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Severe soil frost reduces losses of carbon and nitrogen from the forest floor during simulated snowmelt: A laboratory experiment

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ABSTRACT

Considerable progress has been made in understanding the impacts of soil frost on carbon (C) and nitrogen (N) cycling, but the effects of soil frost on C and N fluxes during snowmelt remain poorly understood. We conducted a laboratory experiment to determine the effects of soil frost on C and N fluxes from forest floor soils during snowmelt. Soil cores were collected from a sugar maple (*Acer saccharum*)–American beech (*Fagus grandifolia*) and a red spruce (*Picea rubens*)–balsam fir (*Abies balsamea*) forest at the Hubbard Brook Experimental Forest in New Hampshire, U.S.A. Soils were exposed to one of three temperature treatments, including severe (−15 °C), mild (−0.5 °C), and no soil frost (+5 °C) conditions. After one week the soils were incubated at +5 °C and snow was placed on top of the soils to simulate spring snowmelt. NO_3^- losses were up to 5.5 mg N kg^{-1} soil greater in the mild soil frost treatment than the severe soil frost treatment. Net losses of NH_4^+ and DON in leachate were up to 19 and 18 mg N kg^{-1} soil greater in the no soil frost and mild soil frost treatments, respectively, than the severe soil frost treatment. In contrast, soil frost did not have a significant impact on dissolved organic C or cumulative gaseous fluxes of C and N throughout the snowmelt period. However, the total cumulative flux of C (i.e. dissolved organic C + CO_2 + CH_4) and N (i.e. dissolved organic N + NH_4 + NO_3 + N_2O) in the severe soil frost treatment were between one quarter and one half that observed in the no soil frost treatment for both forest types. Together, the results of this study show that total fluxes of N in leachate, as well as total cumulative C and N fluxes (gases + leachate), were significantly reduced following severe soil frost. We conclude that the extent to which C and N cycling during snowmelt is altered in response to changes in winter climate depend on both the presence and severity of soil frost.

1. Introduction

Biological processes during winter in seasonally snow-covered ecosystems have been increasingly recognized for their contribution to annual fluxes of carbon (C) and nitrogen (N; Groffman et al., 2006; Monson et al., 2006; Judd et al., 2007; Filippa et al., 2009). The snow pack in winter insulates soil from below-freezing air temperatures, which facilitates a significant amount of biological activity (Groffman et al., 2001; Campbell et al., 2005). Northern forest ecosystems typically experience a deep and persistent snow pack, but future changes in climate may alter snow pack dynamics and thus winter soil temperatures. Climate projections for the northeastern U.S. indicate that annual air temperatures will increase by 2.9–5.3 °C during the 21st century accompanied by a 12–30% increase in winter precipitation with a higher ratio of rain to snow than what is currently observed (Hayhoe et al., 2007). These changes in climate are expected to decrease the depth and duration of the winter snow pack. Snow removal experiments indicate an inverse relationship between snow depth and soil frost (Boutin and Robitaille, 1994; Groffman et al., 2001; Decker et al., 2003). However, analyses of historical data suggest that warmer winters could result in either an increase or decrease in the frequency of soil frost (Lindström et al., 2002; Zhao et al., 2004; Henry, 2008). Models that incorporate soil frost dynamics predict that warmer winters may result in fewer days with soil frost (Venäläinen et al., 2001; Campbell et al., 2010), although mid-winter soil frost may become more common (Venäläinen et al., 2001). Because changes in winter climate are likely to alter belowground temperature regimes, it is important to understand how changes in soil frost dynamics could impact forest processes such as C and N cycling.

Considerable progress has been made in recent years in understanding the potential response of C and N cycling to changes in soil frost. For example, studies show that soil frost results in increased losses of inorganic N at the plot scale (Fitzhugh et al., 2001; Groffman et al., 2006; Callesen et al., 2007; Goldberg et al., 2010), but no clear link has been made between soil frost and watershed export of inorganic N (Fitzhugh et al., 2003). Soil frost

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has been shown to have either no effect on annual CO_2 fluxes (Matzner and Borken, 2008; Groffman et al., 2006) or a reduction in growing season CO_2 fluxes (Muhr et al., 2009). Soil frost may enhance stabilization and retention of soil organic matter (SOM; Matzner and Borken, 2008; Schmitt et al., 2008; Steinweg et al., 2008). Impacts of soil frost on dissolved organic C (DOC) and N (DON) are complex with variable results (Austnes et al., 2008; Austnes and Vestgarden, 2008). For example, soil frost manipulation experiments have shown that severe soil frost (<−8 °C) increases DOC losses in leachate upon thawing of frozen soils (Hentschel et al., 2008), while mild soil frost (>−5 °C) has no impact on losses of DOC in leachate (Fitzhugh et al., 2001; Hentschel et al., 2009). A positive correlation has been observed between soil frost severity and concentrations of soil solution DOC during the growing season (Haei et al., 2010).

In regions with a persistent snow pack, spring snowmelt can be a particularly important time of year for C and N fluxes. During this period solutes such as NO_3^- and DOC that accumulated in the snow pack and soil over the winter are flushed (Rascher et al., 1987) and, coupled with rising soil temperatures, may stimulate microbial biomass production (Brooks et al., 1998), mineralization, nitrification (Rascher et al., 1987), and losses of CO_2 (Monson et al., 2006; Pacific et al., 2008) and N_2O (Goodroad and Keeney, 1984; Maljanen et al., 2007; Desjardins et al., 2010; Goldberg et al., 2010) during the spring snowmelt period. The N and DOC mobilized during snowmelt can make important contributions to the annual stream water export of these elements from northern forest ecosystems (Årgen et al., 2008; Chirstopher et al., 2008; Sebestyen et al., 2008). For example, while soil retention of NO_3^- during the winter can be high (96% of combined deposition and soil production; Judd et al., 2007), Likens and Bormann (1995) found that NO_3^- export during spring snowmelt accounted for 69% of the annual export from a northern New England watershed.

Despite the advances made in our understanding of the relationships between winter climate and C and N cycling, relatively little is known about the effects of soil frost on C and N fluxes in soils during the dynamic and temporally variable spring snowmelt period (Groffman et al., 2006; Hentschel et al., 2009; Muhr et al., 2009). Laboratory experiments provide an opportunity to control environmental variables such as soil temperature, while facilitating frequent sampling, but we are not aware of any controlled laboratory studies that have examined the impacts of soil frost on C and N fluxes during snowmelt. Therefore, we conducted a laboratory experiment to simulate a spring snowmelt after exposing organic forest soils to one of three temperature treatments. Since forest composition can play an important role in C and N cycling (Janssens et al., 1999; Buchman, 2000; Fahey et al., 2005; Templer et al., 2005; Chirstopher et al., 2008) we used soils collected from two dominant forest types at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire, U.S.A. to compare their responses to changes in soil frost. We hypothesized that (1) total losses of C and N (leachate + gaseous losses) would be greatest in the severe soil frost (−15 °C) treatment, intermediate in the mild soil frost (−0.5 °C) treatment, and smallest in the treatment without soil frost (+5 °C), (2) there would be greater fluxes of C and N from the hardwood forest soils compared to the conifer forest soils, and (3) gaseous losses of C and N would increase throughout the snowmelt period as soil temperatures increase.

