initial electrical conductivity measurements) and for 24 h (for final conductivity measurements) to facilitate electrolyte exchange between sample solutes and root tissues. Electrical conductivities were measured using a multi-electrode conductivity bridge (Wavefront Technology, Ann Arbor, WI, USA), and the mean ratio of initial to final conductivities $\times 100$ of the six subsamples per root sample (to calculate the percent electrolyte leakage relative to heat-killed tissue) was used as the indicator of root membrane integrity for each tree.

Woody shoot and foliage collection, growth and carbohydrate analyses

Woody shoots and foliage were collected from the sunlit upper crowns of three sugar maple trees per plot in July 2010 using a shotgun. Samples were collected in pairs of

one snow removal and one reference plot to avoid any potential confounding of treatment with time of collection. The longest terminal shoot per branch was measured (mm) as an indicator of woody growth in 2010. Shoot sections 1–2 cm in diameter were excised from branches and sealed into plastic bags before being placed on dry ice. Half of the foliage from each branch was kept frozen to preserve tissues for carbohydrate analysis and the other half was dried prior to cation analysis.

Tissues not used for growth or cation assessments were stored at -80°C prior to analyses, and then samples were assessed for soluble carbohydrate concentrations using the methods of Hinesley et al. (1992). For shoots, the bark and pith were removed before samples were submerged in 80 % ethanol, evacuated in an oven at -52 kPa for 15 min, and then boiled in a water bath for 15 min. Samples were chopped using a razor blade, homogenized with a PolytronTM (Brinkman Instruments, Westbury, NY, USA), and then extracted twice using 5 ml of fresh 80 % ethanol at 80°C . Finally, samples were centrifuged at 3,000 rpm for 15 min and the ethanol extracts for each sample pooled and filtered through a $0.45\text{-}\mu\text{m}$ syringe filter in preparation for sugar analysis, while the ethanol-insoluble residues were reserved for starch analysis.

For foliage, 20 circular disks (0.5 cm in diameter) were punched from leaves with care taken to avoid sampling major leaf veins. Soluble sugars were extracted from leaf punches using 80 % ethanol. Chlorophyll was removed from the soluble sugar ethanol supernatant using a Waters C₁₈ Sep-Pak Plus Cartridge (Waters, Milford, MA, USA). A subsample of the filtered supernatant was dried at 37°C in a limited volume insert, reconstituted in 200 μL 0.1 mM Ca EDTA, and filtered through a $0.45\text{-}\mu\text{m}$ syringe filter. Samples were analyzed for glucose, fructose, sucrose, stachyose, raffinose, and xylose using high performance liquid chromatography (Alliance 2695; Waters) and a Waters Sugar-Pak column (Hinesley et al. 1992). The column was maintained at 90°C and 0.1 mM Ca EDTA was used as the solvent at a flow rate of 0.6 ml min^{-1} . Sugar concentrations were quantified using Waters Empower software and expressed as mg cm^{-2} leaf area.

For shoot and foliar samples, the pellet from the ethanol extract was gelatinized with 0.2 M KOH, boiled for 30 min in a water bath, and neutralized with 1 M acetic acid. The solubilized starch was hydrolyzed to glucose with amyloglucosidase (no. 10115; Sigma Chemicals) in 0.1 M acetate buffer (pH 4.5) and incubated at 55°C for 30 min. The reaction was terminated by boiling the sample for 4 min. The supernatant was centrifuged for 10 min at 3,000 rpm. Starch content was quantified by assaying for glucose (liquid glucose reagent set; Pointe Scientific, Canton, MI, USA) as described by Hendrix (1993). Samples and glucose standards were read with an Elx800 UV plate reader

(Bio-Tek, Winooski, VT, USA) at 340 nm. Starch concentrations were calculated using glucose standard curves and expressed as mg cm^{-2} leaf area. Total nonstructural carbohydrates (TNC) were calculated by summing all sugars and starch.

