

Increased nitrogen leaching following soil freezing is due to decreased root uptake in a northern hardwood forest

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Abstract

The depth and duration of snow pack is declining in the northeastern United States as a result of warming air temperatures. Since snow insulates soil, a decreased snow pack can increase the frequency of soil freezing, which has been shown to have important biogeochemical implications. One of the most notable effects of soil freezing is increased inorganic nitrogen losses from soil during the following growing season. Decreased nitrogen retention is thought to be due to reduced root uptake, but has not yet been measured directly. We conducted a 2-year snow-removal experiment at Hubbard Brook Experimental Forest in New Hampshire, USA to determine the effects of soil freezing on root uptake and leaching of inorganic nitrogen simultaneously. Snow removal significantly increased the depth of maximal soil frost by 37.2 and 39.5 cm in the first and second winters, respectively ($P < 0.001$ in 2008/2009 and 2009/2010). As a consequence of soil freezing, root uptake of ammonium declined significantly during the first and second growing seasons after snow removal ($P = 0.023$ for 2009 and $P = 0.005$ for 2010). These observed reductions in root nitrogen uptake coincided with significant increases in soil solution concentrations of ammonium in the Oa horizon ($P = 0.001$ for 2009 and 2010) and nitrate in the B horizon ($P < 0.001$ and $P = 0.003$ for 2009 and 2010, respectively). The excess flux of dissolved inorganic nitrogen from the Oa horizon that was attributable to soil freezing was 7.0 and 2.8 kg N ha⁻¹ in 2009 and 2010, respectively. The excess flux of dissolved inorganic nitrogen from the B horizon was lower, amounting to 1.7 and 0.7 kg N ha⁻¹ in 2009 and 2010, respectively. Results of this study provide direct evidence that soil freezing reduces root nitrogen uptake, demonstrating that the effects of winter climate change on root function has significant consequences for nitrogen retention and loss in forest ecosystems.

Keywords: climate change, root uptake, snow, soil frost, soil solution, stable isotopes

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Introduction

In the northeastern United States (US), climate projections indicate that average annual air temperature will increase by 2.1 to 5.3 °C by the end of the century (Hayhoe *et al.*, 2008) and the depth and duration of the snowpack will decline (Hayhoe *et al.*, 2007). Snow is an insulator, and when the snow pack accumulates to sufficient depths, the soil beneath it can remain unfrozen even when air temperatures are below freezing (Edwards *et al.*, 1986; Sharratt *et al.*, 1992). A shallow snowpack or delay in snow pack onset can result in frozen soil when air temperatures are below freezing, which has been demonstrated with snow-removal experiments (e.g., Boutin & Robitaille, 1994; Hardy *et al.*, 2001; Decker *et al.*, 2003). While the duration of soil frost will likely decrease in the Northeast, minimum soil surface temperatures will be colder and soil freezing events may become more common in the

future (Campbell *et al.*, 2010; Brown & DeGaetano, 2011).

Forests of the northeastern United States receive elevated inputs of anthropogenic nitrogen (N) from atmospheric deposition (Aber *et al.*, 1998; Driscoll *et al.*, 2003; Galloway *et al.*, 2004), and a large proportion of this N is currently retained by forest ecosystems (Campbell *et al.*, 2004; Goodale *et al.*, 2005; Templer *et al.*, 2012). However, at the watershed scale, soil freezing has been linked to increased nitrate (NO₃⁻) losses in surface waters (Edwards *et al.*, 1986; Mitchell *et al.*, 1996; Fitzhugh *et al.*, 2003). Similarly, results from experimental reductions in the winter snow pack indicate that the soil freezing leads to soil acidification (Fitzhugh *et al.*, 2001) and increases in N leaching (Boutin & Robitaille, 1994; Neilsen *et al.*, 2001; Hentschel *et al.*, 2009; Brooks *et al.*, 2011), as demonstrated at locations such as the Hubbard Brook Experimental Forest (HBEF) in central New Hampshire, USA (Fitzhugh *et al.*, 2001; Groffman *et al.*, 2001a, 2006, 2011). Soil acidification caused by soil freezing leads to reduced foliar calcium : aluminum ratios and terminal shoot growth of sugar maple (*Acer saccharum* Marsh.) trees

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(Comerford *et al.*, 2013). Thus, the ability of Northeastern forests to retain high atmospheric inputs of N in the future may be affected by altered soil frost regimes arising from climate change.

Increases in N export following experimental soil freezing in mixed hardwood forests at the Fernow Experimental Forest, West Virginia were attributed in part to elevated rates of nitrification (Gilliam *et al.*, 2010), but elevated N leaching could not be explained by increased rates of nitrification alone in the majority of other forests studied (Fitzhugh *et al.*, 2001; Callesen *et al.*, 2007; Christopher *et al.*, 2008; Hentschel *et al.*, 2009). Rather, most studies purport that reduced plant uptake is the primary mechanism for increased N losses (Boutin & Robitaille, 1994; Fitzhugh *et al.*, 2001; Groffman *et al.*, 2001b; Matzner & Borken, 2008; Kreyling, 2010; Brooks *et al.*, 2011). This finding is supported by empirical evidence of root mortality (Tierney *et al.*, 2001; Weih & Karlsson, 2002; Cleavitt *et al.*, 2008; Gaul *et al.*, 2008), decreased root vitality or condition (Cleavitt *et al.*, 2008), and induced root injury (Comerford *et al.*, 2013) following exposure to soil frost, which could impair the ability of trees to take up N. While rates of N uptake by mature trees have been shown to directly impact ecosystem-scale retention and loss of N (Templer *et al.*, 2005), the direct effects of soil frost on plant N uptake have not been demonstrated.

