Soil Freezing Effects on Sources of Nitrogen and Carbon Leached During Snowmelt

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Soil freezing in winter has been shown to enhance growing season losses of C and N in northern forests. However, less is known about effects of soil freezing on C and N retention during snowmelt and the sources of C and N leached, which is important because losses to stream water are greatest during this period. Organic horizon soils (Oi + Oe + Oa) from the Hubbard Brook Experimental Forest in New Hampshire, United States were placed in columns in a laboratory experiment and subjected to one of three different temperature treatments (+5.0, −0.5, and −15.0°C) before they were covered with snow and placed in a +5.0°C cold room to induce snowmelt. Results for all temperature treatments showed that fluxes of all forms of C and N declined over snowmelt, indicating flushing of a limited soil pool. The quality of dissolved organic matter (DOM) became increasingly aromatic, while $\delta^{15}N\text{-}NO_3^-$ declined, indicating that as labile organic matter and N become less available during snowmelt, a greater proportion of N is cycled through the microbial pool. Mild soil freezing had little effect on C and N processing; however, severe soil freezing resulted in delayed leaching and a flush of labile DOM. The severely frozen soils also leached significantly less dissolved inorganic N (DIN; NH_4^+ and NO_3^-), likely due to the inhibitory effect of extremely cold soil temperatures on microbial production. These results highlight the importance of winter climate in regulating fluxes and sources of C and N leached during snowmelt, having implications for stream water quality.

Abbreviations: DIN, dissolved inorganic N; DOC, dissolved organic C; DOM, dissolved organic matter; DON, dissolved organic N; HBEF, Hubbard Brook Experimental Forest; $SUVA_{254}$, specific ultraviolet absorbance measured at a wavelength of 254 nm; SWE, snow water equivalent; TDN, total dissolved N.

In seasonally snow-covered regions, the period of snowmelt is of great hydrological and ecological importance. Much of the annual water and chemical export occurs during this time, having important implications for water quality and nutrient budgets (e.g., Boyer et al., 2000; Sebestyen et al., 2008; Sickman et al., 2003). Soil freezing in winter influences biogeochemical dynamics during snowmelt and into the following growing season by altering both physical and biological controls. Long-term data and shorter term experiments have demonstrated the effects of soil frost on hydrology (Laudon et al., 2004; Shanley and Chalmers, 1999), vegetation (Comerford et al., 2013; Kreyling, 2010), soil fauna (Sulkava and Huhta, 2003; Templer et al., 2012), and decomposition (Christenson et al., 2010; Kreyling et al., 2013). Consequently, changes in soil frost regimes could ultimately affect forest health, soil biodiversity, and water quality.

Recent interest in understanding soil freezing effects stems from the expectation that future changes in climate will alter the temporal patterns and spatial extent of seasonally frozen ground. In the northeastern United States, declines in snowpack depth and duration have been observed, and are projected to continue

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in the future (Burakowski et al., 2008; Campbell et al., 2010; Hayhoe et al., 2007; Hodgkins and Dudley, 2006). Since snow insulates soil, these changes in the snowpack may alter belowground thermal regimes. Most studies indicate that even though climate change results in fewer days with soil frost, the incidence of freezing events may increase because of the shallower and more intermittent snowpack (Brown and DeGaetano, 2011; Campbell et al., 2010; Henry, 2008).

A spate of studies over the last decade has begun to comprehensively address how ecosystems respond to changes in the frequency, duration, and intensity of soil frost (as reviewed by Blankinship and Hart, 2012; Henry, 2007; Matzner and Borken, 2008). Research has shown that C and N are especially responsive to soil freezing since climate-sensitive processes regulate the cycling of these elements (as reviewed by Matzner and Borken, 2008). Past investigations have used multiple research approaches to evaluate how soil freezing influences C and N cycling, including watershed studies (Fitzhugh et al., 2003; Mitchell et al., 1996), climatic gradients (Christopher et al., 2008; Groffman et al., 2009), field manipulations (Blankinship and Hart, 2012; Boutin and Robitaille, 1995; Groffman et al., 2001; Haei et al., 2010), and laboratory experiments (Austnes and Vestgarden, 2008; Elliott and Henry, 2009; Gilliam et al., 2010; Hentschel et al., 2008; Nielsen et al., 2001). The application of these methods across diverse landscapes has led to an improved understanding of the effects of soil freezing on biogeochemical processes.

At the small watershed scale, some evidence indicates that prolonged soil freezing results in elevated concentrations of dissolved organic C (DOC) in streamwater (Edwards et al., 1986; Haei et al., 2010). Similarly, high concentrations of streamwater NO_3^- have been associated with major soil frost events (Fitzhugh et al., 2003; Mitchell et al., 1996; Watmough et al., 2004). However, the relationship between soil freezing and streamwater N and C is not consistently evident, possibly because potential responses are obfuscated by numerous other factors that can affect concentrations, such as the influence of other disturbances, changing flow paths, plant uptake, soil retention, and gaseous efflux (Fitzhugh et al., 2003; Judd et al., 2010). Gradient studies, that utilize natural variation in soil frost across the landscape, have also shown inconsistent responses in N and C losses (e.g., Christopher et al., 2008; Groffman et al., 2010), possibly because of confounding factors that vary across gradients, such as soil properties and tree species composition.

The relationship between soil frost and nutrient leaching is more apparent at the plot scale. Snow manipulation experiments, in which the insulating layer of snow is removed from plots to induce soil freezing, have often shown marked increases in NO_3^- export during the following growing season (Boutin and Robitaille, 1995; Fitzhugh et al., 2001; Hentschel et al., 2009; Kaste et al., 2008). Evidence from the Hubbard Brook Experimental Forest in New Hampshire indicates that increased NO_3^- leaching is at least in part due to a vegetation response, whereby soil freezing damages roots (Cleavitt et al., 2008; Tierney et al., 2001), which in turn reduces inorganic N uptake (Socci, 2012), causing greater leaching losses. Compared to inorganic N, the DOM response is less consistent among snow manipulation experiments. Haei et al. (2010) found that soil solution DOC increased following induced soil frost whereas the response has generally been negligible in other experiments (Austnes et al., 2008; Fitzhugh et al., 2001; Hentschel et al., 2009).

Additional insight into the effects of soil frost on nutrient leaching losses has been obtained from laboratory experiments, with closely controlled environmental conditions. These studies have shown that even without the influence of plants, soil freezing can enhance the release of N and C (Austnes and Vestgarden, 2008; Wang and Bettany, 1993). It has been suggested that enhanced nutrient leaching may be due to physical changes in soil structure (Six et al., 2004; Steinweg et al., 2008) and cellular damage to plant litter and microorganisms (Feng et al., 2007; Schmitt et al., 2008; Skogland et al., 1988). However, responses have been variable, which is not surprising since many of these previous laboratory studies have used soil that has been massively disturbed (e.g., sieving) and have utilized unrealistic freezing treatments (e.g., extreme temperatures and/or more freeze–thaw cycles than possible under natural conditions; Henry, 2007; Lipson et al., 2000).