Foliar cation analysis

Foliar tissue was dried for 2 weeks at 55°C and ground in a Wiley mill with a 2-mm mesh. Approximately 0.5 g of ground foliage from each tree was run through a series of nitric acid and hydrogen peroxide digestions (Jones et al. 1990). Following digestion, samples were analyzed for Ca, Al, potassium (K), and manganese (Mn) using an inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (Perkin-Elmer Optima DV 3000; Perkin-Elmer, Norwalk, CT, USA). National Institute of Standards and Technology (NIST) peach leaf standards were used (SRM 1547) to ensure accuracy. Standards were within 5 % of certified values.

Statistical analysis

Treatment differences in root, shoot, and foliar tissues were tested using a nested analysis of variance (ANOVA) where plot within treatment and tree within plot were the sources of variation (Montgomery 2008). This analysis allowed us to evaluate the consistency in response among plots within a treatment, and differences among plot means associated with snow removal treatment. Treatment differences in minimum soil temperatures were tested using Student's *t* tests. A Box-Cox transformation was used on shoot length data to satisfy the assumption of homogeneity of variances (Montgomery 2008). Data were analyzed using JMP statistical software (SAS Institute, Cary, NC, USA). Unless noted otherwise, results were considered statistically significant when $P \leq 0.05$.

Results

Soil temperatures

Snow removal significantly decreased minimum soil temperatures recorded at all depths measured in the winter of 2008–2009 and the upper four depths measured in 2009–2010 (Table 1). Differences in soil temperature at the 30 and 50 cm depths attributable to treatment were significant at $P \leq 0.10$ during the winter of 2009–2010. Soil temperatures at 1 cm depth fell to almost -6°C on snow removal plots, while soils at the same depth in reference plots did not freeze during either winter (Table 1). Although differences between treatments decreased with

Table 1 Mean (\pm SE) minimum soil temperatures at six depths in reference and snow removal plots during meteorological winter (December 1–February 28) and after treatment initiation

Soil depth (cm)	2008–2009			2009–2010		
	Soil temperature ($^{\circ}$ C)		<i>P</i> value	Soil temperature ($^{\circ}$ C)		<i>P</i> value
	Reference	Snow removal		Reference	Snow removal	
1	-0.47 ± 0.23	-5.88 ± 0.6	0.0007	-0.43 ± 0.06	-5.17 ± 0.67	0.0004
3	0.1 ± 0.38	-4.93 ± 0.44	0.0004	0.26 ± 0.04	-4.46 ± 0.79	0.004
7	0.23 ± 0.35	-3.99 ± 0.33	0.0003	0.28 ± 0.11	-3.63 ± 0.72	0.0061
15	0.66 ± 0.32	-2.52 ± 0.45	0.003	0.54 ± 0.16	-2.55 ± 0.91	0.0362
30	1.16 ± 0.28	-1.13 ± 0.29	0.0027	0.94 ± 0.43	-1.41 ± 0.78	0.0626
50	1.76 ± 0.16	-0.16 ± 0.07	<0.0001	1.68 ± 0.28	-0.88 ± 0.95	0.0766