Sugar maple is a deciduous tree species of commercial and ecological importance in northeastern North America. It is particularly important with regard to N cycling because sugar maple stands have labile litter with high rates of soil nitrification and NO_3^- leaching (Finzi *et al.*, 1998; Lovett & Mitchell, 2004; Templer *et al.*, 2005). Previous studies have shown that compared to other species, sugar maple stands are more responsive to soil freezing, having greater leaching losses of NO_3^- after freezing events (Boutin & Robitaille, 1994; Fitzhugh *et al.*, 2001). The median fine root depth of sugar maple at the HBEF is ~10 cm, with 35% of fine roots occurring within the upper 4.5 cm of the soil organic horizon (Yanai *et al.*, 2008). Thus, even shallow soil frost (<10 cm) could have a substantial impact on root function in stands dominated by sugar maple.

We conducted a snow-removal experiment at the HBEF to determine if soil frost reduces plant uptake of inorganic N. During one growing season prior to, and two consecutive growing seasons following experimental snow removal, we measured uptake of ammonium (NH_4^+) and NO_3^- by fine roots of sugar maple trees. We also evaluated the effect of soil frost on the concentration and flux of NH_4^+ and NO_3^- in soil solution, and used measurements of natural abundance ^{18}O in NO_3^-

to determine the source of NO_3^- (i.e. atmospheric N or soil nitrification) in soil solution. Our hypothesis was that soil freezing increases leaching losses of NO_3^- because of reduced plant uptake of N rather than increases in rates of nitrification in a northern hardwood forest.

Materials and methods

Site description

The HBEF is located in the White Mountain National Forest in central New Hampshire (43°56'N, 71°45'W). The climate is cool, humid, and continental. Mean annual precipitation is 1400 mm, with approximately one-third falling as snow (Bailey *et al.*, 2003). The winter snow pack is typically continuous from midNovember to midApril (165 days, 30 year mean), with winter air temperatures averaging -4.7°C (Campbell *et al.*, 2010). Long-term data from the HBEF indicate that there is measurable soil frost two of every three years with an average annual maximum depth of 6 cm and an all-time maximum depth of 26 cm in 1993 (Campbell *et al.*, 2010). Soils typically consist of base-poor spodosols, mostly Haplopodols, that developed in glacial till, and the bedrock is generally shallow (~1 m; Johnson *et al.*, 2000). Dominant overstorey hardwood tree species include sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Brit.), and coniferous tree species include red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* Mill.).

Snow removal

At the HBEF, four paired reference and treatment plots ($N = 8$ plots total, each 13×13 m) were established in October 2007. Paired plots were adjacent to each other and the pairs were between 60 to 120 m aside from each other. Each plot contained a minimum of three mature sugar maple trees in the overstorey and included a 1 m buffer at the edge of each plot, where we did not sample. Sparse understorey vegetation, consisting primarily of hobblebush (*Viburnum lantanoides* Michx.), was clipped on both the reference and treatment plots to facilitate shoveling in the treatment plots. To induce soil freezing, we removed snow from the treatment plots by shoveling within 48 h of each snow fall event during the first half of winter (i.e. through 31 January; $N = 6$ and 7 shoveling events in 2008/2009 and 2009/2010, respectively). This period of snow removal was long enough to allow frost to develop with minimal impact on the water balance (Hardy *et al.*, 2001). A 3 cm layer of snow was left on the snow-removal plots to avoid disturbance to the forest floor and maintain the surface albedo in winter. Following the end of the snow-removal period, a natural snow pack accumulated on the treatment plots. During the snow-covered period, disturbance on all plots was minimized by using snowshoes to disperse weight.

Snow depth, soil frost depth, and soil temperature

Snow and soil frost depths were measured every 7–10 days from late October to early May during the pretreatment (2007/2008) and treatment (2008/2009, 2009/2010) winters. Snow depth was measured using a stainless steel snow sampling tube (Model 3600 Federal, $N = 4$ per plot per date). Soil frost depth was measured with frost gages constructed of flexible polyethylene tubes filled with a 0.05 percent solution of methylene blue dye that were inserted into PVC pipes installed vertically in the soil (Ricard *et al.*, 1976). As the solution freezes, the dye is excluded from the ice, creating a discrete demarcation between the frozen (clear) and liquid (blue) portions. During measurement, the tubes were removed from the PVC pipe and the depth of the soil frost was recorded.

Soil temperature was measured at one location in each plot continuously during the study period. Copper-constantan thermocouples (± 0.7 °C) were inserted at six depths in the soil (1, 3, 7, 15, 30 and 50 cm). Soil temperature was measured at 10 s intervals and the hourly mean values were recorded with dataloggers. A thermistor (± 0.4 °C) was used as a reference temperature for the thermocouples. Depths of 0 °C isotherms were determined using linear interpolation between depths where soil temperature was measured (i.e. 1, 3, 7, 15, 30, and 50 cm).

Plant nitrogen uptake

Two commonly used methods to measure N uptake by trees include the *ex situ* excised root method (Epstein *et al.*, 1963) and the *in situ* N depletion method (BassiriRad *et al.*, 1999). Although both methods are useful in comparative studies, we used the N depletion method in this study because the roots remain connected to the plant, thus potentially yielding a more accurate measurement of root N uptake than roots that have been excised and measured in the laboratory (Bloom & Caldwell, 1988; Soggi & Templer, 2011).

Measurements of fine root uptake of NH_4^+ and NO_3^- by sugar maple trees were made three times throughout the growing season in 2008 (prior to snow removal; late May, early July, midSeptember, $N = 1$ per plot) and eight times throughout each growing season in 2009 and 2010 (post snow-removal treatment, $N = 2$ per plot). We expected to see the greatest effects in the early growing season and sampled more intensively during that time, with six sampling dates spread between late April and late May (from bud burst to full leaf expansion), followed by one measurement in the peak growing season (July) and one in the late growing season (midSeptember). We did not measure rates of organic forms of N uptake since it has been shown to account for <20% total N uptake by sugar maple trees (Gallet-Budynek *et al.*, 2009).