Despite improved understanding of soil frost effects, we still know little about how it influences sources of C and N, especially during the snowmelt period, which is critically important since nutrient export is typically greatest during this time (Ågren et al., 2010; Rascher et al., 1987; Sebestyen et al., 2008). Additionally, while tree species composition has been shown to strongly control C and N cycling (e.g., Finzi et al., 1998), it is unclear how soil freezing effects are influenced by tree species. Knowledge of species influences on soil freezing effects will enable better predictions across the landscape and improve understanding of future changes that may be expected as a result of possible shifts in forest cover type.

We conducted a laboratory study to evaluate the influence of soil freezing on C and N retention in soil collected from hardwood and coniferous forest stands. Organic forest soils were placed in columns and subjected to one of three different temperature treatments (+5.0, −0.5, and −15.0°C) before being covered with snow and placed in a +5.0°C cold room to induce snowmelt. Great care was taken to minimize artifacts of experimental design identified in previous laboratory studies (Henry, 2007). For example, intact soil cores were used rather than homogenized soil samples; soil cores remained in the field over winter to equilibrate under natural conditions; simulated snowmelt was timed to occur during actual spring snowmelt; and soil was leached with real snowmelt rather than water or chemical extracts.

Prior published results from this laboratory experiment established that total cumulative C and N fluxes (gases + leachates) during snowmelt were significantly lower in the severe soil frost treatment (Reinmann et al., 2012). In all treatments, gaseous efflux of N was low relative to N losses in leachate during snowmelt, and remained low for more than a week after the snow had melted. Gaseous C efflux was also low relative to C losses in leachate during

snowmelt, but increased significantly after the snow had melted. These results show that solute leaching is the dominant pathway for N and C loss from these soils during snowmelt.

The present article evaluates the effect of soil freezing on the sources and flux of DOM and dissolved inorganic N (DIN = NO_3^--N + NH_4^+-N) leached from the soil during snowmelt. Leachate losses were subtracted from snowmelt inputs to determine how much DOM and DIN was retained in the soil. We demonstrate how measurements of DOM quality and stable isotopes of NO_3^- can provide insight into the origin and processing of C and N that cannot be obtained by measuring concentrations or fluxes alone. Improved understanding of how soil freezing influences the interplay between C and N can be used to make predictions about water quality and how it might be affected by changes in climate.

MATERIALS AND METHODS

Site Description

Field sampling was conducted at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of central New Hampshire (43°56′ N lat, 71°45′ W long). Vegetation at the HBEF is dominated by northern hardwoods comprised primarily of American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marshall), and yellow birch (*Betula alleghaniensis* Britton). Red spruce (*Picea rubens* Sarg.) and balsam fir [*Abies balsamea* (L.) Mill.] are the dominant coniferous species and are typically found at higher elevations and on steeper slopes. Soils are predominantly well-drained Spodosols (Typic Haplorthods) with sandy loam textures and a 3- to 15-cm thick organic soil horizon (Oi + Oe + Oa).

The climate is cool, humid, and continental with an average monthly low air temperature of −9°C in January and a high of 18°C in July. Average annual precipitation is 140 cm and is distributed fairly evenly throughout the year. About one-third of the precipitation falls as snow, and a snowpack usually persists from late December until mid-April. The snowpack typically reaches a peak in March, with a long-term (53 yr) average annual maximum depth of 72 cm [19 cm snow water equivalent (SWE); Campbell et al., 2010]. Measureable soil frost (i.e., soil moisture in a frozen state) is present 2 in every 3 yr, depending largely on early winter air temperatures and the timing and amount of snowfall. The long-term average annual maximum frost depth is 6 cm, and the deepest annual frost depth during the 47-yr record of measurement is 26 cm in the year 1993 (Campbell et al., 2010).

Intermittent measurements of soil temperature from 1965–1998 indicate that the long-term mean annual minimum soil temperature measured at depths of 3 (14 yr of data) and 8 cm (26 yr of data) is −0.4 and −0.5°C, respectively (Federer, 1973). The coldest annual soil temperature at depths of 3 and 8 cm is, respectively, −3.8°C in the year 1965 and −3.4°C in the year 1992. These minimum temperature values are likely warmer than the actual values because they are based on measurements made at 1- to 3-wk intervals during the daytime at a low elevation site.

Field Sampling

Thirty soil columns were constructed of 7.6 cm diameter by 55 cm long PVC tubes. A rounded sealed cap was installed at the bottom with a 1-cm hole so that water could drain through them freely. On 11 Nov. 2008, 15 organic soil horizon cores were randomly sampled from a site at the HBEF dominated by red spruce and balsam fir (hereafter referred to as spruce–fir) and 15 others were randomly sampled from a site dominated by sugar maple and American beech (hereafter referred to as maple–beech). Soils were collected from sites with these species because they represent the spruce-fir and northern hardwood cover types that most of northern New England is composed of. Of the 15 cores collected at each site, nine were used for sampling soil leachate (three replicates for each of the three temperature treatments) and three for soil temperature measurements; the remaining three cores were used to determine soil moisture (measured as gravimetric water content as reported in Reinmann et al., 2012). We chose to examine only the organic soil horizon because it is where much of the biological activity occurs and is most prone to freezing. A beveled PVC pipe that was the same diameter as the soil columns (7.6 cm) was used to extract intact organic soil cores. The pipe was hammered into the ground, and the top 5 cm of organic soil was removed and placed into the PVC columns. The columns containing the organic soil were then inserted in the holes in the ground and were left to equilibrate under field conditions over winter to minimize disturbance effects on soil chemistry. One hundred twenty days later, on 3 Mar. 2009, the columns were collected from the field, and the snow that had accumulated in them was removed. The columns were immediately transported in an insulated container to a laboratory facility at the USDA Forest Service in Durham, NH.

Laboratory Procedures

Upon returning to the laboratory, the columns were randomly allocated to one of three open-top, insulated boxes. Each box contained four columns with soil from the spruce–fir site and four from the maple–beech site (eight columns total). Six of the columns (three spruce–fir and three maple–beech) were designated for collecting soil leachate and the other two columns (one spruce–fir and one maple–beech) were designated for monitoring temperature. A hose barb was installed in the bottom of each column, a plug of acid washed polyfill was inserted to prevent clogging, and drain tubes were connected. Once the soil columns were installed in the boxes, they were insulated by filling soil around them to a depth that was consistent with the soil inside the column.