2. Methods

2.1. Field sampling

This study was conducted using soils from HBEF that were collected from two common northern forests; a low elevation (500 m) hardwood forest dominated by sugar maple (*Acer saccharum* Marsh.) and American beech (*Fagus grandifolia* Ehrh.) and a high elevation (1000 m) conifer forest dominated by red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* [L.] Mill). Soils at HBEF are well-drained, base-poor spodosols with a mor organic horizon that averages 7 cm thick (Johnson et al., 1997). The climate is cool, humid, and continental with a continuous winter snow pack that typically lasts from December to mid-April. The average soil temperature in the organic horizon during winter is ~0 °C.

In November 2008, soil cores (7.6 cm diameter; 5 cm depth) were excavated from the organic horizon ($O_i + O_e + O_a$) in one stand from each of the two forest types ($n = 15$ cores for each forest type). Following excavation, soil from each core was placed in a PVC pipe (55 cm height; 7.6 cm diameter; hereafter referred to as 'PVC column') that was fitted at the bottom with a slip cap. Each PVC column was placed vertically in the soil in the same location where its respective sample was excavated. The slip cap had a 1 cm diameter hole drilled at the bottom to allow water to drain freely from each PVC column. Each PVC column was left *in situ* to allow the soils to equilibrate in the PVC columns prior to harvesting in March 2009.

2.2. Experimental treatments

Upon removal from the field, the PVC columns were transported to a laboratory where soils from three of the columns from each forest type were removed to determine soil moisture content (see Section 2.5 for details). The remaining 12 PVC columns from each forest type were exposed to one of three temperature treatments ($n = 4$ PVC columns for each temperature treatment for each forest type). These treatments included severe (−15 °C), mild (−0.5 °C), and no soil frost (+5 °C) conditions. Within each temperature treatment, the four PVC columns per forest type were placed in open-top bins that were surrounded by foam insulation to enable top-down changes in soil temperature similar to field conditions. A layer of organic soil filled the space around each PVC column to a depth that was consistent with the top of the soils within each PVC column. Following one week of treatment, all of the columns were moved into a +5 °C refrigerator and 857 g of snow (equivalent to 35 cm snow pack depth) was placed on top of the soil in each PVC column to simulate spring snowmelt. Snow was also added around the PVC columns to the same depth as the snow within the columns to create an insulating snow pack.

2.3. Gas sampling during laboratory experiment

Fluxes of CH_4, CO_2, and N_2O from soils and snow were quantified using the closed static chamber method (Lundegårdh, 1927). Headspace air samples were collected from each PVC column during the following times: immediately after snow was placed on the soil surface on March 11, 2009 (i.e. the start of the snowmelt; Day 0), after one-third of the snow had melted (Day 3), after two-thirds of the snow had melted (Day 6), immediately after all of the snow had melted (Day 10), and 13 and 19 days after the start of snowmelt (Days 13 and 19, respectively). Gases were sampled by placing caps over the PVC columns to create a closed chamber. Four 40 mL samples were collected during a 1 h incubation (0, 20, 40, and 60 min) using a polyethylene syringe and needle that was inserted into the chamber through a septum. After collecting each sample, 10 mL were ejected into the air and the remaining 30 mL were injected into evacuated 20 mL glass vials and stored at room temperature prior to analysis by gas chromatography on a Shimadzu GC-2014. CH_4, CO_2, and N_2O were measured with flame ionization, thermal conductivity, and electron capture detectors, respectively.

Headspace was quantified as the volume between the top of the snow pack and the top of the chamber; headspace volume therefore

varied as the snow melted, but was taken into account. Trace gas fluxes were calculated based on the linear rate of change in the mass of each gas in the chamber. Fluxes were not corrected for temperature and pressure at the time of sampling since these variables did not vary among treatments or throughout the experiment. Regressions that were not significant were included to avoid any biases that could be caused by setting non-significant regressions to zero (*sensu* Groffman et al., 2010). The cumulative flux of each gaseous compound throughout the experimental period was calculated by summing the estimated gas flux of each compound between sampling periods. The gas flux between sampling periods was calculated by multiplying the mean flux of two consecutive sampling periods by the time that elapsed between them.

The closed chamber method is commonly used to measure fluxes of multiple gases, but it can create artifacts that need to be considered when calculating fluxes. These artifacts include the disruption of natural pressure gradients across the soil–atmosphere interface (Hutchinson and Mosier, 1981; Livingston et al., 2006) and possible reductions in headspace volume when air samples are removed. We used a micromanometer (Infiltec DM-4) with an independent set of chambers to quantify differences in pressure between inside and outside the chamber. Our test results indicate that capping the chamber produced a short-lived internal pressure change that may have affected our T_0 (0 min) sample, but was alleviated before collection of the T_1 (20 min) sample. T_0 samples were, therefore, omitted from analyses and only data from the remaining three sampling times were included. Also, when soil temperatures were less than 0 °C, each air sample that was removed resulted in an internal pressure change that was not completely alleviated before the next air sample was collected, which effectively reduced the headspace volume. We therefore corrected the headspace volume based on soil temperature.

2.4. Leachate and snow sampling during laboratory experiment

Aqueous losses of C and N were measured by capturing leachate that drained from the hole at the bottom of each PVC column through tygon tubing and into a sample bottle located below the bin. Leachate samples were collected throughout the snowmelt period. We determined the amount of aqueous C and N in snow by melting three samples that were equivalent to the amount of snow we placed in each of the PVC columns (857 g).

Leachate and snow samples were filtered through pre-combusted (450 °C) glass-fiber filters (0.7 μm pore size) immediately after they were collected and stored at 4 °C until they were analyzed for DOC, total dissolved nitrogen (TDN), NO_3^-, and NH_4^+ (14 days maximum). 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). DON was determined as the difference between TDN and dissolved inorganic nitrogen (DIN = NO_3^-–N + NH_4^+–N). Leakage from a subset of lysimeters prevented complete recovery of leachate from some of the PVC columns, resulting in a sample size equal to two for total leachate fluxes for the three temperature treatments for red spruce–balsam fir and the severe soil frost temperature treatment for sugar maple–American beech.