We excavated live terminal root branches at the soil surface (0 to 10 cm in the Oa horizon) and from within 1 m of the base of a mature sugar maple tree, taking care to minimize disturbance and ensure that roots remained connected to the tree. Therefore, 'intact' refers to the connection to the terminal root branch of the tree, but may not include

complete mycorrhizal associations. We used first- and second-order fine roots (<2 mm) because these are the most active in nutrient acquisition (Eissenstat, 1992; Nadelhoffer & Raich, 1992). Sugar maple roots were identified by the club-shaped appearance of the root tips, their pinnate branching with a gradual decrease in size with root order, and their lack of fragrance (this latter trait distinguishes them from yellow birch roots, which smell like wintergreen; Yanai *et al.*, 2008).

Following excavation, roots were rinsed with deionized water and dabbed with low-lint, nonabrasive cellulose paper to remove soil particles from the root surface. Roots were then incubated for 90 min in a 40 mL solution containing 25 and 38 $\mu\text{mol N L}^{-1}$ as NH_4Cl and KNO_3 , respectively. The concentration of dissolved inorganic N (DIN) used in this study ($63 \mu\text{mol N L}^{-1}$) was about 3.5 times higher than the mean growing season concentration measured in the Oa horizon of the reference plots, was nearly equal to the maximum monthly growing season DIN concentration in the reference plots ($59 \mu\text{mol N L}^{-1}$ in July 2008), and was about half the maximum monthly growing season DIN concentration in the treatment plots ($131 \mu\text{mol N L}^{-1}$ in May 2009). Each solution was covered with parafilm during incubation to prevent evaporation and contamination. On each date, solutions of the same nutrient concentrations, but containing no roots, were uncapped and covered with parafilm for 90 min under field conditions to serve as a control. Additional fine root samples were incubated for 90 min in a deionized water control. At the end of the 90-min incubation period, roots were removed from solution and the incubated portion of the root was cut, oven-dried at 55 °C, and weighed (dry root mass mean \pm SE = 83.3 ± 2.2 mg). Treatment solutions were filtered and analyzed using automated colorimetry (Lachat QuickChem 8500) at Boston University to determine the concentrations of NH_4^+ and NO_3^- in each nutrient solution following the 90-min incubation.

Net rates of NH_4^+ and NO_3^- uptake by intact roots using the *in situ* depletion method were calculated as:

$$N_{\text{up}} = \frac{(N_{\text{initial}} \times \text{Vol}_{\text{solution}}) - (N_{\text{final}} \times \text{Vol}_{\text{solution}})}{(\text{Mass}_{\text{root}} \times T)} \quad (1)$$

where N_{up} is the rate of N taken up by roots as NH_4^+ or NO_3^- ; $\text{Mass}_{\text{root}}$ = dry root mass; N_{initial} = concentration of N in the treatment solution at the start of incubation; N_{final} = concentration of N in the treatment solution at the end of incubation; $\text{Vol}_{\text{solution}}$ = volume of the treatment solution; T = incubation time. Rates of N uptake are expressed as positive values where there is net uptake of N by roots, or negative values where there is net efflux of N from the roots.

Nitrogen in soil solution

Soil water was collected with porous cup, tension lysimeters (5 cm diameter) installed horizontally in the Oa and B soil horizons at one location in each plot. Soil lysimeters were connected by tubing to sample bottles housed in containers that were buried in the ground (see Campbell *et al.*, 2006). The storage containers were accessible through the surface of the snow pack and were insulated to prevent the drainage water

from freezing. Soil water lysimeters were installed during fall 2007 and were left overwinter (7 months) to minimize disturbance effects. Sample collection began the following summer, to ensure that there was adequate time for the lysimeters to equilibrate following installation disturbance. Soil water samples were collected at 2 weeks intervals and tension was applied (30 kPa) the day before the samples were collected. When available, snow melt samples were collected at the same 2 weeks intervals with a snowmelt lysimeter located in each of the reference plots. Polyethylene pans (76 cm diameter, 7.6 cm height) placed on the surface of the forest floor collected melt water, which drained by gravity through a PVC pipe into sample bottles housed in the same buried containers used for soil water sample collection.

Immediately upon returning from the field, snow melt and soil lysimeter samples were passed through precombusted (450 °C) glass-fiber filters (0.7 µm nominal pore size). Samples were transported on ice to the laboratory at the USDA Forest Service, Durham, New Hampshire and stored frozen until analysis. Concentrations of NO_3^- were measured with ion chromatography (Metrohm 761), and NH_4^+ with automated colorimetry (Lachat QuickChem 8500). A subset of water samples (snowmelt and Oa horizon lysimeters) was analyzed for $\delta^{18}\text{O}\text{-NO}_3^-$ to evaluate the source of NO_3^- leached from the soil. Frozen aliquots were thawed and prepared for $\delta^{18}\text{O}\text{-NO}_3^-$ analysis using the denitrifier method (Sigman *et al.*, 2001; Casciotti *et al.*, 2002). Samples of $\delta\text{N}_2^{18}\text{O}$ gas produced with this method were shipped to the Stable Isotope Facility at the University of California Davis where they were analyzed on a SerCon Cryoprep trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, UK). Ten percent of samples were run in duplicate, providing an estimate of precision (0.0093 ‰ SE). Our detection limit was approximately 10 nmol N mL⁻¹. Consequently, only snow melt and Oa horizon lysimeter samples were analyzed for $\delta^{18}\text{O}\text{-NO}_3^-$ because most of the lysimeter samples from the B horizon did not have a sufficient N for analysis.

To calculate DIN export from the Oa and B soil horizons, we modeled soil water using the Brook90 hydrologic model (Federer, 2012). Brook90 simulates water movement through multiple soil layers, making it possible to approximate the flux of soil water from different horizons. Brook90 was run on a daily timestep using daily precipitation, minimum and maximum air temperature, vapor pressure, solar radiation, and wind speed measurements from the HBEF. Modeled water fluxes from Oa and B horizon were multiplied by the average monthly concentration of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ measured in those horizons. The excess N export due to soil freezing was calculated as the difference in N flux between the snow removal and reference plots for each growing season.