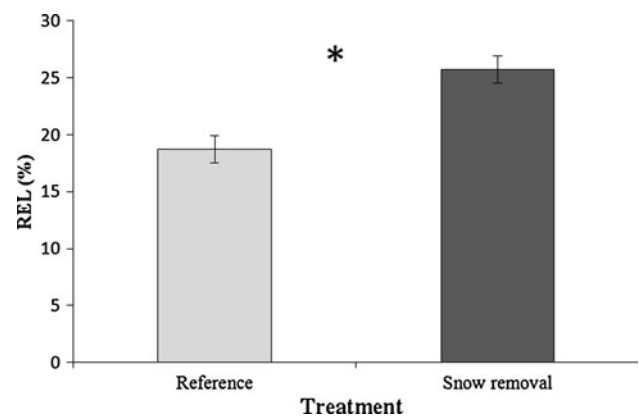
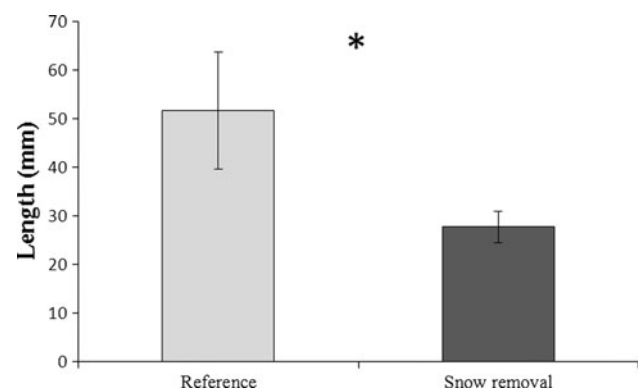
soil depth (Table 1), snow removal resulted in soil freezing as deep as 0.5 m (Table 1). In general, temperatures varied more in soils within snow removal plots relative to reference plots (see SE values in Table 1). There was a trend for temperature variation to be greater in surface than deeper soils in 2008–2009 (the colder of the two winters), with little pattern with depth evident in 2009–2010. Overall, there were few shifts in temperature across the freeze–thaw threshold for soils from either treatment during the two winters (Comerford 2011).

Root injury

Fine roots collected from the snow removal plots showed significantly higher REL values than roots from reference plots ($P = 0.005$; Fig. 1). Root REL was 37 % higher in the snow removal plots than in reference plots. However, even in snow removal plots, average REL values were below the 50 % threshold that is typically associated with tissue mortality (Strimbeck et al. 2007). For root and other tree-based data, no differences in response attributable to plot within treatment were detected, indicating that the influence of treatment was robust and not plot-dependent.

Shoot growth and shoot/foiar carbohydrates

Terminal shoots from trees on snow removal plots were approximately 46 % shorter than terminal shoots from trees on reference plots (51.75 ± 12.02 vs. 27.83 ± 3.23 mm respectively, $P = 0.058$; Fig. 2). However, there were no significant differences between snow removal and reference plots in shoot starch concentrations ($P = 0.84$; Table 2). Mean starch concentrations of shoots from trees in reference plots and snow removal plots were 17.1 and 16.63 mg g⁻¹, respectively. There were also no differences among individual or combined soluble sugars between the shoots from trees on reference and snow removal plots (Table 2).

**Fig. 1** Mean relative electrolyte leakage (REL) (\pm SE) for sugar maple (*Acer saccharum* Marsh.) fine roots from reference and snow removal plots. Asterisk means are statistically different at $P \leq 0.05$ based on ANOVA analysis**Fig. 2** Mean terminal shoot lengths (\pm SE) for sugar maple trees from reference and snow removal plots. Asterisk means are statistically different at $P = 0.058$ based on ANOVA analysis

In contrast to shoot carbohydrate data, foliage from trees on snow removal plots had 86 % higher starch concentrations (102.72 mg g⁻¹) than foliage from the reference plots (55.15 mg g⁻¹; $P = 0.004$; Table 2). No differences in foliar sugar concentration between trees in snow removal

Table 2 Mean (\pm SE) carbohydrate concentrations of *woody shoots* and *foliage* from sugar maple (*Acer saccharum* Marsh.) trees on reference and snow removal plots