2.5. Soil analyses

After the laboratory experiment, inorganic N was extracted from a 10 g subsample of soil from each PVC column ($n = 9$ from each forest type) with 60 mL of 2 M KCl. Soils with KCl were placed on a shaker table for 30 min and then filtered through a pre-rinsed Whatman 42 filter. The solution was measured on a Quik Chem 8500 Lachat Autoanalyzer for NH_4^+, and NO_2^- and NO_3^- (hereafter NO_3^-). Soil C and N content in each PVC column were quantified after drying soils at 60 °C for 48–72 h, homogenizing the soil, and analyzing on a Costech ECS4010 Elemental Analyzer.

Soil moisture was determined on both field moist samples harvested immediately upon sampling from the field ($n = 3$ from each forest type) and on soils harvested from the PVC columns at the end of the laboratory experiment ($n = 9$ from each forest type). The soils were dried at 60 °C until a constant weight was achieved (approximately 48–72 h) and soil moisture was determined with the following equation:

$$\text{Soil moisture (\%)} = 100*[(\text{wet soil mass} - \text{dry soil mass})*(\text{wet soil mass})^{-1}].$$

Soil bulk density was calculated by dividing the dry mass of each soil sample by its volume.

Air and soil temperature within each of the three temperature treatments were measured throughout the experiment with thermistors and copper-constantan thermocouples, respectively, connected to a data logger. Soil temperature was measured by inserting a thermocouple horizontally into the center of one side of one PVC column for each forest type in each temperature treatment ($n = 3$ for each forest type). Temperature measurements were made at 10 s intervals and the 5 min averages were logged. Only PVC columns without thermocouples were used for collecting gas and leachate samples ($n = 9$ from each forest type).

2.6. Statistical analysis

We examined the impact of sampling period, forest type, and temperature treatment on trace gas fluxes using repeated measures analysis of variance (ANOVA). Temperature treatment was designated as a random factor and sampling period and forest type as fixed factors. A two-way ANOVA was used to examine differences in cumulative trace gas fluxes, cumulative flux of C and N species in leachate, total C and N fluxes (i.e. sum of gaseous and aqueous fluxes), soil moisture, soil bulk density, and soil C and N content with treatment, forest type, and their interaction as main effects. Tukey's HSD test was used to determine significant differences among the means. Total C and N fluxes were calculated only for PVC columns with leachate data. We subtracted C and N in leachate (aqueous losses) from snow (inputs) and considered there to be significant 'net loss' (negative values) or 'net retention' (positive values) of aqueous forms of C or N in soils if the values were significantly different than zero. Normality was tested with the Shapiro–Wilk test and equal variance with the Bartlett test. Non-normally distributed data were analyzed using a non-parametric Kruskal–Wallis test if transforming the data was unsuccessful in correcting non-normality. All statistical analyses were conducted using SAS JMP software version 8.0.2 (2009) and $\alpha = 0.05$ was used to determine significance. Standard error values are reported throughout this paper.

3. Results

3.1. Soil moisture and temperature

The soils reached their target temperatures within two days of being placed in their respective laboratory treatments. Within 24 h of moving the PVC columns from their respective treatments to the +5 °C refrigerator and adding snow (Day 0), the temperature of the soils converged between −1 and 0 °C (Fig. 1a). Soil temperatures in the no soil frost and mild soil frost treatments had risen above freezing by Day 8 (<2 days prior to 100% snowmelt in all

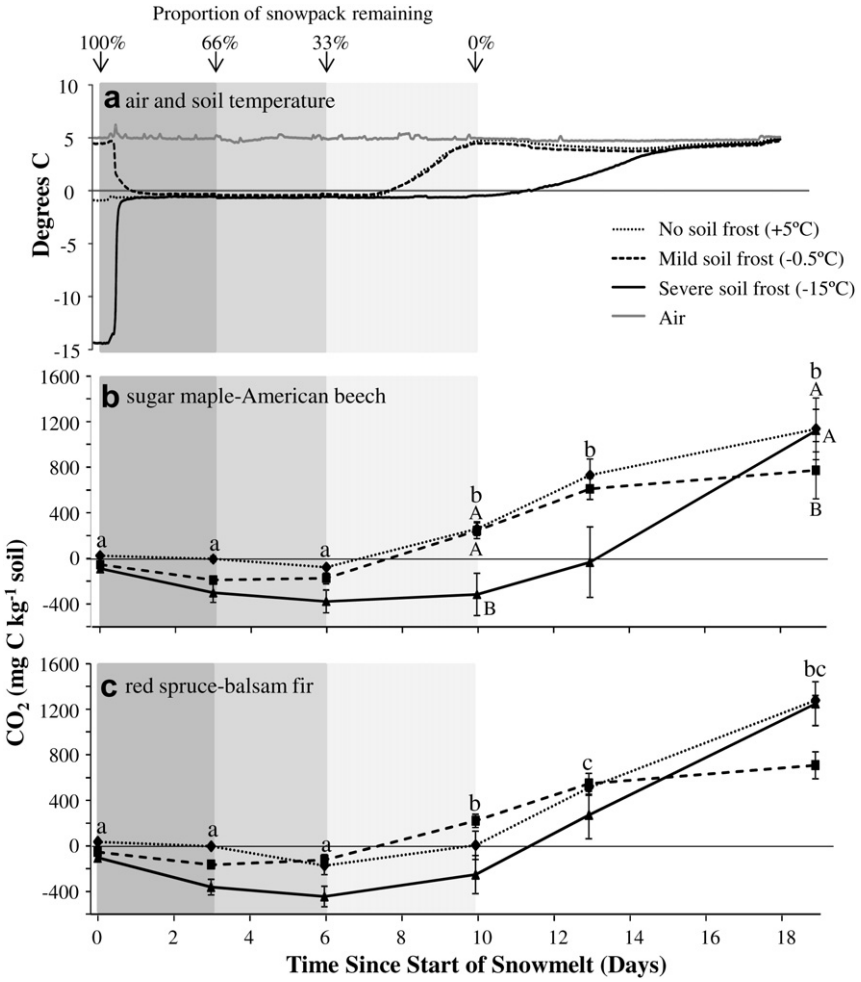

Fig. 1. (a) Air and soil temperature with (b) CO_2 flux from sugar maple–American beech and (c) red spruce–balsam fir soils during and after snowmelt. PVC columns were placed in their respective temperature treatments one week prior to the start of snowmelt. The means are the cumulative flux at each sampling date. However, different upper case letters denote significant differences ($P < 0.05$; $n = 3$) in the daily flux (i.e. not the cumulative flux) among temperature treatments within a sampling period. Different lower case letters denote significant differences ($P < 0.05$) in the daily flux among sampling dates. Values are means with standard error.

columns) and equilibrated with the air temperature (~5 °C) by Day 10. By contrast, soil warming in the severe soil frost treatment lagged behind the other two treatments by approximately one week.