Each of the three boxes containing the columns was placed in a cold room with a soil temperature treatment representing a hard frost (−15.0°C), a mild frost (−0.5°C), or above freezing (+5.0°C). Temperature of air and soil cores in the cold rooms was measured with Type T (copper-constantan) thermocouples. Temperature was scanned at 10-s intervals, and 12-hr averages were calculated for comparison among treatments. The columns were subjected to respective cold treatments for 8 d, which was

the amount of time it took soil in the -15.0°C treatment to reach temperature equilibrium (soil in the -0.5°C and +5.0°C treatments took less than 1 d to reach temperature equilibrium). Once the cores were at the target temperatures, 857 g of snow (857 mL or 19 mm SWE) was added on top of the soil in each of the PVC columns, which is equivalent to the long-term average maximum SWE at the HBEF (Campbell et al., 2010). The snow was sampled randomly from a forested site adjacent to the laboratory, homogenized with a paddle mixer, and weighed to ensure that each column received equal amounts. For insulation, snow was also filled in around each column to the same depth within the column. The boxes were then moved from their respective cold rooms to a central cold room that was maintained at +5.0°C to mimic spring temperatures during snowmelt. The melting snow leached through the soil columns into sample bottles. Three samples of snow were also melted to determine the chemical and isotopic composition of snowmelt before infiltration. Cumulative samples of soil leachate were collected for chemical analyses seven times (Days 2, 3, 4, 6, 8, 9, 11), and snow depth within each PVC column was recorded at the same time.

Laboratory Analyses

Aqueous samples were filtered through precombusted (450°C) glass-fiber filters (0.7 μm nominal pore size) immediately after they were collected. Total dissolved N (TDN), NO_3^-, NH_4^+, DOC, and ultraviolet-visible absorption were measured at the Forest Service Laboratory within several days of collection. Concentrations of DOC and TDN were measured simultaneously using high temperature catalytic oxidation with chemiluminescent N detection (Shimadzu TOC-VCSH/TNM-1 analyzer), NO_3^- with ion chromatography (Metrohm 761), and NH_4^+ with automated colorimetry (SmartChem 200 Discrete Analyzer). Dissolved organic N (DON) was determined as the difference between TDN and DIN. Ultraviolet-visible absorption of DOM was measured on a spectrophotometer (Hitachi U-2010) using a quartz cell with a 1.0-cm path length. Specific ultraviolet absorbance ($SUVA_{254}$) values were determined by dividing the ultraviolet absorbance measured at a wavelength of 254 nm by the DOC concentration. Specific ultraviolet absorbance measured at a wavelength of 254 nm is a good indicator of aromaticity and, therefore, provides insight into the composition and source of DOM (Inamdar et al., 2012; Jaffé et al., 2008; Weishaar et al., 2003). High aromaticity is indicative of plant-derived sources of DOM that tend to be relatively refractory due to their higher lignin content. In contrast, low $SUVA_{254}$ values are indicative of more labile DOM composed of aliphatic, microbially-derived sources (Inamdar et al., 2012; Jaffé et al., 2008). Both inorganic Fe ($Fe^{2+}$and Fe^{3+}) and NO_3^- can absorb light in the near UV, causing possible interference with the UV absorbance of DOC. We did not measure Fe in this study; however, Fuss et al. (2011) found that total inorganic Fe in soil solution of the Oa horizon at the HBEF averaged 2.2 mg Fe L^{-1}, which would cause negligible interference (Weishaar et al., 2003). Similarly, the maximum soil solution NO_3^- concentration measured in this study (1.0 mg N L^{-1}) was far less than concentrations that would cause interference (>9.0 mg N L^{-1}; Weishaar et al., 2003).

Water samples were prepared for stable isotopic analyses of NO_3^- at Boston University using the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001). We incubated 1 mL of leachate overnight in 20-mL headspace vials containing denitrifying bacteria (*Pseudomonas aureofaciens*) that lack active N_2O reductase. Before injecting the samples into the test tubes, 1 mL of antifoam B was added to the vials containing bacteria, which were then crimp-sealed and flushed with N_2 for 3 h. Following 24 h of incubation, five drops (approximately 0.5 mL) of 12M NaOH were added to kill the bacteria, thereby stopping the conversion of NO_3^- to N_2O. The $\delta^{15}N_2{}^{18}O$ gas produced by the bacteria was measured on a SerCon Cryoprep trace gas concentration system interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility. Standard solutions (USGS standard #32, 34, and 35 = KNO_3, KNO_3, and $NaNO_3$, respectively) were processed with the same method as internal standards. Samples were run in increasing mass of N to correct for mass based isotope effects within each batch. Ten percent of samples were run in duplicate, providing an estimate of precision (0.015‰ for $\delta^{15}N$ and 0.0093‰ for $\delta^{18}O$; standard error). Isotopic values were used for samples whose beam areas (Beam 44) on the isotope ratio mass spectrometer were at least 10 times greater than the beam area of the blanks. The minimum detection limit for this method is approximately 10 nmol of N. All the leachate samples in the −15.0°C treatment, and most of the spruce-fir soil leachate samples were below this limit. Consequently, NO_3^- isotopic analyses were primarily restricted to maple–beech soil leachate in the two warmer temperature treatments (i.e., +5.0 and −0.5°C).

To make reporting of isotope ratios more tractable, they are expressed in the typical delta notation (d) in per mil (‰):

$$\delta_{\text{sample}}(‰) = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000$$

where R_{sample} and R_{standard} are the $^{15}N/^{14}N$ or $^{18}O/^{16}O$ ratio of the sample and the standard, respectively. The internationally accepted standards are atmospheric air for N isotopes and Vienna standard mean ocean water for O isotopes.

The soil in the columns was analyzed for total C and N and $\delta^{15}N$ at the end of the experiment. Soil cores were removed from the columns, air-dried, pulverized into a fine powder with a shatterbox, and packed into tin capsules. The samples were combusted with a Costech ECS 4010 elemental analyzer and $^{15}N/^{14}N$ ratios were determined with a DeltaPlus XP mass spectrometer at the University of New Hampshire Stable Isotope Laboratory. The $^{15}N/^{14}N$ ratios of the soil are reported in the standard delta notation described previously. Measurement precision for $\delta^{15}N$ in soil samples was ±0.2‰.

A two-end member mixing model equation was used to estimate the range in the percentage of NO_3^- in leachate derived

from nitrification vs. atmospheric deposition (i.e., snow) based on $\delta^{18}O$ analyses as follows:

$$\%\ \text{contribution from nitrification} = \left(\frac{\delta^{18}O_{\text{leachate}} - \delta^{18}O_{\text{snow}}}{\delta^{18}O_{\text{nitrification}} - \delta^{18}O_{\text{snow}}}\right) \times 1000$$

where $\delta^{18}O_{\text{nitrification}}$ is based on the range of values from published literature (−5 to +15‰; Kendall, 1998; Mayer et al., 2001; Pardo et al., 2004), and $\delta^{18}O_{\text{snow}}$ is the mean of measured values from snow samples collected in this study (+73‰).