Treatment	Starch (mg g ⁻¹)	Stachyose (mg g ⁻¹)	Raffinose (mg g ⁻¹)	Sucrose (mg g ⁻¹)	Glucose (mg g ⁻¹)	Xylose (mg g ⁻¹)	Fructose (mg g ⁻¹)	Total sugars (mg g ⁻¹)	TNC ^a (mg g ⁻¹)
Woody shoots									
Reference	17.1 \pm 1.59	0 \pm 0 ^b	0.14 \pm 0.02	6.3 \pm 0.43	0.11 \pm 0.02	0 \pm 0	0.05 \pm 0.02	6.6 \pm 0.45	23.7 \pm 1.75
Snow removal	16.63 \pm 2.04	0 \pm 0	0.13 \pm 0.02	5.76 \pm 0.42	0.13 \pm 0.03	0 \pm 0	0.08 \pm 0.04	6.1 \pm 0.43	22.73 \pm 2.21
Treatment	Starch (mg cm ⁻²)	Stachyose (mg cm ⁻²)	Raffinose (mg cm ⁻²)	Sucrose (mg cm ⁻²)	Glucose (mg cm ⁻²)	Xylose (mg cm ⁻²)	Fructose (mg cm ⁻²)	Total sugars (mg cm ⁻²)	TNC ^a (mg cm ⁻²)
Foliage									
Reference	55.15 \pm 5.61	0 \pm 0	0 \pm 0	0.06 \pm 0.01	0.01 \pm 0	0 \pm 0	0.01 \pm 0	0.09 \pm 0.01	55.24 \pm 5.62
Snow removal	102.72 \pm 8.05*	0 \pm 0	0 \pm 0	0.07 \pm 0.01	0.01 \pm 0	0 \pm 0	0.02 \pm 0	0.1 \pm 0.01	102.82 \pm 8.06*

* Significant differences between means, $P \leq 0.05$

^a Total nonstructural carbohydrates (TNC)

^b Values of zero indicate concentrations below instrumental detection levels

and reference plots for individual or total sugars were found (Table 2). Total nonstructural carbohydrates values were also higher in leaves from snow removal plots (102.82 mg g⁻¹) than those from reference plots (55.24 mg g⁻¹; $P = 0.004$), and reflected the dominance of starch values as a component of TNC during the growing season.

Foliar cations

Foliage from trees on snow removal plots had significantly higher Al concentrations than foliage from trees on reference plots ($P = 0.0074$; Table 3). Foliage from trees on snow removal plots tended to have lower Ca concentrations ($P \leq 0.12$; Table 3) and had significantly lower Ca:Al molar ratios than foliage from trees on reference plots ($P = 0.049$). No differences in foliar K or Mn associated with treatment were detected (Table 3).

Discussion

Companion research at our study site has verified that the snow removal treatment reduced the depth and duration of snowpack during the winters of 2008–2009 and 2009–2010, which resulted in significantly lower soil temperatures on snow removal plots relative to reference plots during winter (Templer et al. 2012). The snowpack

depth varied among years, with maximum mean depths of 98.6 and 48.7 cm on the reference plots and 53.9 and 19.8 cm on the treatment plots during 2008–2009 and 2009–2010, respectively. During the winter of 2008–2009, the maximum mean frost depth on reference plots was 6.6 cm (25 November 2008), whereas it averaged 37.2 cm (13 February 2008) on treatment plots. During the winter of 2009–2010, the maximum mean frost depths on the reference and treatment plots were 5.4 cm (10 February 2010) and 38.0 cm (3 March 2010), respectively. Snow removal increased the mean duration of soil frost among the plots by 16 days in 2008–2009 and 29 days in 2009–2010 (Templer et al. 2012). Treatment-induced reductions in soil temperature increased the average maximum depths and duration of soil frost to 37.2 and 38.0 cm and 16 and 29 days in 2008–2009 and 2009–2010, respectively (Templer et al. 2012). Because temperature minima are particularly important in inducing species-specific freezing injury, here we focus on these minima for the 6 soil depths sampled (Table 1). The lowest soil temperature measured for snow removal plots was -5.88 ± 06 °C at the 3 cm depth.