The snowmelt period lasted approximately 10 days for all treatments (Fig. 1a). Melted snow drained freely from soils in the no soil frost and mild soil frost treatments. However, pooling occurred inside the PVC columns of the severe soil frost treatment, and the melted snow drained from these PVC columns within a 24 h period by Day 10. Soil moisture after the laboratory experiment was significantly higher than before the soils were placed in their respective temperature treatments ($P < 0.0001$), but these differences were small (<15%; Table 1). Following the laboratory experiment, soil moisture in the red spruce-balsam fir soils was significantly higher than in the sugar maple–American beech soils ($P = 0.014$), but these differences were also small (<5%).

3.2. Soil carbon and nitrogen content

Total soil C concentration following the laboratory experiment was significantly higher in the red spruce-balsam fir soils than the sugar maple–American beech soils ($P = 0.028$; Table 1), while there was no significant difference in total N concentration or soil C:N among temperature treatments ($P = 0.79$ and 0.42, respectively) or forest types ($P = 0.12$ and 0.65, respectively). Bulk density ($P = 0.0006$) and soil NH_4^+ ($P = 0.001$) and NO_3^- ($P = 0.022$) concentrations were significantly greater in the sugar maple-American beech soils than the red spruce–balsam fir soils. Across both forest types, soil NO_3^- concentrations were significantly higher in the no soil frost treatment than the severe soil frost treatment ($P = 0.011$), while the mild soil frost treatment did not differ from the other treatments ($P > 0.05$). Soil NH_4^+ concentrations were significantly higher in the severe soil frost treatment than the other two temperature treatments for both forest types ($P < 0.0001$).

3.3. Trace gas fluxes

In both forest types, fluxes of CO_2 were small and often negative when the soil was covered with snow, but were consistently positive and significantly greater after the snow had melted ($P < 0.05$). CO_2 fluxes from sugar maple-American beech soils were significantly higher in the no soil frost and mild soil frost temperature treatments compared to the severe soil frost temperature treatment immediately after snowmelt (Day 10; $P < 0.05$). However, nine days after snowmelt (Day 19) this pattern was reversed and the CO_2 flux was significantly higher in the severe soil frost temperature treatment relative to the mild soil frost treatment ($P = 0.0059$; Fig. 1b).

Table 1
Soil moisture, soil bulk density, total soil C and N, soil C:N, extractable N in soil, N and C in snow and leachate, and flux of aqueous forms of N from soils (snow minus leachate N; e.g., Soil $NH_4^+{}_{flux}$). Pre-incubation moisture refers to soil cores collected from the field at the same time as the soil used in the laboratory experiment. Positive values for soil flux indicate net retention in soil and negative values for soil flux indicate net loss from soils. Different lower case letters within a row denote significant differences ($P < 0.05$) among temperature treatments. Values are means with standard error except for $NH_4^+{}_{leachate}$, $NO_3^-{}_{leachate}$, Soil $NO_3^-{}_{flux}$, and Soil $NH_4^+{}_{flux}$ where values are medians with minimum and maximum values since these data were not normally distributed and therefore non-parametric analyses were conducted using rank-transformed data.

	Red Spruce–balsam Fir			Sugar Maple–American Beech			ANOVA *P*-value		
	Severe Soil Frost (−15 °C)	Mild Soil Frost (−0.5 °C)	No Soil Frost (+5 °C)	Severe Soil Frost (−15 °C)	Mild Soil Frost (−0.5 °C)	No Soil Frost (+5 °C)	Treatment	Forest type	Treatment × Forest type
Pre-incubation soil moisture (%)		68.1 ± 0.7			72.6 ± 1.8		–	0.0797	–
Post-incubation soil moisture (%)	82.7 ± 1.5	83.7 ± 0.8	82.5 ± 0.6	82.4 ± 1.2	78.2 ± 1.4	80.9 ± 0.1	0.35	0.0143	0.071
Soil bulk density (g cm^{-3})	0.26 ± 0.04	0.24 ± 0.01	0.24 ± 0.01	0.27 ± 0.02	0.43 ± 0.03	0.34 ± 0.03	0.10	0.0006	0.055
Total C (%)	46.8 ± 2.9	47.1 ± 0.7	46.9 ± 0.7	44.6 ± 2.6	37.5 ± 3.6	43.9 ± 1.7	0.12	0.028	0.24
Total N (%)	2.01 ± 0.03	2.09 ± 0.06	2.09 ± 0.07	2.10 ± 0.17	1.79 ± 0.06	1.96 ± 0.08	0.79	0.12	0.28
Soil C:N	23.4 ± 1.7	22.5 ± 0.5	22.5 ± 1.0	21.6 ± 2.6	21.2 ± 2.8	22.4 ± 0.5	0.42	0.65	0.92
Extractable NH_4^+ (mg N Kg^{-1}soil)	9.6 ± 1.15[a]	1.0 ± 0.14[b]	1.5 ± 0.08[b]	29.10 ± 4.6[a]	5.60 ± 2.2[b]	2.90 ± 1.3[b]	<0.0001	0.001	0.23
Extractable NO_3^- (mg N Kg^{-1}soil)	0.14 ± 0.11[a]	0.04 ± 0.03[ab]	0.27 ± 0.07[b]	0.05 ± 0.02[a]	0.79 ± 0.27[ab]	0.67 ± 0.30[b]	0.014	0.022	0.031
$NH_4^+{}_{snow}$ (mg N kg^{-1} soil)	1.75 ± 0.08	1.58 ± 0.04	1.53 ± 0.07	1.40 ± 0.18	0.88 ± 0.07	1.09 ± 0.09	0.086	0.85	0.33
$NO_3^-{}_{snow}$ (mg N kg^{-1} soil)	4.24 ± 0.19	3.82 ± 0.10	3.71 ± 0.02	3.39 ± 0.44	2.13 ± 0.18	2.63 ± 0.21	0.33	0.95	0.32
DON_{snow} (mg N kg^{-1} soil)	0.92 ± 0.04	0.83 ± 0.02	0.81 ± 0.05	0.74 ± 0.10	0.46 ± 0.04	0.57 ± 0.57	0.072	0.24	0.75
DOC_{snow} (mg C kg^{-1} soil)	58.9 ± 2.6	53.1 ± 1.4	51.5 ± 3.1	47.0 ± 6.1	29.6 ± 2.5	36.5 ± 2.9	0.10	0.28	0.72
$NH_4^+{}_{leachate}$ (mg N kg^{-1} soil)	0.37 (0.25/0.49)[a]	1.38 (1.16/1.59)[b]	1.90 (0.76/3.03)[b]	0.79 (0.28/1.30)[a]	13.0 (12.1/16.0)[b]	19.6 (3.2/24.2)[b]	0.0059	0.0056	0.40
$NO_3^-{}_{leachate}$ (mg N kg^{-1} soil)	0.92 (0.88/0.97)	1.00 (0.97/1.03)	0.60 (0.46/0.73)	1.29 (1.00/1.58)	5.36 (5.18/8.94)	4.54 (1.33/15.5)	0.047	0.0002	0.18
$DON_{leachate}$ (mg N kg^{-1} soil)	5.85 ± 1.5[a]	18.9 ± 0.17[b]	23.3 ± 4.1[b]	11.9 ± 1.8[a]	13.7 ± 1.8[b]	11.9 ± 1.8[b]	0.0042	0.71	0.070
$DOC_{leachate}$ (mg C kg^{-1} soil)	564 ± 132	1069 ± 177	1116 ± 173	938 ± 369	556 ± 87	649 ± 109	0.68	0.16	0.060
Soil $NH_4^+{}_{flux}$ (mg N kg^{-1} soil)	1.38 (1.18/1.58)[a]	−0.20 (−0.05/0.45)[b]	−0.36 (−1.6/0.87)[b]	0.61 (0.28/0.94)[a]	−12.2 (−15.2/−11.1)[b]	−18.4 (−23.0/−2.24)[b]	0.0038	0.0024	0.55
Soil $NO_3^-{}_{flux}$ (mg N kg^{-1} soil)	3.32 (3.18/3.46)[a]	2.82 (2.70/2.95)[b]	3.11 (3.02/3.21)[ab]	2.10 (1.95/2.25)[a]	−3.41 (−6.45/−3.23)[b]	−1.65 (−12.7/0.89)[ab]	0.02	<0.0001	0.54
Soil DON_{flux} (mg N kg^{-1} soil)	−4.3 ± 1.44[a]	−17.3 ± 0.96[b]	−22.5 ± 4.2[b]	−11.2 ± 1.71[a]	−13.2 ± 1.81[b]	−17.0 ± 2.41[b]	0.004	0.59	0.083
Soil DOC_{flux} (mg C kg^{-1} soil)	−505 ± 129	−1016 ± 175	1115 ± 176	−891 ± 363	−526 ± 86.0	−613 ± 109	0.65	0.19	0.060