Statistical Analyses

Changes in fluxes and forms of N and C, $SUVA_{254}$, and $\delta^{18}O\text{-}NO_3^-$, and $\delta^{15}N\text{-}NO_3^-$ in soil leachate over the course of the experiment were evaluated with a generalized linear mixed model (GLIMMIX procedure in SAS 9.2; SAS Institute, 2008) fitted with residual pseudo-likelihood estimation. Models were evaluated with a log link function and Γ distribution, which yielded the best fit among candidate distributions (Γ, Gaussian, lognormal). We utilized a repeated measures completely randomized factorial design and treated time, temperature treatment, forest type, and associated interactions as fixed effects and soil column replicate as a random effect. To account for the dependence of measurements on time, we selected a spatial power covariance structure, which accommodates data collected at unequally spaced intervals. Denominator degrees of freedom were adjusted using the Kenward–Roger approximation. Post hoc analyses were done using the Tukey–Kramer test, and differences were considered significant at $\alpha = 0.05$. The total amount of N and C leached from the soil by the end of the experiment was also analyzed with a generalized linear mixed model using the same specifications described previously but without the repeated measures component of the model since it is not applicable to these cumulative values.

RESULTS

Physical Measurements

After being placed in cold rooms, soils reached target temperatures in less than 1 d of exposure in the +5.0 and −0.5°C temperature treatments and 8 d in the −15.0°C temperature treatment. The snowmelt simulation experiment began when the soils were simultaneously removed from their respective temperature treatments, covered with snow, and placed in the +5.0°C cold room (indicated as Day 1 in Fig. 1). Soil temperatures changed rapidly on the first day of the experiment, converging near 0°C by Day 2, and then remaining fairly constant through Day 8 (Fig. 1a). On Day 9, soil temperatures in the +5.0 and −0.5°C treatments began to rise and reached equilibrium with cold room air temperature (+5.0°C) by Day 12 whereas soil in the −15.0°C treatment remained near 0°C.

Snow depth declined steadily throughout the experiment in both the +5.0 and −0.5°C treatments until complete ablation on Day 11 (Fig. 1b). Snow depth in the −15.0°C temperature treatment declined concomitantly with snow depth in the other two treatments to Day 4, after which it began to level off due to ponding of partially melted snow on top of the frozen soil. Sometime during the 42-h intervening period between sample collections on Day 8 and 9, the snow in the −15.0°C temperature treatment completely melted and passed through all of the columns. Measurements of snowmelt leachate volume draining from the bottom of the columns (Fig. 1c) reflected patterns in snow depth. Cumulative leachate volume increased steadily throughout the experiment in both the +5.0 and −0.5°C treatments. In the −15.0°C treatment, snowmelt water ponded on the soil surface until Day 8. By the time of sample collection on Day 9, nearly all the snowmelt water in the −15.0°C treatment had drained through the columns. Across all temperature treatments and forest types, an average of 97% (range 94–99%) of the 857 mL SWE added to each column had leached through the soil by the end of the experiment.

Chemical and Isotopic Measurements

During the course of the experiment, the chemical composition of leachate showed consistent changes over time (Fig. 2). In the −15.0°C treatment, $SUVA_{254}$, DOC, DON, NH_4^+,

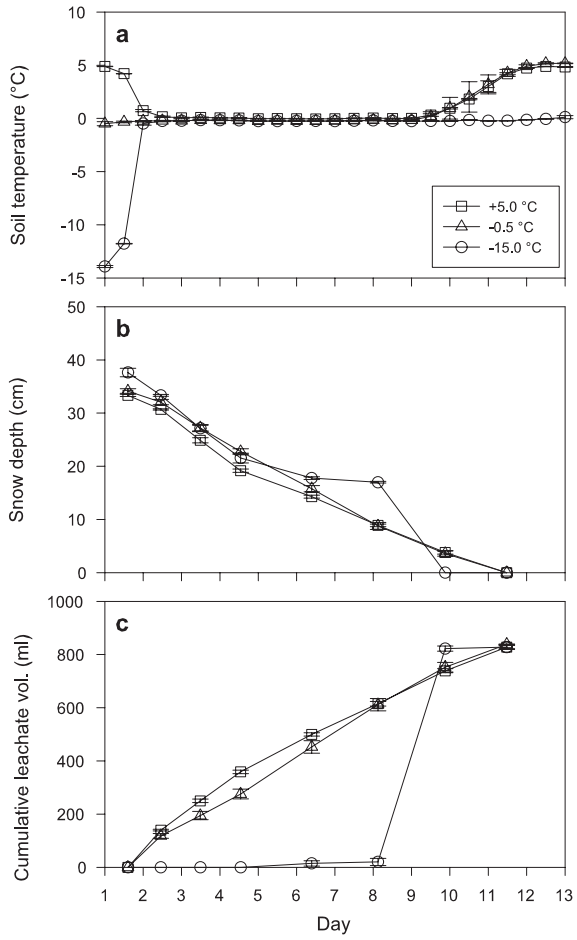

Fig. 1. Soil temperature (a), snow depth (b), and cumulative leachate water volume (c) for each temperature treatment (+5.0, −0.5, or −15.0°C) during the experimental period. Error bars indicate standard error. The starting point at Day 1 is when the soil columns were topped off with snow and placed in the cold room maintained at +5.0°C. Before that, the soils had been subjected to their respective cold treatments for 8 d.

and NO_3^- values were all higher for the samples collected on Day 9 compared to the other sampling periods, which had values of zero due to negligible leaching. In the +5.0 and −0.5°C temperature treatments, $SUVA_{254}$ increased over time in both forest soil types, indicating a shift toward more aromatic DOM. These increases were significant ($p < 0.01$) in all but one case ($p = 0.18$ for spruce–fir, +5.0°C treatment). In contrast, fluxes of DOC, DON, NH_4^+, and NO_3^- declined significantly ($p < 0.02$) throughout the 11-d period.