Soil bulk density

After snow melt on 4 May 2010, we collected soil cores (10 cm depth; 6.5 cm diameter; $N = 3$ per plot; Oe and Oa horizons combined) to determine the impact of the snow-removal treatment on soil bulk density. Soil cores were dried in an

oven at 50 °C for 48 h and weighed. Soil bulk density was calculated as:

$$\text{Soil Bulk Density} = \text{Mass}_{\text{dry}} \times \text{Vol}_{\text{soil}}^{-1} \quad (2)$$

where Mass_{dry} is the mass of the oven-dried soil and Vol_{soil} is the volume of the soil core.

Statistical analysis

The effects of the snow removal treatment on snow depth, soil frost depth, soil bulk density, root N uptake, and N leaching, were evaluated with a generalized linear mixed model fitted with residual pseudolikelihood estimation (GLIMMIX procedure in SAS 9.2; SAS Institute, Inc. Cary, NC, USA, 2008). Treatment effects were analyzed by sample period within each year using a repeated measures randomized block design, with each block consisting of a treatment/reference plot pair. Soil frost treatment, sample period, and their interaction were treated as fixed effects, and block as a random effect. For N uptake, the data were normalized prior to analysis to make the values positive. The model was analyzed with a gamma distribution and log link function. Denominator degrees of freedom were adjusted using the Kenward–Roger approximation. Since N uptake measurements were made at unequally spaced intervals, we used a spatial power covariance structure to account for the correlation between sampling periods within a year. For other models (i.e. snow depth, soil frost depth and soil lysimeter), an autoregressive covariance structure was used. Soil bulk density was analyzed with a generalized linear mixed model, like that described previously, but without the repeated measures component of the model since it was only measured one time. Soil bulk density data were fit with a Gaussian distribution and identity link function. A Tukey–Kramer test was used for all *post hoc* analyses, and differences were considered significant at $\alpha = 0.05$.

Results

Snow manipulation

The snow pack depth varied among years, with a mean (\pm SE) annual maximum depth of 113.2 ± 2.3 , 98.6 ± 2.6 , and 48.7 ± 2.6 cm on the reference plots during the winters of 2007/2008, 2008/2009, and 2009/2010, respectively (Fig. 1a). No significant difference in snow pack depth between the reference and snow-removal plots was observed in the pretreatment year ($P = 0.998$); however, there were significant differences during the winters of 2008/2009 ($P < 0.001$) and 2009/2010 ($P < 0.001$). The duration of the snow pack also varied among years, with 95, 152, and 113 days of continuous snow cover on the reference plots during the winters of 2007/2008, 2008/2009, and 2009/2010, respectively. Snow removal resulted in 78 and 71 fewer days of snow cover during the winters of 2008/2009 and 2009/2010, respectively.