The only significant differences in N_2O fluxes were observed on Day 19 when fluxes from soils in the no soil frost and severe soil frost temperature treatments were significantly higher than the mild soil frost temperature treatment for both forest types ($P = 0.026$ and 0.0079, respectively; Fig. 2); however, none of the means were significantly different than zero ($P > 0.05$).

Neither temperature treatment nor forest type had a significant effect on CH_4 fluxes ($P = 0.79$ and 0.98, respectively; Fig. 3). Fluxes of CH_4 were significantly higher on Day 19 than Day 6 across both forest types ($P = 0.035$). In addition, CH_4 fluxes from the severe soil frost treatment on Day 19 were significantly higher than on Day 10 across both forest types ($P = 0.0084$).

3.4. Carbon and nitrogen in snow and leachate

Inputs of DOC from snow ranged between 5 and 10% DOC lost in leachate ($P < 0.0001$; Table 1). DOC fluxes (snow minus leachate) tended to be lower in the severe soil frost treatment relative to the other treatments in red spruce-balsam fir forest soils and higher in sugar maple–American beech forest soils, but differences were not significant among temperature treatments or forest type ($P > 0.05$).

Averaged across temperature treatments, NO_3^- and NH_4^+ concentrations in snow were significantly greater than they were in leachate for the red spruce-balsam fir forest type ($P = 0.0002$ and 0.001, respectively; Table 1). In contrast, in the sugar maple-American beech forest type NO_3^- and NH_4^+ concentrations in leachate were significantly greater than they were in snow ($P = 0.0013$ and 0.014, respectively). Contributions of DON in snow were on average only 6% of the content in leachate ($P < 0.0001$).

Net retention and loss of N varied among temperature treatments and forest types. For example, both extractable NH_4^+ and NO_3^- and losses of NH_4^+ and NO_3^- in soil (e.g., soil $NH_4^+{}_{flux}$ in Table 1; snow minus leachate) were significantly greater in sugar maple-American beech than red spruce-balsam fir soils ($P = 0.001$, 0.022, 0.0024 and <0.0001, respectively), but there was no significant difference in DON lost from soils among the two forest types ($P = 0.59$). Losses of NO_3^- in soils were significantly greater for the mild soil frost treatment than the severe soil frost treatment ($P = 0.02$), while the no soil frost treatment did not differ from the other two treatments for both forest types ($P > 0.05$). Losses of NH_4^+ were significantly greater in the no soil frost and mild soil frost temperature treatments compared to the severe soil frost temperature treatment ($P = 0.0038$), across both forest types. Net losses of DON were significantly greater following mild soil frost and no soil frost compared to the severe soil frost temperature treatments ($P = 0.004$) across both forest types. Net losses of TDN (i.e. NH_4^+–N + NO_3^-–N + DON) from soils were greater from sugar maple-American beech soils than red spruce-balsam fir soils, but this trend was only marginally significant ($P = 0.05$; data not shown). Net losses of TDN from soils were significantly greater in the no soil frost treatment compared to the severe soil frost treatment ($P = 0.015$), but the mild soil frost treatment was not significantly different than the other two temperature treatments ($P > 0.05$).

3.5. Total carbon and nitrogen fluxes

The total cumulative losses of C (i.e. DOC + CO_2–C + CH_4–C) and N (i.e. DON + NO_3–N + NH_4–N + N_2O–N) from soils were both significantly lower in the severe soil frost treatment compared to the no soil frost treatment for both forest types ($P < 0.05$; Fig. 4a and b). Total C losses tended to be greater in red spruce-balsam fir forest soils, while total N losses tended to be greater in the sugar maple–American beech forest soils, but there was no significant difference between the two forest types for either total C or N fluxes ($P = 0.44$ and 0.13, respectively). While the cumulative fluxes of CO_2