We found some significant interactions between the main effects of temperature and forest type with time on $SUVA_{254}$, DOC, DON, NO_3^-, and NH_4^+ (Table 1). For $SUVA_{254}$ and NO_3^-, there was a significant three-way temperature by forest type by time interaction ($p < 0.001$ for $SUVA_{254}$ and $p = 0.001$ for NO_3^-). For DOC, all three two-way interactions were

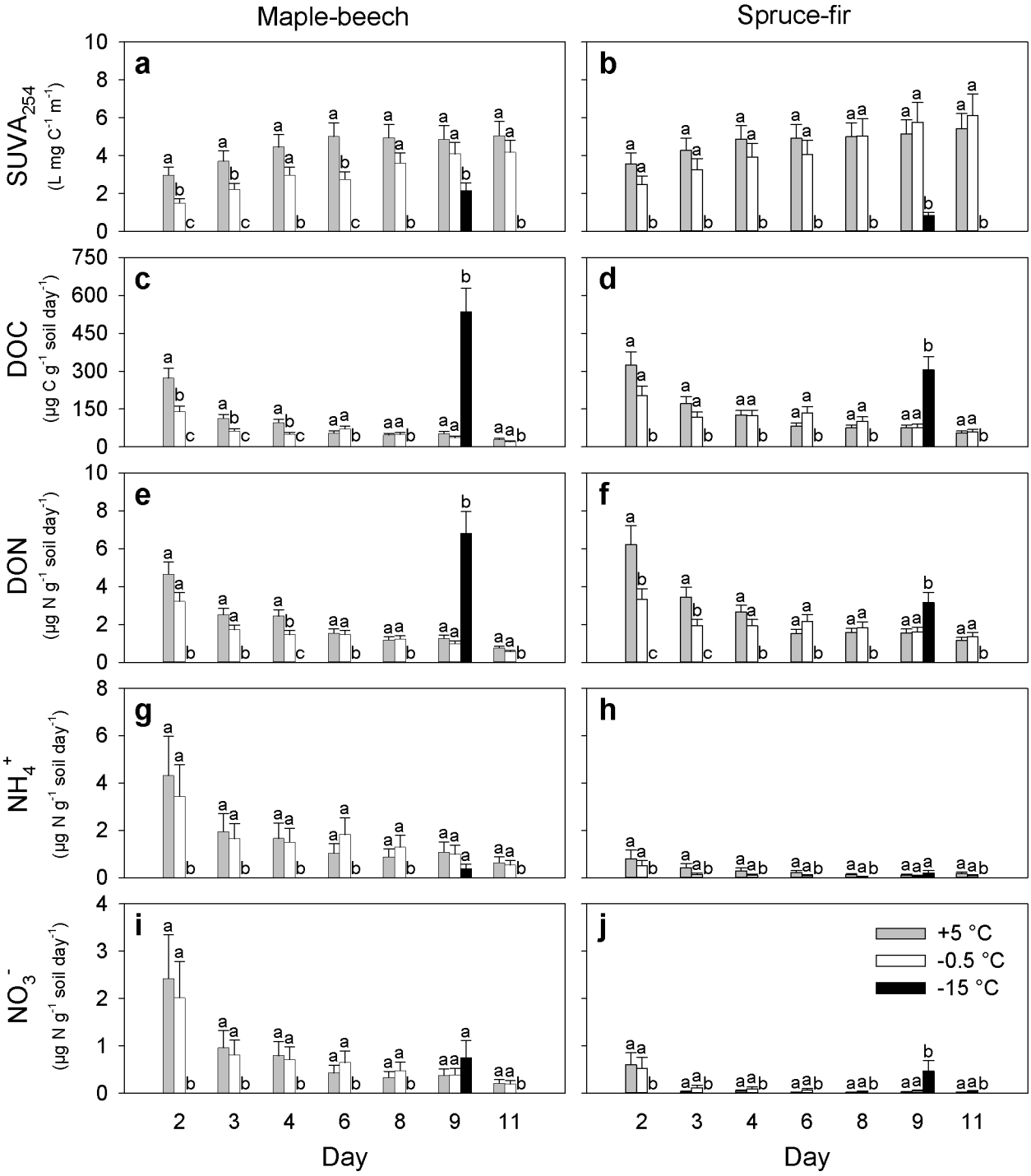

Fig. 2. Time series of soil water specific ultraviolet absorbance measured at a wavelength of 254 nm ($SUVA_{254}$; L mg C^{-1} m^{-1}) and fluxes of dissolved organic C (DOC; µg C g^{-1} soil d^{-1}), dissolved organic N (DON), NH_4^+ and NO_3^- (µg N g^{-1} soil d^{-1}) of soil leachate for maple–beech and spruce–fir under three temperature treatments (+5.0, −0.5, and −15.0°C). Letters indicate significant differences among temperature treatments within each time period. Error bars indicate standard error.

significant ($p = 0.016$ for temperature by forest type, $p < 0.001$ for temperature by time, and $p = 0.020$ for forest type by time). Lastly, there were two significant two-way interactions for DON ($p < 0.001$ for temperature by time and $p = 0.002$ for forest type by time) and NH_4^+ ($p = 0.005$ for temperature by forest type and $p < 0.001$ for temperature by time). In the +5.0 and −0.5°C temperature treatments for both forest types, there were no significant differences in $SUVA_{254}$, DOC, DON, NH_4^+, and NO_3^- within each time interval, except for several instances where the fluxes were greater in the warmer (+5.0°C) temperature treatment (Fig. 2). In the maple–beech forest type, $SUVA_{254}$ was significantly higher in the +5.0°C temperature treatment compared to the −0.5°C temperature treatment for three out of the four initial sampling periods (Day 2, 3, and 6). Fluxes of DOC were also significantly higher in +5.0°C temperature treatment for the maple–beech forest type at the beginning of the experiment (Days 2, 3, and 4). Fluxes of DON were higher in the −0.5°C temperature treatment on Day 4 of the maple-beech forest type and on Days 2 and 3 in the spruce–fir forest type.

Isotopic results are limited to the two warmer temperature treatments in the maple–beech soil type because these were the only samples that had sufficient NO_3^- concentrations for isotopic analyses. In the maple–beech soil, $\delta^{18}O$-NO_3^- in leachate showed no significant difference between the +5.0 and −0.5°C temperature treatments ($F = 0.37$, $p = 0.60$), no detectable change in $\delta^{18}O$-NO_3^- over time ($F = 1.13$, $p = 0.38$), and no temperature treatment by time interaction ($F = 0.16$, $p = 0.98$; Fig. 3a). Soil solution $\delta^{18}O$-NO_3^- at the beginning of the experiment was highly variable (e.g., range of 0 to +55‰ for Day 2) compared to the latter stages of the experiment (e.g., range of +4 to +15‰ for Day 11). Throughout the experiment, the range of $\delta^{18}O$-NO_3^- values in leachate were below the range measured in the snowpack (+69 to +80‰) and were above or within the range of published $\delta^{18}O$-NO_3^- values produced by nitrification (−5 to +15 ‰; Kendall, 1998; Mayer et al., 2001; Pardo et al., 2004).

The two end-member mixing model analysis indicated that NO_3^- leached in the two warmer treatments was predominantly from nitrification rather than directly from the melting snow. We estimate that on the initial sampling date (Day 2) for the +5.0 and −0.5°C temperature treatments, respectively, 59–79% and 72–96% of the NO_3^- in leachate came from nitrification. By the last sampling date on Day 11, the contribution of NO_3^- from nitrification was 80–100% and 84–100% in the +5.0 and −0.5°C treatments, respectively.