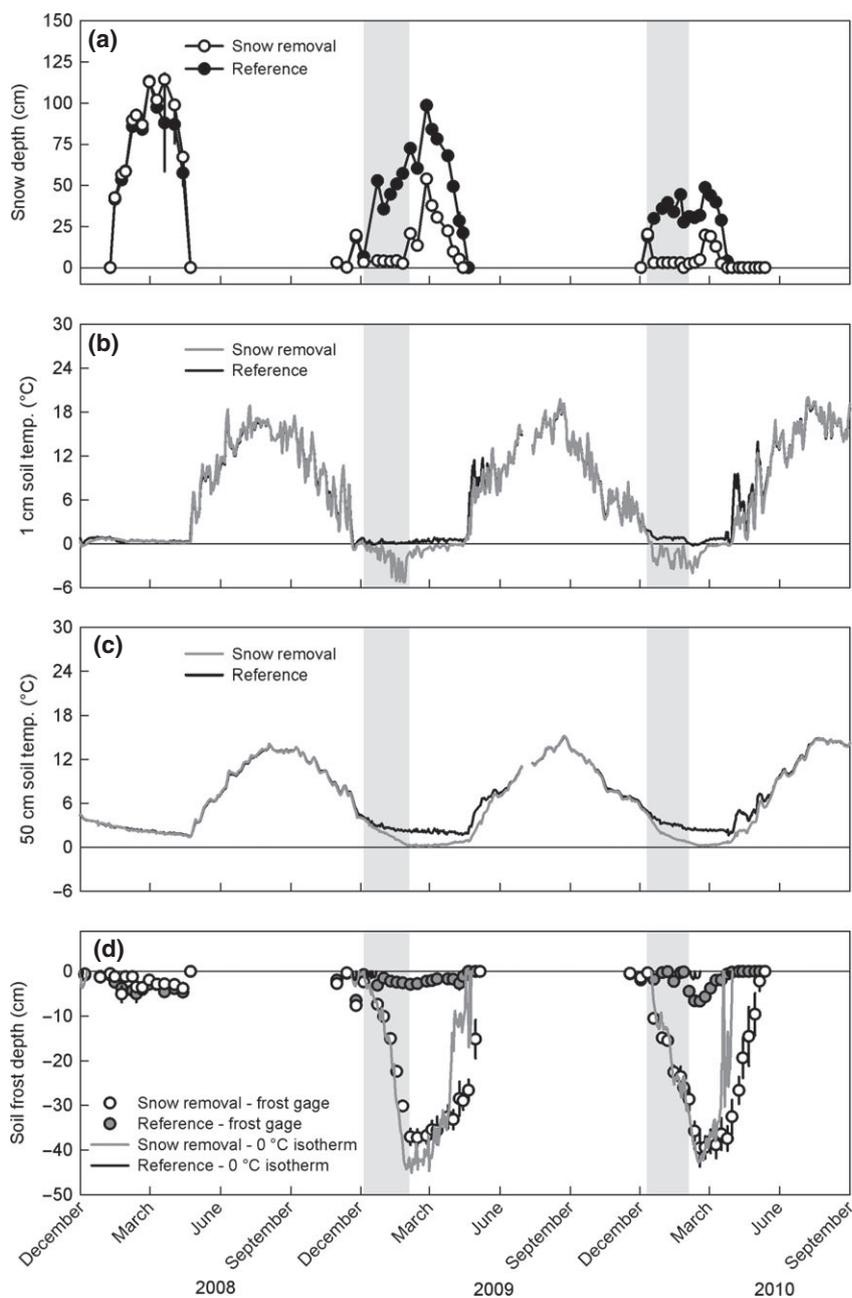


Fig. 1 Comparison between snow-removal and reference plots showing: (a) mean snow depth (cm); (b) soil temperature (°C) at 1 cm and (c) 50 cm depths; and (d) soil frost depth (cm) measured with frost gages and the 0 °C isotherm interpolated from the soil temperature data. The shaded areas denote the experimental snow-removal period. Error bars for the snow and soil frost depth measurements show the standard error of the mean, which in most cases is very small and therefore is not visible. Soil frost depth (d) as determined with the 0 °C isotherm is not apparent in the pretreatment year and in the reference plots during both posttreatment years because soil temperatures were nearly always above freezing in these cases.

Although soil temperature was measured at six depths (1, 3, 7, 15, 30, and 50 cm), only results for the extremes (1 and 50 cm depths) are presented since they encompass the range of response to snow removal (Fig. 1b, c). Soil temperatures at 1 cm depth were up to 5.3 and 4.2 °C colder in the snow-removal plots during the snow-covered period of the first and second

treatment years, respectively (Fig. 1b). At the 50 cm depth, the maximum difference in soil temperature between snow-removal and reference plots was smaller by 2.2 and 2.1 °C during the snow-covered period of the first and second treatment years, respectively (Fig. 1c). At both the 1 cm depth and 50 cm depth, the greatest difference in temperature between the snow-

removal and reference plots occurred after the snow had melted in spring (6.4 °C on 3 April 2010 for the 1 cm depth; 3.1 °C on 8 May 2009 for the 50 cm depth). After snow melt, temperature differences persisted for 1 to 2 months at both the 1 and 50 cm depths (Fig. 1b, c). The 0 °C isotherm depth calculated from soil temperature measurements (i.e. thermocouples) was comparable to the soil frost depth measured with frost gages during the freezing front of both winters of snow removal (Fig. 1d). However, soil frost estimates for these methods diverged as the soils thawed, with the thermocouple-based frost measurements showing more rapid thawing. This difference between methods may be due to a lag in thawing ice in the frost gages relative to thawing of frozen soil (McCool & Molnau, 1984).

Although we observed no significant differences in soil frost depths measured with frost gages among all study plots during the pre-treatment winter (2007/2008; Fig. 1a; $P = 0.93$), snow removal during both treatment winters induced significant differences in soil freezing depths relative to reference plots (Fig. 1d; $P < 0.001$ in 2008/2009 and 2009/2010). During the winter of 2008/2009, the mean annual maximum frost depth for the reference and snow-removal plots were 6.5 ± 0.7 cm (25 November 2008) and 37.2 ± 1.9 cm (13 February 2008), respectively. During the winter of 2009/2010, the mean annual maximum frost depths on the reference and snow-removal plots were 6.7 ± 0.8 cm (17 February 2010) and 39.5 ± 4.2 cm (17 February 2010), respectively.

Soil bulk density

There were no significant differences in soil bulk density between the reference and treatment plots following 2 years of snow removal (0.11 ± 0.03 and 0.18 ± 0.03 g cm⁻³ on the reference and treatment plots, respectively, $P = 0.21$). These results indicate that the snow-removal treatment did not compact the forest floor significantly.

Root nitrogen uptake

Prior to snow removal, there were no significant differences in DIN uptake between the reference and treatment plots (in 2008, $P = 0.98$ and 0.98 for NH_4^+ uptake and $P = 0.999$ and 0.99 for NO_3^- uptake when roots were provided 0 and $63 \mu\text{mol DIN L}^{-1}$ (i.e. $25 \mu\text{mol NH}_4^+\text{-N L}^{-1} + 38 \mu\text{mol NO}_3^-\text{-N L}^{-1}$), respectively). Following snow removal, rates of NH_4^+ uptake by roots were significantly lower in the treatment plots compared to the reference ($P = 0.023$ in 2009 and $P = 0.005$ in 2010, Fig. 2a, b). In contrast to NH_4^+ , NO_3^- uptake by roots was not significantly different between reference and treatment plots ($P = 0.74$ in 2009 and $P = 0.56$ in 2010, Fig. 2c, d). We found no significant differences in efflux of NH_4^+ or NO_3^- between treatments when the roots were provided with deionized water ($P = 0.40$ and 0.21 for NH_4^+ uptake and $P = 0.97$ and 0.16 for NO_3^- uptake, in 2009 and 2010, respectively).

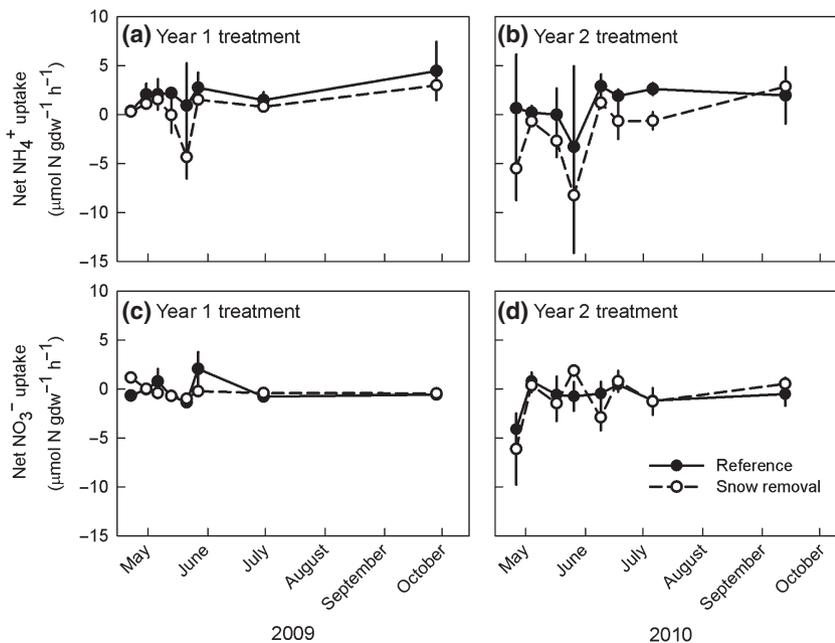


Fig. 2 Mean uptake of NH_4^+ (a = treatment year 1, b = treatment year 2) and NO_3^- (c = treatment year 1, d = treatment year 2) by sugar maple roots provided with $63 \mu\text{mol N L}^{-1}$ on reference and snow-removal plots. Error bars show the standard error of the mean. Negative values indicate net efflux of NH_4^+ and NO_3^- from the roots.