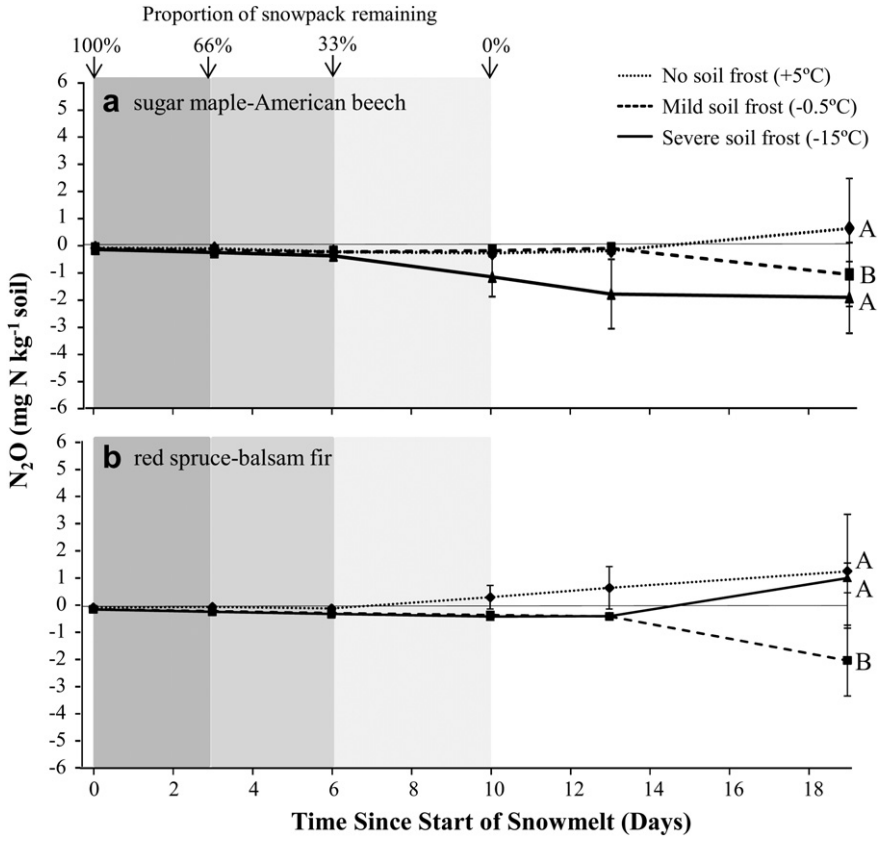

Fig. 2. N_2O flux from (a) sugar maple–American beech and (b) red spruce–balsam fir soils during and after snowmelt. The means are the cumulative flux at each sampling date. However, different upper case letters denote significant differences ($P < 0.05$; $n = 3$) in the daily flux (i.e. not the cumulative flux) among temperature treatments within a sampling period. There were no significant differences between sampling periods or between forest types ($P > 0.05$; $n = 3$). Fluxes on Day 19 from the no soil frost (+5 °C) and severe soil frost (−15 °C) treatments were significantly higher than the mild soil frost (−0.5 °C) treatment ($P = 0.026$ and 0.0079, respectively). Values are means with standard error.

during the incubation tended to increase with decreasing soil frost intensity, differences were not statistically significant ($P = 0.13$; Fig. 4a). Cumulative fluxes of CH_4 were orders of magnitude smaller than CO_2 and DOC fluxes ($P < 0.0001$), and therefore had a negligible impact on total C fluxes.

4. Discussion

Results from this study show that soil frost can significantly impact the cycling of C and N in northern forest ecosystems during the snowmelt period. While we expected that total losses of C and N would increase with increasing severity of soil frost, we found the opposite pattern with severe soil frost resulting in lower losses in both forest types. The lower losses of N in the severe soil frost treatment were surprising since the opposite pattern has been found in field experiments (e.g., Fitzhugh et al., 2001). Results from this laboratory experiment are limited given that we did not include plant roots or mineral soil and we examined fluxes over a short time period (19 days). However, the differences we found suggest that severe soil frost could lead to lower losses of C and N during the snowmelt period equivalent to up to 4.6% of C (heterotrophic respiration + DOC) and 22% of N (TDN) exported annually from the forest floor of northern forests (Fahey et al., 2005; Dittman et al., 2007).

4.1. Effects of soil frost on aqueous carbon and nitrogen

The lower losses of NH_4^+ and NO_3^- following severe soil frost may be attributed to colder soil temperatures and a longer duration of soil frost relative to the other two temperature treatments. For example, in the severe soil frost treatment soils were still below freezing (~−1 °C) during the flush of water which could have limited microbial activity such as mineralization, and hence the amount of C and N produced by soil microbes and then subsequently leached.

Our results are in contrast to Fitzhugh et al. (2001) and Austnes and Vestgarden (2008) and, who both observed an increase in inorganic N leaching in response to increased soil frost in forest and heathland soils, respectively. The difference in relationship between soil frost and inorganic N leaching in our study compared to others could be due to a variety of factors, including (1) reductions in metabolic activity in the comparatively colder soil temperatures of our study (e.g., −15 °C in our severe soil frost soils vs. −7 °C in field experiments; Hardy et al., 2001), (2) the limitation of microbial activity caused by pooling of water and hence reduced diffusion of oxygen, substrates, or enzymes in frozen soil (Mikan et al., 2002), or (3) differences in timing of measurements. Fitzhugh et al. (2001) observed an increase in rates of NO_3^- leaching during the growing season, but not during the snowmelt period. Increased NO_3^- leaching during the growing season has been attributed to decreased plant N uptake due to root mortality (Tierney et al., 2001) rather than changes in microbial activity (Groffman et al., 2001). We evaluated C and N fluxes with high temporal resolution only during snowmelt when root activity is typically low (Tierney et al., 2003). While the absence of roots in our study contrasts with previous field experiments, this difference is likely of little importance to fluxes during the snowmelt period.

Nitrate export from forests at HBEF has been declining in recent decades even though rates of atmospheric deposition have not changed significantly (Driscol et al., 2001; Goodale et al., 2003).

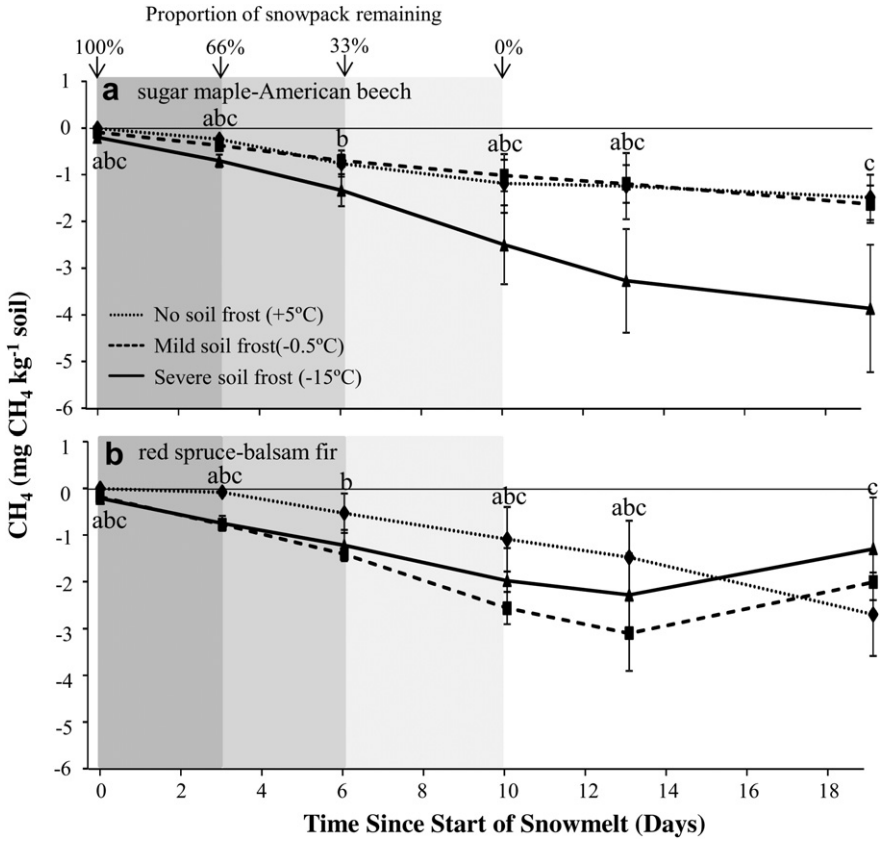

Fig. 3. CH_4 flux from (a) sugar maple–American beech and (b) red spruce–balsam fir soils during and after snowmelt. The means are the cumulative flux at each sampling date. There were no significant differences between temperature treatments within sampling periods or between forest types ($P > 0.05$; $n = 3$). Daily CH_4 fluxes in response to severe soil frost were significantly greater on Day 19 than Day 10 ($P = 0.043$). Values are means with standard error.