The proportion of the root system damaged by soil freezing depends on the freezing event (how cold soils become and how deep frosts reach), and the tree species (which differ in cold tolerance and the proportion of fine roots that populate upper soil horizons). For example, yellow-cedar [*Callitropsis nootkatensis* (D. Don) Florin ex

Table 3 Mean (\pm SE) element concentrations and Ca:Al molar ratios of foliage from reference and snow removal plots

Treatment	Al (mg kg ⁻¹)	Ca (mg kg ⁻¹)	K (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Ca:Al molar ratio
Reference	29.47 \pm 1.09	7,020.42 \pm 673.41	6,706.36 \pm 361.81	966.56 \pm 123.03	207.84 \pm 22.09
Snow removal	38.8 \pm 2.78*	5,942.59 \pm 596.55	6,620.19 \pm 284.3	849.74 \pm 126.33	135.63 \pm 13.7*

* Significant differences between means, $P \leq 0.05$

D. P. Little] trees in Alaska appear particularly vulnerable to root freezing injury because they have limited hardiness and a high proportion of superficial roots (Schaberg et al. 2011). Compared to co-occurring American beech and yellow birch, sugar maple trees have a higher proportion of fine roots in shallow organic and mineral soil horizons (Fahey and Hughes 1994), which should put maples at greater risk of root damage following soil freezing.

Although no single low temperature threshold has been established for the induction of root mortality in sugar maple, Calmé et al. (1994) reported a gradual decrease in the dry mass of live roots below -10°C . Thus, the temperatures of injury noted by Calmé et al. (1994) were well below the minima we recorded on snow removal plots (Table 1). Others have reported elevated mortality for sugar maple roots when snow removal increased soil freezing in the field, although the temperatures associated with this damage were not reported (Tierney et al. 2001). A separate study found no clear influence of snow manipulation on sugar maple root mortality, but detected a significant reduction in the vitality (assessed using a colorimetric assay) of cells in first and second order roots (Cleavitt et al. 2008). The REL values recorded in our study are consistent with these findings of sub-lethal injury, and indicate that membrane damage can occur for the fine roots of sugar maples even when the temperature thresholds that induce mortality are not reached. Even if mortality thresholds are not exceeded, sub-lethal injury has a metabolic cost as energy and other resources are used to support tissue repair and maintain electrolyte balance across damaged (leaky) membranes (Morsomme and Boutry 2000; Schapire et al. 2009). Nutrient uptake through injured roots can be reduced (Marschner 2002), increasing opportunities for leaching loss from soils. Although various datasets highlight tree root injury following snow removal and soil freezing (Tierney et al. 2001; Cleavitt et al. 2008; Table 1), Cleavitt et al. (2008) found that snow manipulation had no effect on mycorrhizal colonization of sugar maple.

In addition to root damage, snow removal was significantly associated with increases in foliar Al concentrations (Table 3), reductions in terminal shoot lengths (Fig. 2), and increases in foliar starch and TNC concentrations (Table 2). Similar changes in root and foliar parameters have been noted in other plants following root freezing injury that directly reduced water and nutrient uptake and led to altered carbon relationships (Marschner 2002). However, a diminished capacity for root uptake would reduce the foliar incorporation for all cations transported via the transpiration stream, not just the Al and Ca:Al concentrations that we report.

In contrast, the indirect effects of soil freezing on nutrient availability may better account for the changes in

aboveground physiology that we detected. Specifically, we propose that freeze-induced soil acidification increased Al uptake and reduced foliar Ca:Al to levels that hindered woody growth, thereby altering carbon source–sink relationships that allowed for build-ups of foliar starch. Past work has shown that a reduced snowpack led to root freezing damage, increased N leaching, acidified soils, and altered soil cation availability (Tierney et al. 2001; Groffman et al. 2001; Fitzhugh et al. 2001). Our foliar cation data show that this alteration in soil nutrition also leads to a specific increase in foliar Al and a reduction of Ca:Al molar ratios—patterns that are characteristic of soil acidification (Cronan and Grigal 1995). Furthermore, the Al concentrations (almost 40 mg kg^{-1}) and Ca:Al ratios (under 150) that we report for maple foliage from the snow removal plots are consistent with levels associated with reduced basal area growth (and thus reduced carbon sink strength) in sugar maple trees (Schaberg et al. 2006b; Huggett et al. 2007). The reduced length of terminal shoots that we document indicate that reduced crown growth can accompany snow removal. Whether this reflects more substantial reductions in carbon sequestration in tree boles has yet to be determined. A reduction in distal sink strength with reduced growth would slow phloem transport away from leaves and shunt foliar photosynthate production toward starch accumulation—a biochemically neutral form of localized carbon storage that would limit the feedback inhibition of photosynthesis (Herold 1980).