Nitrogen leaching

Concentrations of soil solution NH_4^+ were significantly higher in the Oa horizon in both years of snow-removal treatment ($P = 0.001$ for treatment year 1 and 2), and showed no significant difference in the pretreatment year ($P = 0.16$). In the snow-removal plots, NH_4^+ concentrations in the Oa horizon were highest in early spring and declined thereafter (Fig. 3a). Additionally, the average monthly peak NH_4^+ concentration in the Oa horizon during the first year of the snow-removal treatment ($97 \mu\text{mol N L}^{-1}$) was more than double the peak concentration in the second year ($46 \mu\text{mol N L}^{-1}$). In the B horizon, con-

centrations of NH_4^+ remained low ($<13 \mu\text{mol N L}^{-1}$) throughout the measurement period in both the treatment and reference plots (Fig. 3b). Concentrations of NH_4^+ in the B horizon showed no significant difference between treatment and reference plots in the pretreatment year ($P = 0.42$) or in both snow-removal years ($P = 0.69$ for year 1 and $P = 0.68$ for year 2).

Concentrations of soil water NO_3^- in the Oa horizon were highly variable, and although the values were typically higher in the snow-removal treatment compared to the reference plots, there were no significant differences in either of the study years (Fig. 3c; $P > 0.05$). In the B horizon, there was no significant dif-

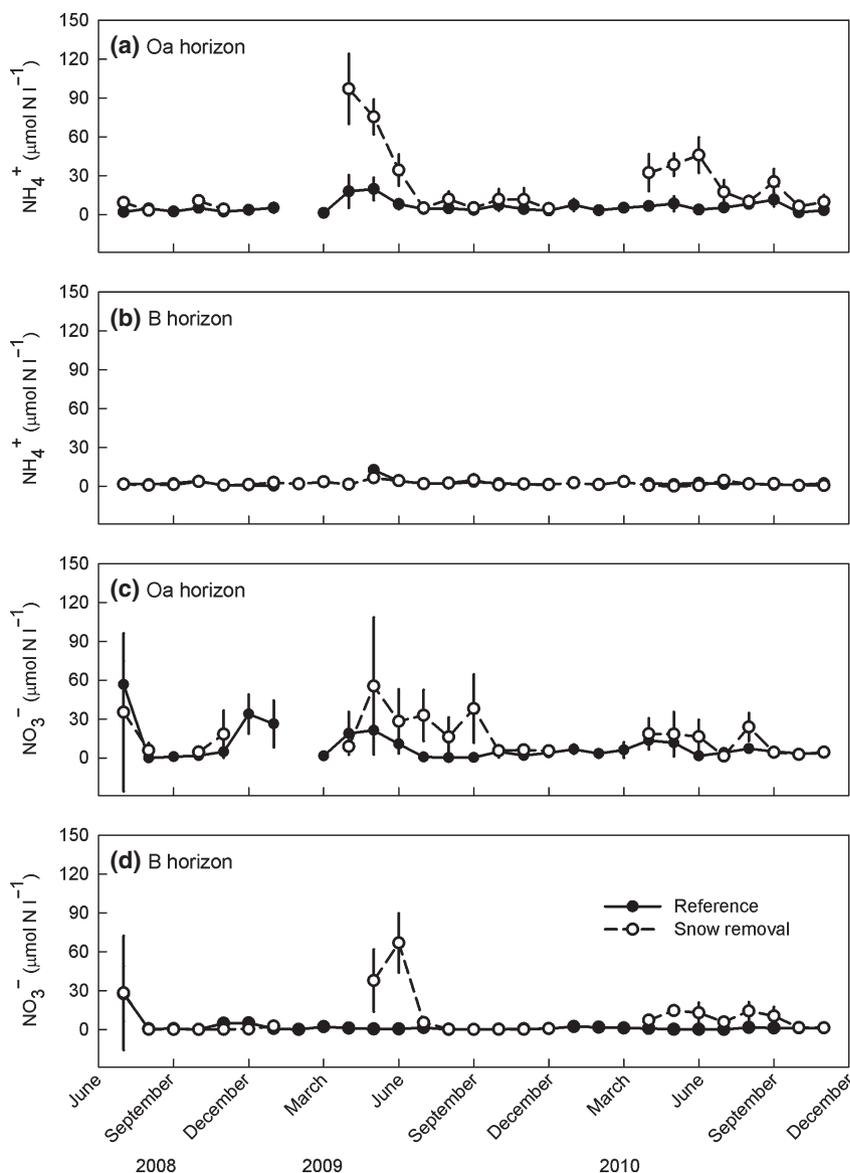


Fig. 3 Concentrations of soil solution NH_4^+ in the Oa (a) and B (b) horizon and NO_3^- in the Oa (c) and B (d) horizon in reference and snow-removal plots. Error bars show the standard error of the mean.

ference in soil water NO_3^- between the reference and the snow-removal plots in the pretreatment year ($P = 0.78$; Fig. 3d). However, NO_3^- concentrations were significantly greater in the snow-removal treatment plots in treatment years 1 and 2 ($P < 0.001$ and $P = 0.003$, respectively). Much like NH_4^+ , the NO_3^- peak in the first year of the treatment was higher than the second year (66 and 14 $\mu\text{mol N L}^{-1}$, respectively).