For example, on an annual basis, forests at HBEF lost on average 6.9 kg N ha^{-1} yr^{-1} in 1973–74 (Likens and Bormann, 1995), but only 0.4 kg N yr^{-1} ha^{-1} in 1996–97 (Campbell et al., 2000). While the role of soil frost in mediating N export remains uncertain (e.g., Fitzhugh et al., 2003) the results from this study suggest that soil frost during snowmelt may mitigate export of N by reducing losses from the forest floor during this critical time of the year.

Our observation that mild soil frost had no significant impact on DON losses is in agreement with past snow removal experiments in forest (Fitzhugh et al., 2001) and boreal heathland (Austnes et al., 2008) ecosystems, although laboratory experiments with boreal heathland soils suggest an increase in DON leaching following mild soil frost (Austnes and Vestgarden, 2008). We are unaware of any other studies that have measured DON fluxes in response to severe soil frost (<−8 °C). The fact that we observed no impact of severe soil frost on DON fluxes suggests that DON may be well buffered from changes in soil temperature in northern forest soils. Despite our low level of replication, we had sufficiently high statistical power (power = 0.95) to detect treatment-level differences if there were any.

Annual fluxes of DOC in northern forests are typically much smaller than CO_2 fluxes (Fahey et al., 2005). In contrast, the relative contributions of CO_2 and DOC to total C fluxes observed in this study were comparable (CO_2–C:DOC ratios ranged from 0.8 to 2.2, depending on treatment). High DOC export during the snowmelt period (Sebestyen et al., 2008) coupled with low rates of soil respiration in this study (Figs. 1 and 4a) likely explain the small differences between fluxes of DOC and CO_2 that we observed. Similar to DON, the absence of a soil frost effect on DOC fluxes suggests that organic pools of C may also be well buffered against changes in soil frost or temperature. However, low statistical power (power = 0.10) could have prevented detection of treatment-level differences.

4.2. Effects of soil frost on gaseous losses of carbon and nitrogen

We predicted that increasing soil moisture during snowmelt would stimulate N_2O production since denitrification is an anaerobic process and a major pathway for gaseous N losses. However, N_2O fluxes were highly variable throughout the experiment and there were no significant differences among the sampling periods, although one week after all snow had melted fluxes were highest in soils experiencing mild soil frost compared to no or severe soil frost. The greater flux of N_2O from soils exposed to greater soil frost in the field has been attributed to greater rates of denitrification (Groffman et al., 2006). However, soil extractable NO_3^- was not significantly different among the temperature treatments in our laboratory experiment, suggesting that either denitrification cannot explain the differences in N_2O that we observed or that rates of N_2O losses kept up with production of NO_3^- in soils.

Fluxes of CO_2 from soil tended to be negative when snow cover was present. However, CH_4 fluxes were also generally negative indicating that redox conditions throughout the experiment favored CO_2 production and CH_4 consumption, which is commonly observed in upland forest soils (Crill, 1991; Fahey et al., 2005). It is possible that negative fluxes of CO_2 were due to low rates of soil respiration that were outpaced by CO_2 storage in the wet snow pack (see Sommerfeld et al., 1996). The increase in CO_2 fluxes following snowmelt compared to the pre-melt period was likely due to a combination of warming soil temperatures and absence of a snow pack to store CO_2. Carbon inputs from snow accounted for less than

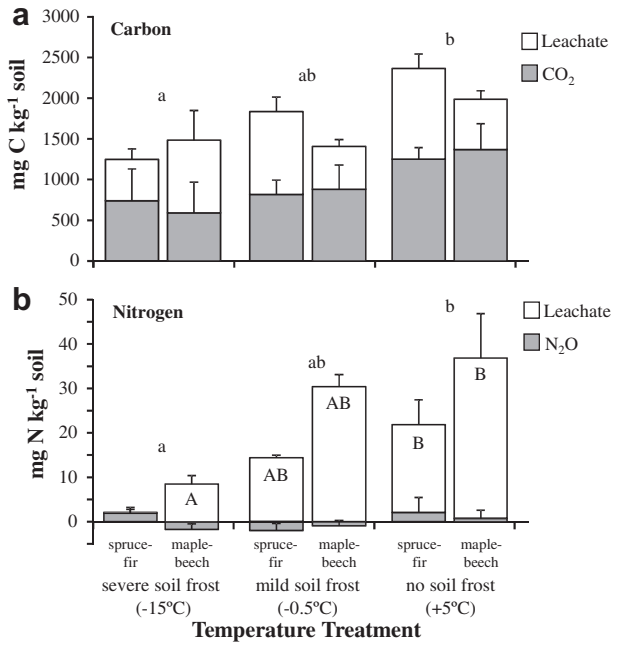

Fig. 4. (a) Total C fluxes in leachate (DOC) and gases ($CO_2 + CH_4$) and (b) total N fluxes in leachate ($NO_3^- + NH_4^+ +$ DON) and gases (N_2O) among temperature treatments and forest types. Different lower case letters denote significant differences ($P < 0.05$) in total flux of C or N among temperature treatments. Different upper case letters denote significant differences ($P < 0.05$) in loss in leachate among temperature treatments. Positive values indicate net loss of C or N from soils and negative values indicate net retention of C or N in soils. Values for CH_4 (range equal to −2.7 to −1.2 mg C kg^{-1}) were too small to be viewed on the scale of this figure. There were no significant differences between forest types for total C or N fluxes. Total N flux in leachate was significantly higher in sugar maple–American beech than red spruce–balsam fir ($P = 0.05$). There were no significant differences in CO_2 or N_2O fluxes among temperature treatments or between forest types. Due to insufficient leachate volume our sample size for fluxes shown here was $n = 2$ for the three temperature treatments for red spruce-balsam fir and the severe soil frost temperature treatment for sugar maple–American beech. Values are means with standard error.

5% of total respired CO_2 and less than 6% of DOC export in this study. It therefore seems probable that substrate contributions from snowmelt played a small role in stimulating soil respiration.