Our data also show that increased soil freezing could exacerbate the effects of acid deposition and push soils further toward acidification that can deplete Ca when it is available, and mobilize Al when Ca availability is low. Indeed, in a manipulation study where snow was excluded from soils all winter in a sugar maple forest in Quebec, Canada, Boutin and Robitaille (1995) found that soil acidification the year after treatment was the equivalent to decades of acidic deposition. Altered Ca and Al nutrition from inputs of acidic deposition have already been associated with the declines of sugar maple (Schaberg et al. 2006b; Huggett et al. 2007), red spruce (*Picea rubens* Sarg.) (Schaberg et al. 2006a; Halman et al. 2008), and paper birch (*Betula papyrifera* Marsh.) (Halman et al. 2011) in the northeastern USA. If climate change results in increased soil freezing that further acidifies sites altered by acidic deposition, then more forests and tree species may be put at risk of injury and decline. A significant decrease in sugar maple canopy dominance over the next 100 years has already been predicted as a result of Ca depletion associated with acid deposition (Kobe et al. 2002). Any additional susceptibility to root damage or greater sensitivity to lower Ca:Al ratios in soils could lead to a gradual replacement of sugar maple by other tree species in many northern hardwood forest ecosystems.

Intra-system cycling of Ca is dominated by vegetation uptake and eventual return to the forest floor with litterfall (Likens et al. 1998). Our data and the results of others (Groffman et al. 2001; Fitzhugh et al. 2003) indicate that, at least in northern hardwood forests dominated by sugar maple, reduced snow cover can damage tree roots, acidify soils, alter soil and foliar cation nutrition, reduce woody growth, and perturb tree carbohydrate relations. Similar alterations caused by acid deposition have been shown to move beyond soil and plant populations to influence dependent animal populations (Schaberg et al. 2001). Notably, acid-induced depletions of Ca have led to declines in snail populations that then reduce Ca intakes of dependent breeding birds—resulting in elevated rates of eggshell defects, clutch desertions, and empty nests (Graveland and Van Gijzen 1994; Graveland and van der Wal 1996). It is not yet known if soil acidification following soil freezing when snowpack depths are low may similarly cause cation perturbations that cascade up through food webs.