Isotopic analyses indicated that the snow melt samples collected were within the range of published values for natural abundance atmospheric $^{18}\text{O}\text{-NO}_3^-$ (Fig. 4; Pardo *et al.*, 2004; Elliott *et al.*, 2005; Kendall *et al.*, 2007). Many of the soil solution samples had concentrations of NO_3^- too low for isotopic analysis ($<10 \text{ nmol N mL}^{-1}$). Soil solution $\delta^{18}\text{O}\text{-NO}_3^-$ values in both the treatment and reference plots were within the range produced by nitrification (Mayer *et al.*, 2001; Kendall *et al.*, 2007), with the exception of one outlier in the reference plot that was affected by a rainfall event, resulting in a relatively high $\delta^{18}\text{O}\text{-NO}_3^-$ value suggesting some direct contribution from rain (Fig. 4). Values of $\delta^{18}\text{O}\text{-NO}_3^-$ were similar for the snow-removal treatment and reference plots indicating that soil freezing had no effect on the proportion of NO_3^- produced via nitrification in the forest floor.

The excess flux of DIN from the Oa horizon that was attributable to soil freezing was 7.0 and 2.8 kg N ha^{-1} in 2009 and 2010, respectively (Table 1). The excess flux of DIN from the B horizon was lower, amounting to 1.7 and 0.7 kg N ha^{-1} in 2009 and 2010, respectively. Of

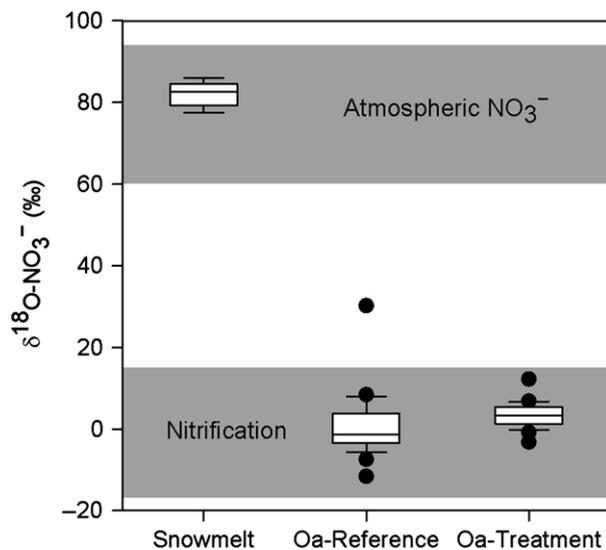


Fig. 4 Snow melt and Oa horizon soil solution $\delta^{18}\text{O}\text{-NO}_3^-$ in the reference and treatment (snow removal) plots. The shaded areas show literature values of the range in $\delta^{18}\text{O}$ for NO_3^- from atmospheric sources and NO_3^- produced by microbial nitrification in the soil (Mayer *et al.*, 2001; Kendall *et al.*, 2007). The horizontal line indicates median value.

the total excess DIN flux from the Oa horizon, 71–75% consisted of NO_3^- and 25–29% was NH_4^+ . The total excess DIN flux from the B horizon was comprised almost entirely of NO_3^- . The patterns of DIN export reflected soil solution concentrations; soil freezing had a greater effect on NH_4^+ in the Oa horizon and on NO_3^- in the B horizon.

Discussion

Freezing effects on root nitrogen uptake

Soil freezing decreased NH_4^+ uptake by roots, providing direct evidence that soil freezing significantly reduces plant N uptake, which likely accounts for increases in N export from forest ecosystems following soil freezing events. The underlying cause of reduced plant uptake is not well established and may be attributed to fine root mortality or cellular damage as indicated by previous snow-removal experiments at the HBEF. Using minirhizotrons, Tierney *et al.* (2001) observed elevated fine root necromass in the early spring after soil frost treatment. Cleavitt *et al.* (2008) used triphenyl-tetrazolium chloride (TTC) assays to show that soil freezing causes cellular damage to first- and second-order roots (as were used in this study), compromising root vitality and function. Additionally, Comerford *et al.* (2013) found greater tree electrolyte leakage, an indication of root injury, in snow-removal plots compared to reference plots. In our study, soil temperature may have also influenced uptake, since plant uptake and soil temperatures have been shown to be positively correlated (Gessler *et al.*, 1998), and temperatures remained colder in the snow-removal plots into the growing season. The temporal synchrony of NH_4^+ uptake between snow-removal and reference plots suggests that factors, such as environmental conditions, influence uptake throughout the growing season, in addition to relative differences due to soil freezing.

In addition to effects on fine roots, it is also possible that soil freezing affected mycorrhizal fungi, which form a symbiotic association with roots, providing them with water and nutrients in return for carbohydrates. We did not evaluate the effects of soil freezing on mycorrhizal fungi in this study; however, Cleavitt *et al.* (2008) showed no difference in colonization of sugar maple roots by arbuscular mycorrhizae (AM) between reference and snow-removal plots at the HBEF. Other studies have shown that the external mycelium of some AM species can survive winter freezing without an impact on colonization after thawing (Addy *et al.*, 1994), while others have shown negative impacts on colonization (Klironomos *et al.*, 2001).

Table 1 Growing season fluxes of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and DIN ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (SD) for the snow-removal treatment and reference plots. Excess flux is calculated as the difference between the treatment (snow removal) and reference plots

	2009			2010		
	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	DIN	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	DIN
Oa horizon						
Treatment	7.3 (2.2)	4.2 (3.1)	11.5 (3.8)	2.8 (0.8)	1.3 (0.8)	4.2 (1.3)
Reference	2.3 (1.4)	2.2 (1.8)	4.5 (3.1)	0.7 (0.3)	0.6 (0.2)	1.4 (0.5)
Excess flux	5.0 (2.6)	2.0 (3.6)	7.0 (4.9)	2.1 (0.8)	0.7 (0.8)	2.8 (1.4)
B horizon						
Treatment	0.5 (0.2)	1.7 (1.2)	2.2 (1.2)	0.2 (0.1)	0.7 (0.4)	0.9 (0.4)
Reference	0.4 (0.1)	0.1 (0.1)	0.5 (0.1)	0.2 (0.1)	0.1 (0.0)	0.3 (0.1)
Excess flux	0.1 (0.2)	1.6 (1.2)	1.7 (1.2)	0.0 (0.1)	0.7 (0.4)	0.7 (0.4)

Further work is needed to better establish the effect of soil freezing on mycorrhizal communities and the influence on plant nutrient uptake.