As with soil solution $\delta^{18}O$-NO_3^-, $\delta^{15}N$-NO_3^- showed no significant difference between the warmer two temperature treatments ($F = 0.00$, $p = 0.96$) or interactive effect of temperature and time ($F = 1.35$, $p = 0.28$). The main effect of time was significant ($F = 9.39$, $p < 0.001$), with $\delta^{15}N$-NO_3^- decreasing as snowmelt progressed. At the beginning of the experiment, $\delta^{15}N$-NO_3^- was similar to the range measured in soil (+2 to +5‰) and snowpack (-1 to 0‰) and declined below the range thereafter.

We compared the initial C and N content in snow at the beginning of the experiment with the amount measured in leachate at the end of the experiment to evaluate patterns of retention and loss (Fig. 4). Total cumulative leachate losses of DOC and DON greatly exceeded inputs in snow in all temperature treatments for

Table 1. Results of a generalized linear mixed model to evaluate the effects of temperature, forest type, and time on soil water specific ultraviolet absorbance measured at a wavelength of 254 nm ($SUVA_{254}$; L mg C^{-1} m^{-1}), dissolved organic C (DOC; μg C g^{-1} soil d^{-1}), dissolved organic N (DON), NH_4^+, and NO_3^-(μg N g^{-1} soil d^{-1}).

Effect	df	F	$p > F$
$SUVA_{254}$			
Temperature	2	3335.3	<0.001
Forest type	1	0.7	0.432
Time	6	2137.3	<0.001
Temperature by forest type	2	1.9	0.221
Temperature by time	12	1930.6	<0.001
Forest type by time	6	4.7	0.001
Temperature by forest type by time	12	4.1	<0.001
DOC			
Temperature	2	6848.5	<0.001
Forest type	1	14.8	0.003
Time	6	2354.4	<0.001
Temperature by forest type	2	6.2	0.016
Temperature by time	12	2023.6	<0.001
Forest type by time	6	2.8	0.020
Temperature by forest type by time	12	1.0	0.446
DON			
Temperature	2	3552.6	<0.001
Forest type	1	3.3	0.101
Time	6	1422.1	<0.001
Temperature by forest type	2	2.3	0.155
Temperature by time	12	1189.5	<0.001
Forest type by time	6	4.1	0.002
Temperature by forest type by time	12	1.8	0.081
NH_4^+			
Temperature	2	502.2	<0.001
Forest type	1	43.7	<0.001
Time	6	110.0	<0.001
Temperature by forest type	2	10.5	0.005
Temperature by time	12	94.1	<0.001
Forest type by time	6	2.0	0.083
Temperature by forest type by time	12	0.6	0.823
NO_3^-			
Temperature	2	228.9	<0.001
Forest type	1	37.2	<0.001
Time	6	402.5	<0.001
Temperature by forest type	2	7.3	0.012
Temperature by time	12	276.8	<0.001
Forest type by time	6	6.7	<0.001
Temperature by forest type by time	12	3.6	0.001

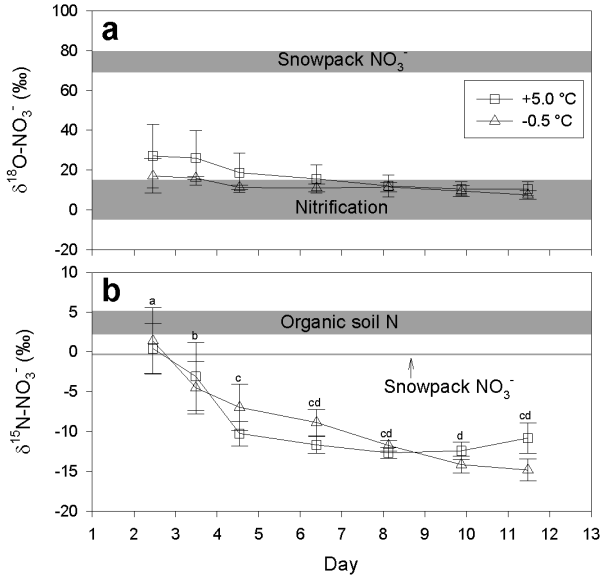

Fig. 3. Time series of $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$ in maple–beech soil leachate under two temperature treatments (+5.0 and −0.5°C). The shaded areas show the range of (a) $\delta^{18}O\text{-}NO_3^-$ measured in the snowpack in this study and literature values for NO_3^- produced by microbial nitrification, and (b) $\delta^{15}N\text{-}NO_3^-$ measured in the snowpack and $\delta^{15}N$ measured in maple–beech soil. Letters denote significant (α = 0.05) changes in $\delta^{15}N\text{-}NO_3^-$ over time for both temperature treatments.

both forest types (Fig. 4a and 4b). Net loss of DOC and DON ranged from −2120 to −1000% and −1940 to −390%, respectively. For DOC, there were no significant differences in leachate losses across treatments and forest types (Fig. 4a). Losses of DON from the spruce–fir soil subjected to the −15.0°C treatment were significantly lower than losses from the +5.0 and −0.5°C treatments for both forest types but were not significantly different than losses from the maple–beech soil subjected to the −15.0°C treatment (Fig. 4b).

In general, the inorganic forms of N in snow exceeded leachate losses in all spruce–fir soil treatments and in the −15.0°C temperature treatment of the maple–beech soil (Fig. 4c, 4d, and 4e). In these treatments, 52–75% of the DIN in snow was retained whereas the warmer two temperature treatments of the maple–beech forest type showed a −506 to −571% net loss of DIN. Leachate losses of DIN were significantly greater in the +5.0 and −0.5°C temperature treatments for the maple–beech soil compared to the other treatments. In all cases, there was a net loss of TDN (DON + DIN) that ranged from −20 to −880%, with the greatest net losses in the warmer two temperature treatments (Fig. 4f).

DISCUSSION

Sources of DOM in Leachate

In all treatments, the amount of DOC and DON measured in leachate far exceeded the initial content in snow. These results show that DOC and DON in melting snow could only account for a small fraction of the organic matter export in leachate, suggesting that nearly all of the DOM was produced in the soil. We used measurements of DOM quality, as indicated by $SUVA_{254}$ of soil leachate, to evaluate the effect of soil freezing on sources of DOC and DON during snowmelt. The snow placed on top of the soil at the beginning of the experiment had low DOC concentrations (0.8–1.6 mg L^{-1}), and the absorbance was undetectable as expected with DOM from an atmospheric source whereas the $SUVA_{254}$ of soil leachate was more aromatic and, thus, characteristic of a soil-derived source.

Soil leachate in the +5.0 and −0.5°C treatments showed steady increases in $SUVA_{254}$ during snowmelt, while DOC and DON concentrations declined, suggesting flushing of a more labile pool of organic C that became increasingly more aromatic as snowmelt progressed. In the two warmer temperature treatments, the decline in DOC during snowmelt suggests that a limited amount of DOC was flushed from the soil. This DOC was leached more readily than could be replaced through mineralization, resulting in a gradual decline in fluxes over time. Other factors, such as sorption and microbial processes, may have influenced this trend as well.