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References

- Bailey AS, Hornbeck JW, Campbell JL, Eager C (2003) Hydrometeorological database for Hubbard Brook experimental forest, 1955–2000. In: General technical report NE305. US Department of Agriculture, Forest Service, Northeastern Research Station, Newtown Square, Pennsylvania
- Boutin R, Robitaille G (1995) Increased soil nitrate losses under mature sugar maple trees affected by experimentally induced deep frost. *Can J For Res* 25:588–602
- Brown PJ, DeGaetano AT (2011) A paradox of cooling winter soil surface temperatures in a warming northeastern United States. *Agric For Meteorol* 151:947–956
- Calmé S, Bigras FJ, Margolis HA, Hébert C (1994) Frost tolerance and bud dormancy of container-grown yellow birch, red oak and sugar maple seedlings. *Tree Physiol* 14:1313–1325
- Campbell JL, Ollinger SV, Flerchinger GN, Wicklein H, Hayhoe K, Bailey A (2010) Past and projected future changes in snowpack and soil frost at the Hubbard Brook experimental forest, New Hampshire, USA. *Hyrol Process* 24:2465–2480
- Cleavitt NL, Fahey TJ, Groffman PM, Hardy JP, Henry KS, Driscoll CT (2008) Effects of soil freezing on fine roots in a northern hardwood forest. *Can J For Res* 38:82–91
- Comerford DP (2011) Influence of snow removal on sugar maple physiology and snow and vegetation removal on ground dwelling insects. Master thesis, University of Vermont, Burlington
- Cronan CS, Grigal DF (1995) Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *J Environ Qual* 24:209–226
- Decker K, Wang D, Waite C, Scherbatskoy T (2003) Snow removal and ambient air temperature effects on forest soil temperatures in northern Vermont. *Soil Sci Soc Am J* 67:1234–1242
- Driscoll CT, Lawrence GB, Bulger AJ, Butler TJ, Cronan CS, Eagar C, Lambert KF, Likens GE, Stoddard JL, Weathers KC (2001) Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *Bioscience* 51:180–198
- Fahey TM, Hughes JW (1994) Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *J Ecol* 82:533–548
- Federer C (2001) Effects of warming on snow at the Hubbard Brook Experimental Forest. In: Rock B (ed) New England regional assessment group. Preparing for a changing climate: the potential consequences of climate variability and change for New England regional overview. US Global Change Research Program, University of New Hampshire, Durham
- Fitzhugh RD, Driscoll CT, Groffman PM, Tierney GL, Fahey TJ, Hardy JP (2001) Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry* 56:215–238
- Fitzhugh RD, Driscoll CT, Groffman PM, Tierney GL, Fahey TJ, Hardy JP (2003) Soil freezing and the acid-base chemistry of soil solutions in a northern hardwood forest. *Soil Sci Soc Am J* 67:1897–1908
- Graveland J, van der Wal R (1996) Decline in snail abundance due to soil acidification causes eggshell defects in forest passerines. *Oecologia* 105:351–360
- Graveland J, Van Gijzen T (1994) Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. *Ardea* 82:299–314
- Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001) Colder soils in a warmer world: a snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* 56:135–150
- Halman JM, Schaberg PG, Hawley GJ, Eagar C (2008) Calcium addition at the Hubbard Brook Experimental Forest increases sugar storage, antioxidant activity and cold tolerance in native red spruce (*Picea rubens*). *Tree Physiol* 28:855–862
- Halman JM, Schaberg PG, Hawley GJ, Hansen CF (2011) Potential role of soil calcium in recovery of paper birch following ice storm injury in Vermont, USA. *For Ecol Manag* 261:1539–1545
- Hendrix DL (1993) Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Sci* 33:1306–1311
- Herold A (1980) Regulation of photosynthesis by sink activity—the missing link. *New Phytol* 86:131–144
- Hinesley L, Pharr D, Snelling L, Funderburk S (1992) Foliar raffinose and sucrose in four conifer species: relationship to seasonal temperature. *J Am Soc Hortic Sci* 117:852–855
- Huggett BA, Schaberg PG, Hawley GJ, Eagar C (2007) Long-term calcium addition increases growth release, wound closure, and health of sugar maple (*Acer saccharum*) trees at the Hubbard Brook Experimental Forest. *Can J For Res* 37:1692–1700
- IPCC (2007) Executive summary of the Intergovernmental Panel on Climate Change. <http://www.ipcc.com.ch>. Accessed Feb 2007