In contrast to NH_4^+ uptake, no significant differences in NO_3^- uptake were observed during the first or second year of treatment, and NO_3^- uptake remained near zero throughout the study. This lack of NO_3^- uptake by sugar maples is consistent with previous N uptake studies (Knoepp *et al.*, 1993; Gessler *et al.*, 1998; Templer & Dawson, 2004; Lucash *et al.*, 2005). Some evidence suggests that NO_3^- uptake is inhibited in the presence of NH_4^+ (Knoepp *et al.*, 1993; Kronzucker *et al.*, 1999), which could be a factor since the solution used in this study included both NO_3^- and NH_4^+ . Preferential uptake of NH_4^+ by plants may be a result of the lower energetic costs of NH_4^+ uptake and assimilation relative to NO_3^- uptake (Pate & Layzell, 1990).

In this study, there were instances of net efflux of both NH_4^+ and NO_3^- by roots in all of the plots. Net efflux of NH_4^+ and NO_3^- has been observed in previous studies using the *in situ* depletion method (Lucash *et al.*, 2005, 2008; Soggi & Templer, 2011) and could be attributed to several factors. Even though the concentrations of NH_4^+ and NO_3^- provided in this study were similar to concentrations that occur under natural field conditions, it is possible that concentrations of NH_4^+ and NO_3^- in the solution induced efflux by altering the osmotic potential of roots (Rygiewicz & Bledsoe, 1986). While we attempted to limit disturbance to roots, some physical disturbance during excavation could have contributed to root N efflux (Aslam *et al.*, 1996; Lucash *et al.*, 2008). The greater NH_4^+ efflux in the snow-removal treatment compared to the reference suggests that soil freezing may have further exacerbated this response.

Freezing effects on nitrogen in soil solution

Increased N leaching during the growing season after winters with severe soil frost has been observed in

many forested ecosystems (as reviewed by Blankinship & Hart, 2012; Matzner & Borken, 2008). Results from our study are consistent with these findings, showing significantly higher concentrations of soil water NH_4^+ in the Oa horizon of snow-removal plots compared to the reference plots (Fig. 3a), and significantly higher concentrations of NO_3^- in the B horizon (Fig. 3b). In the reference plots, the low concentrations of NH_4^+ and NO_3^- in the B horizon suggest that the DIN present in the O horizon was retained, resulting in low DIN leachate losses from the B horizon (Table 1). Losses of NO_3^- in the snow-removal plots were greatest in early spring during both years of treatment, and overlapped temporally with reduced uptake of NH_4^+ by trees when roots were provided with near-ambient concentrations of N, suggesting that the NO_3^- response is driven by vegetation.

It is possible that increases in nitrification following soil frost may also contribute to NO_3^- leaching (Gilliam *et al.*, 2010). In our study, the low concentrations of NH_4^+ and high concentrations of NO_3^- in the B horizon of the snow-removal plots suggest that most of the excess NH_4^+ in the Oa horizon was nitrified. This finding is supported by the $\delta^{18}\text{O}\text{-NO}_3^-$ data, which showed that nearly all of the NO_3^- in the snow-removal plots was produced by nitrification. The isotopic data also show that nearly all the NO_3^- in the reference plots was produced by nitrification, indicating that soil freezing had little impact on the percentage of NO_3^- produced by nitrification, rather than coming directly from precipitation. While it is not possible to ascertain relative differences in the quantity of NO_3^- produced by nitrification with these isotopic data, measurements of nitrification in prior snow-removal experiments at the HBEF have shown no significant difference in rates of nitrification between reference and snow-removal plots (Groffman *et al.*, 2001b, 2011).

Our results also show that soil freezing results in an excess flux of DIN leaching from both the Oa and B horizons. The excess flux of DIN was greater during

the first year of treatment compared to the second year in both the Oa and B horizons despite nearly identical amounts of frost during each year (maximum soil frost depth was 37.2 cm in 2009 and 39.5 cm in 2010). It is unclear what caused these differences in DIN flux between years, but may be due to differences in climatic conditions or pretreatment N stores in soil. The total excess DIN flux from the B horizon due to soil freezing (i.e. 1.7 kg N ha⁻¹ in 2009 and 0.7 kg N ha⁻¹ in 2010) was less than the 2.2 kg N ha⁻¹ yr⁻¹ reported by Fitzhugh *et al.* (2001), likely due to factors such as differences in site conditions, tree species composition, and antecedent freezing events. Nevertheless, the N losses are substantial relative to annual DIN inputs in precipitation (5.3 kg N ha⁻¹) and losses in stream water (0.5 kg N ha⁻¹) at the HBEF.

Implications for forest nitrogen retention and loss

Like many other areas, the northeastern United States is projected to experience changes in climate over the 21st Century that include warmer temperatures and longer growing seasons (Hayhoe *et al.*, 2007, 2008). These changes may lead to enhanced plant productivity and function, thereby increasing N uptake. However, shallower snow packs and associated increases in soil freezing events are also expected in the future, which could partially offset these changes. This study provides the first direct measurement of plant N uptake following experimental soil freezing that we are aware of. Our results suggest that soil freezing reduces the rates of N uptake by plants, thus making more N available in the soil to be lost via leaching. It is still unclear how well plants will adapt to repeated soil freezing in the future. The short-term effects of soil freezing have significant implications for the ability of forests of the northeastern United States to retain excess atmospheric N inputs. Our data provide further evidence that climate change-induced soil freezing could significantly limit N uptake, thereby reducing the ability of northern forests to retain N.

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