The $SUVA_{254}$ values in the −15.0°C treatment were among the lowest measured in our study, indicating that severely frozen soil is a source of less aromatic and likely more labile DOM. Previous soil frost studies have shown a similar soil freezing effect on $SUVA_{254}$ and attributed it to frost-induced cell lysis of plant material and soil microfauna, which releases more bioavailable DOC (Austnes and Vestgarden, 2008; Austnes et al., 2008; Haei et al., 2012). Our results imply that severe soil freezing results in the mobilization of predominantly labile C in forest soils during snowmelt and substantially less aromatic soil-derived C relative to unfrozen or mildly frozen soil. Even though the amount of DOC leached in the −15.0°C treatment was not significantly different than the total cumulative amount leached over the entire snowmelt period in the +5.0 and −0.5°C treatments, it occurred a week later as a single pulse (Fig. 2c and 4a). These results suggest that severe soil freezing can result in the delayed release of a relatively large quantity of labile DOC, potentially having important implications for biotic cycling and water quality.

Sources of Inorganic N in Leachate

In the two warmest temperature treatments of both forest types, fluxes of inorganic N declined over time. Much like organic matter fluxes, these declines in DIN suggest flushing of a limited supply of N. The natural abundance of $^{18}O\text{-}NO_3^-$ in maple-beech soil leachate for the warmer two temperature treatments indicated that throughout the snowmelt period most of the NO_3^- came from nitrification rather than directly from snowmelt. The lack of difference in natural abundance $\delta^{18}O\text{-}NO_3^-$ values between the two temperature treatments evaluated (+5.0 and −0.5°C) is consistent with the flux of NO_3^-, which also showed no significant differences between these two treatments. Unfortunately, the low concentrations of NO_3^- in the −15.0°C treatment precluded isotopic evaluation of the influence of severe soil freezing on NO_3^- sources. Similarly, it was not possible to compare isotopic values between forest types throughout the study because of the low NO_3^- concentrations in spruce–fir soil

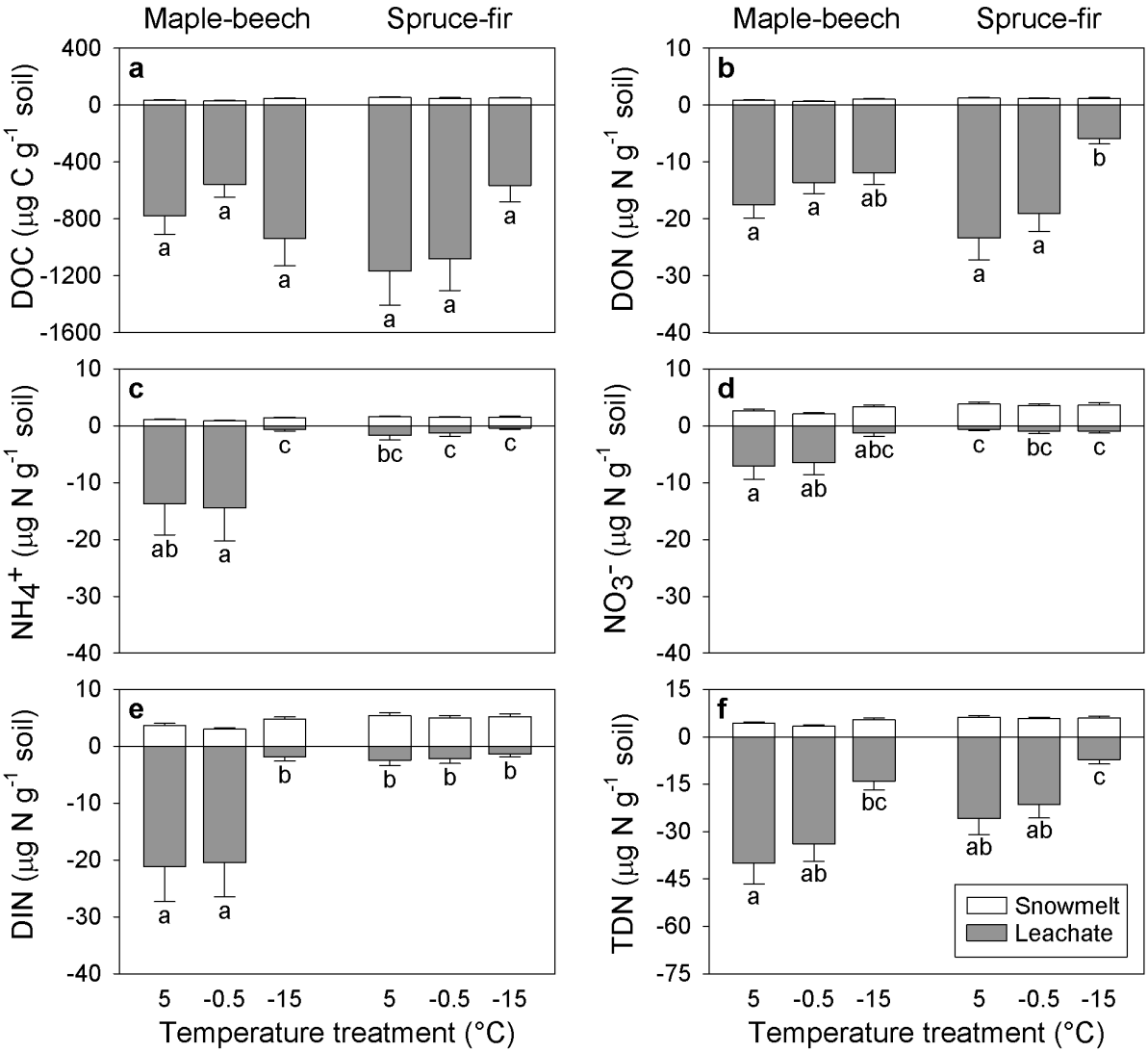

Fig. 4. Snowmelt inputs and leachate losses of dissolved organic C (DOC; μg C g^{-1} soil), dissolved organic N (DON), NH_4^+, NO_3^-, dissolved inorganic N (DIN), and total dissolved N (TDN; μg N g^{-1} soil) from maple–beech and spruce–fir soil under three temperature treatments (+5.0, −0.5, and −15.0°C). Error bars indicate standard error. Leachate losses with the same letter are not significantly different (α = 0.05).

leachate. However, two individual samples of spruce–fir leachate collected in the +5.0°C treatment on Day 2 and the −0.5°C treatment on Day 3 had concentrations that were adequate for isotopic analyses, and like the maple–beech soil leachate, indicated that most of the NO_3^- (>88%) was from nitrification rather than directly from snowmelt. These results suggest that most of the atmospheric NO_3^- stored in the snowpack cycles through the biota before it is leached from soil. This finding is consistent with studies that have shown that streamwater NO_3^- during snowmelt is derived primarily from nitrification, rather than directly from the melting snowpack (Kendall et al., 1996; Pardo et al., 2004; Piatek et al., 2005).