- Johnson CE, Driscoll CT, Siccama TG, Likens GE (2000) Element fluxes and landscape position in a northern hardwood forest watershed ecosystem. *Ecosystems* 3:159–184
- Jones J Jr, Case VW, Westerman R (1990) Soil testing and plant analysis. Soil Science Society of America, Madison
- Kobe RK, Likens GE, Eagar C (2002) Tree seedling growth and mortality responses to manipulations of calcium and aluminum in a northern hardwood forest. *Can J For Res* 32:954–966
- Koricheva J, Larsson S, Haukioja E (1998) Insect performance on experimentally stressed woody plants: a meta-analysis. *Annu Rev Entomol* 43:195–216
- Kuhns MR, Stroup WW, Gebre GM (1993) Dehydration tolerance of five bur oak (*Quercus macrocarpa*) seed sources from Texas, Nebraska, Minnesota, and New York. *Can J For Res* 23:387–393
- Likens GE, Driscoll CT, Buso DC (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* 272:244–246
- Likens G, Driscoll C, Buso D, Siccama T, Johnson C, Lovett G, Fahey T, Reiners W, Ryan D, Martin C (1998) The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* 41:89–173
- Marschner H (2002) Mineral nutrition of higher plants. Academic, San Diego
- McKay H (1998) Root electrolyte leakage and root growth potential as indicators of spruce and larch establishment. *Silva Fenn* 32:241–252
- McLaughlin S (1999) Calcium physiology and its role in terrestrial ecosystem processes. *New Phytol* 142:211–222
- Montgomery DC (2008) Design and analysis of experiments. Wiley, New York
- Morsomme P, Boutry M (2000) The plant plasma membrane H⁺-ATPase: structure, function and regulation. *Biochem Biophys Acta* 1464:1–16
- Ruter JM (1996) High-temperature tolerance of heritage river birch roots decreased by pot-in-pot production systems. *Hortic Sci* 31:813–814
- Sakai A, Larcher W (1987) Frost survival of plants. Responses and adaptation to freezing stress. Springer, New York
- Schaberg PG, DeHayes DH, Hawley GJ (2001) Anthropogenic calcium depletion: a unique threat to forest ecosystem health? *Ecosyst Health* 7:214–228
- Schaberg PG, Eagar C, Borer CH, Hawley GJ (2006a) Calcium addition at the Hubbard Brook Experimental Forest reduced winter injury to red spruce in a high-injury year. *Can J For Res* 36:2544–2549
- Schaberg PG, Tilley JW, Hawley GJ, DeHayes DH, Bailey SW (2006b) Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. *For Ecol Manag* 223:159–169
- Schaberg PG, Hennon PE, D'Amore DV, Hawley GJ (2008) Influence of simulated snow cover on the cold tolerance and freezing injury of yellow-cedar seedlings. *Glob Change Biol* 14:1282–1293
- Schaberg PG, D'Amore DV, Hennon PE, Halman JM, Hawley GJ (2011) Do limited cold tolerance and shallow depth of roots contribute to yellow-cedar decline? *For Ecol Manag* 262:2142–2150
- Schapiro AL, Valpuesta V, Botella MA (2009) Plasma membrane repair in plants. *Trends Plant Sci* 14:645–652
- Shanley J, Chalmers A (1999) The effect of frozen soil on snowmelt runoff at Sleepers River, Vermont. *Hydrol Process* 13:1843–1857
- Stadler D, Wunderli H, Auckenthaler A, Fluehler H, Bruendl M (1996) Measurement of frost-induced snowmelt runoff in a forest soil. *Hydrol Process* 10:1293–1304
- Strimbeck GR, Kjellsen TD, Schaberg PG, Murakami PF (2007) Cold in the common garden: comparative low-temperature tolerance of boreal and temperate conifer foliage. *Trees* 21:557–567
- Sutinen M, Ritari A, Holappa T, Kujala K (1998) Seasonal changes in soil temperature and in the frost hardiness of Scots pine (*Pinus sylvestris*) roots under subarctic conditions. *Can J For Res* 28:946–950
- Templer PH, Schiller AF, Fuller NW, Soggi AM, Campbell JL, Drake JE, Kunz TH (2012) Impact of a reduced winter snowpack on litter arthropod abundance and diversity in a northern hardwood forest ecosystem. *Biol Fertility Soils* 48:413–424
- Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry* 56:175–190
- Waisel Y, Eshel A, Kafkafi U (1996) Plant roots: the hidden half. Marcel Dekker, New York
- Yanai R, Fisk M, Fahey T, Cleavitt N, Park B (2008) Identifying roots of northern hardwood species: patterns with diameter and depth. *Can J For Res* 38:2862–2869
- Zwiazek JJ, Blake TJ (1991) Early detection of membrane injury in black spruce (*Picea mariana*). *Can J For Res* 21:401–404