It is likely that the snow pack indirectly influenced rates of soil respiration by insulating the underlying soil as indicated by the relatively constant soil temperatures prior to and during snowmelt and the increase once all of the snow melted. Soil warming after snowmelt was delayed in the severe soil frost temperature treatment, which corroborates findings from field experiments (Groffman et al., 2001). In the present study, the slower soil warming in response to severe soil frost was likely responsible for the delayed increase in soil respiration after snowmelt. The increase in CO_2 fluxes following snowmelt in both forest types may be attributable to several possible factors including the release of CO_2 that accumulated beneath the frozen soil, warming soil temperatures, and an increase in availability of labile C from the lysing of microbial cells in response to severe soil frost conditions (i.e. < -5 °C; Schimel and Clein, 1996). Microbes appear to be less adversely affected by mild soil frost (>-5 °C; Groffman et al., 2001), which may explain the smaller increase in CO_2 flux upon thawing. We did not observe any significant differences in CH_4 fluxes among temperature treatments during snowmelt.

4.3. Tree species composition and C and N fluxes

We expected that total C ($CO_2 + CH_4 +$ DOC) fluxes would be driven primarily by soil respiration which is typically higher in sugar maple–American beech forests compared to red spruce-balsam fir forests at HBEF (Fahey et al., 2005) because hardwood litter tends to be more labile than conifer litter (Friedland et al., 1986). However, both total C and CO_2 fluxes did not differ between forest types. CO_2 fluxes from the soil to the atmosphere were small among both forest types. Cumulative fluxes of CO_2 in this study (Fig. 4a) amounted to less than 5% of the annual CO_2 flux from heterotrophic respiration measured from the forest floor at the HBEF (Fahey et al., 2005). In this experiment soil temperature was an important factor controlling rates of soil respiration ($R^2 = 72, 62, 39$, and 43% for no soil frost, mild soil frost, severe soil frost, and all temperature treatments and forest types together, respectively). It is possible that cold soil temperatures and C substrate limitation were responsible for the low rates of CO_2 fluxes observed in this study and masked differences associated with forest type.

DOC export during snowmelt can comprise a large proportion of annual DOC flux in northern forest ecosystems (Sebestyen et al., 2008). DOC fluxes in this experiment (Table 1) were equivalent to 15–35% of annual fluxes of DOC from the forest floor measured *in situ* at the HBEF (Fahey et al., 2005). It is surprising that there were no significant differences in DOC fluxes between forest types since DOC leaching from the forest floor at the HBEF has been observed to be greater from red spruce-balsam fir forests than sugar maple–American beech forests (Johnson et al., 2000). Red spruce-balsam fir forest soils tended to have higher leaching losses of DOC than sugar maple–American beech forest soils in this experiment and it is possible that high variability and low statistical power (power = 0.24) masked detection of differences.

As predicted, the sugar maple–American beech forest soils had greater concentrations of soil extractable NH_4^+ and NO_3^-, as well as losses of NH_4^+ and NO_3^- in leachate, compared to the red spruce-balsam fir forest soils. The red spruce–balsam fir forest soils exhibited net retention of NO_3^- across all treatments, while there was a net loss of NO_3^- from sugar maple-American beech forest soils in the no soil frost and mild soil frost treatments. These results suggest that rates of nitrification could have been higher in the sugar maple–American beech forest soils, which is often observed in forests dominated by sugar maple compared to forests dominated by conifers (Pastor et al., 1984; Templer et al., 2003; Lovett et al., 2004). In addition, microbial immobilization of inorganic N could have been higher in the red spruce–balsam fir forest soils than the sugar maple–American beech forest soils.

We expected that greater NO_3^- availability in the sugar maple–American beech forest soils would result in higher N_2O fluxes compared to the red spruce–balsam fir forest soils, but we found no differences in N_2O fluxes between forest types. Rates of N_2O fluxes measured in this study were highly variable but comparable to *in situ* measurements made at the HBEF (Groffman et al., 2006). The processes mediating these fluxes are complex and it is likely that during the snowmelt period other factors such as C availability and redox potential overshadowed the importance of forest type or NO_3^- availability in determining N_2O fluxes. Surprisingly, total N fluxes also did not differ between forest types. It is possible that low statistical power (power = 0.32) and the high variability in N fluxes masked any differences between the forest types.

4.4. Additional considerations

In contrast to previous laboratory experiments (e.g., Austnes and Vestgarden, 2008), we did not allow the soils to thaw prior to adding water to simulate snowmelt. This was intentional in order to mimic soil thawing that occurs during snowmelt in the field (e.g., Hardy et al., 2001). Additionally, few field experiments have explicitly quantified the effects of soil frost on C and N fluxes during the snowmelt period (e.g., Fitzhugh et al., 2001). Therefore, it is important to acknowledge that these differences in experimental design

and temporal scale of sampling may have contributed to the observed discrepancies in the response of C and N fluxes to soil frost.

Air temperature was held constant at 5 °C during the simulated snowmelt in this experiment. Diurnal and daily fluctuations that occur in the field could result in freezing and thawing of snowmelt water and the soil underlying the melting snow pack. Freeze-thaw cycles have been shown to increase losses of C and N from soils (see Henry, 2007); however, it remains uncertain how freezing and thawing during snowmelt might affect C and N cycling during this period and should be investigated in future research.

Drainage of water in this experiment may differ from what occurs *in situ* during snowmelt because the experimental design does not address the influence of horizontal flow. Soils typically become saturated during snowmelt, which could impede vertical movement of water in favor of horizontal flow and result in lateral redistribution of dissolved C and N. This could potentially reduce *in situ* losses from the forest floor during snowmelt compared to those observed in the present study and reduce differences in losses between frozen and unfrozen soils. Additionally, in the severe soil frost treatment infiltration of snowmelt water through frozen soils could have resulted in water following preferential flow paths and reducing interaction between water and soil. This may have reduced microbial activity in areas of the soil profile where water was less available, thereby decreasing C and N leaching. Similarly, in field conditions when the soil is frozen snowmelt water likely has limited interaction with the soil as water moves as overland flow or is channeled through preferential flow paths in the soil.

5. Conclusions

The results from this experiment indicate that severe soil frost (−15 °C) resulted in lower losses of C, NH_4^+, and NO_3^- compared to unfrozen soils during snowmelt, while we found no significant impact of mild soil frost (−0.5 °C), which suggests that severity of soil frost plays an important role in potentially reducing losses of C and N during spring snowmelt. While microbial activity can continue in frozen soils, many microbial processes have a lower temperature limit of approximately −5 °C (Coxson and Parkinson, 1987; Dorland and Beauchamp, 1991; Clein and Schimel, 1995; Brooks et al., 1997). Lower rates of microbial activity could have contributed to the lower losses of C and N in severely frozen soils. Therefore, it is likely that the extent to which C and N cycling during snowmelt is altered in response to changes in winter climate depend on both the presence of soil frost and the temperature to which soils freeze.

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