Similar to $\delta^{18}O\text{-}NO_3^-$, the lack of difference in $\delta^{15}N\text{-}NO_3^-$ between the two warmest temperature treatments (+5.0 and −0.5°C) suggests similar N cycling processes in these two treatments. However, unlike $\delta^{18}O\text{-}NO_3^-$, $\delta^{15}N\text{-}NO_3^-$ decreased significantly over time in both treatments. During nitrification, oxygen from ambient water and gas are incorporated into NO_3^-; therefore, the change in the $\delta^{18}O\text{-}NO_3^-$ is immediate and does not show continued depletion with additional biological cycling. In contrast, $\delta^{15}N\text{-}NO_3^-$ can show continued incremental depletion of $\delta^{15}N$ due to fractionation during biological cycling. Thus, nitrification produces NO_3^- with a lower $\delta^{15}N$ than the $\delta^{15}N$ of the soil or NH_4^+ from which it is derived. This effect can be offset by denitrification, which produces gases that are depleted in $\delta^{15}N$, causing $\delta^{15}N$ enrichment of the residual soil water NO_3^-. However, the companion study by Reinmann et al. (2012) found that denitrification (measured as N_2O efflux) was negligible throughout the period of snowmelt, suggesting that fractionation due to denitrification is unlikely. Consequently, these isotopic values suggest that the depletion of $\delta^{15}N\text{-}NO_3^-$ in leachate that occurred over the snowmelt period is likely a result of enhanced microbial N cycling with time. Even though we were unable to use isotopic analyses to evaluate the effect of severe soil freezing on NO_3^- sources, these results indicate that microbial

cycling of N influences NO_3^- retention during the snowmelt period, but appears to be unaffected by mild soil freezing.

A comparison of inputs of DIN in snowmelt to outputs in leachate indicated that the maple–beech soil exposed to the warmer two temperature treatments was a net source of DIN, and that the severely frozen maple–beech soil and all treatments of the spruce–fir soil were a net sink for DIN. The significantly greater leaching losses of DIN ($NO_3^- + NH_4^+$) from the maple–beech soil in the two warmer temperature treatments (−0.5 and +5.0°C) suggests that the maple–beech forest type is more sensitive to soil temperature than the spruce–fir, which showed no significant differences in DIN among temperature treatments. Concentrations of NO_3^- and NH_4^+ in spruce–fir soil solution remained low throughout the entire experimental period, which may have hampered detection of a temperature treatment response. Greater rates of N cycling and loss among maple–beech forests compared to spruce–fir forests have been attributed to factors such as soil moisture, temperature, pH, litter quality, and biotic controls related to competition between plants and microbes (Booth et al., 2005; Lovett et al., 2004; Templer et al., 2003). The higher net DIN losses in the maple–beech forest type in our study are characteristic of soil from sugar maple stands, which tend to have high rates of nitrification and NO_3^- leaching compared to other forest types (e.g., Finzi et al., 1998; Lovett and Mitchell, 2004; Templer et al., 2005). These factors contributed to the low N retention in the sugar maple forest soils, causing them to be a net source of DIN, compared to the much greater N retention in the spruce–fir forest soils, which are a net sink of DIN.

The effect of soil temperature on DIN losses varied among treatments within the soil of maple–beech; with the two warmer temperature treatments having leached similar amounts of NH_4^+ and NO_3^- even though one was frozen at the beginning of the experiment and the other was not. The soil temperatures of all three treatments were close to 0°C after the snow was placed on top of the soil, and remained relatively constant from Day 3 through Day 8. The range in temperatures across treatments during this period (−0.31 to −0.66°C) was within the accuracy of the thermocouples (±0.7°C), which hampered the ability to determine whether the soil was frozen based on temperature measurements alone. However, the soil temperature data clearly indicated that the soil in the +5.0 and −0.5°C temperature treatments differed at the beginning of the experiment and warmed at the same rate after the insulating layer of snow melted, suggesting that the soil in the −0.5°C temperature treatment thawed during the study. Leachate volume data also indicate that the soil in the −0.5°C treatment thawed because the water leached at similar rates as the +5.0°C treatment. Thus, similarities in temperature in the warmer two treatments may have resulted in similar amounts of DIN losses.

In the −15.0°C treatment, the lack of snowmelt infiltration due to frozen soil caused ponding to occur. Temperature measurements in the −15.0°C treatment indicated that the soil remained frozen even after the snow melted; suggesting that by Day 9, portions of the soil thawed enough to allow all the ponded snowmelt water to pass through the partially frozen soil. Since some of the soil in the −15.0°C temperature treatment remained frozen, it is plausible that freezing limited N mineralization and nitrification (Schimel et al., 2004; Zhao et al., 2010), resulting in lower cumulative N losses relative to the other two temperature treatments (Fig. 4). It is also possible that nitrification in the unfrozen portions of soil was limited by a lack of available oxygen due to the presence of ponded water on the soil surface (Öquist et al., 2007). However, the companion study by Reinmann et al. (2012) showed that N_2O efflux was negligible across treatments and was not significantly greater in the −15.0°C treatment, indicating that even in these oxygen depleted soils, denitrification was likely an unimportant pathway for N loss.

CONCLUSIONS

Results from this study show that in the mild frost and no frost temperature treatments, fluxes of C and N declined during the snowmelt period, suggesting flushing of a limited supply of these two elements. As the quantity of DOC declined, the quality also changed, becoming increasingly aromatic over time. As DOC became more recalcitrant and hence less bioavailable, all forms of N declined (i.e., NH_4^+, NO_3^-, and DON). The concomitant depletion in $\delta^{15}N\text{-}NO_3^-$ in leachate suggests that as the availability of N declines, biotic cycling of N becomes tighter, resulting in the depletion of $\delta^{15}N\text{-}NO_3^-$. These results demonstrate how changes in the quantity and quality of C influence N availability and loss during snowmelt.

Mild soil freezing had little effect on the source and amount of C and N leached. In contrast, severe soil freezing strongly influenced the timing, quality, and quantity of C and N leached. It appears that severe soil freezing releases DOC with low aromaticity, possibly derived from freezing-induced cell lysis, which is a potential source of labile C. Additionally, following severe soil freezing, the DOC released during snowmelt occurs as a pulse and is delayed, which could have implications for biotic cycling and water quality. Cumulative DIN losses from the severely frozen soils were low relative to the mildly frozen and unfrozen soils, likely due to the effect of frozen soil on microbial N production.

Results from this laboratory experiment indicate that soil freezing strongly regulates C and N production, transport, and export during snowmelt, having important implications for water quality and ecosystem budgets. Future studies should evaluate whether these patterns, which were observed in a controlled laboratory environment, occur under field conditions. A better understanding of these soil freezing effects will aid in making predictions about how C and N export will change in response to changes in climate.

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