Freezing Effects on Carbon and Nitrogen Cycling in Northern Hardwood Forest Soils

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ABSTRACT

We evaluated the effects of freeze–thaw events on soil respiration, nitrogen mineralization, nitrification, and nitrous oxide production in soils from a northern hardwood-dominated forest in New Hampshire, USA. Soil samples from three horizons (O_e, O_a, A) from nearly monospecific stands of sugar maple (*Acer saccharum* Marshall) and yellow birch (*Betula alleghaniensis* Britton) were placed in 200-mL incubation vessels (microlysimeters), subjected to severe (−13°C) and mild (−3°C) freeze treatments for 10 d, and then incubated at laboratory temperature (20–25°C) for 3 wk. Evolution of CO_2 and N_2O and concentrations of leachable NH_4^+ and NO_3^- were measured at weekly intervals. Freezing increased rates of C and N cycling in these soils, but the effects varied with species, horizon, and freeze treatment. Whereas severe freezing stimulated respiration, N_2O flux, and mineralization, mild freezing had very few effects. Nitrification did not appear to be affected by either freeze treatment, but increases in denitrification may have masked freeze effects on this process. Freeze effects were much more marked in maple than in birch soils and in the O_a and A horizons. Maple consistently had higher rates of nitrification and N_2O production than did birch. The species and horizon differences were likely driven by higher levels of available C in the birch soils and O_e horizon at both sites. These results suggest that changes in climate and snow cover that influence soil freezing could increase N and C losses from northern hardwood forest ecosystems with potential effects on soil fertility and carbon storage, receiving water quality, and atmospheric chemistry.

THE EFFECTS of climate-driven physical perturbations on soil microbial biomass and activity have been a topic of enduring interest in soil biology. Disturbances caused by wetting and drying cycles and freeze–thaw events have long been known to stimulate microbial activity via killing of microbial biomass, followed by a pulse of activity as surviving organisms use the killed cells as substrate (Birch, 1958; Soulides and Allison, 1961; Kieft et al., 1987; Edwards and Cresser, 1992; DeLuca et al., 1992; Burton and Beauchamp, 1994; Schimel and Clein, 1996). Freezing also causes physical disturbance to the soil, releasing substrates physically protected in soil aggregates (Edwards and Cresser, 1992).

Interest in freeze–thaw events has been particularly marked because of the recognition that overwinter processes account for a significant portion (20–70%) of annual ecosystem C and N cycling and soil–atmosphere trace gas fluxes (Goodroad and Keeney, 1984; Sommerfeld et al., 1993; Brooks et al., 1997; Nyborg et al., 1997; Röver et al., 1998; Alm et al., 1999; van Bochove et al., 2000). Research on freeze–thaw events has increased in recent years because of concerns that climate change may alter their frequency and intensity (Moore and McKendry, 1996; Williams et al., 1998; Murdoch et al., 1998; Boutin and Robitaille, 1995; Groffman et al., 1999).

Assessing the importance of freeze–thaw events in ecosystem C and N cycling is hindered by great variation in the intensity and length of these events under field conditions. As a result, there have been few studies to evaluate ecosystem-scale controls on their effects on C and N processes (Mitchell et al., 1996; Stenberg et al., 1998; Lipson and Monson, 1998; Jones, 1999). There is a strong need for systematic experiments that examine the key climatic (freeze length, intensity, frequency) and ecosystem (soil and vegetation type) factors that control the effects of freeze–thaw events on these processes. Data from these experiments can then be combined with field measurements of the response of soil processes to freezing events and/or incorporated into soil and ecosystem biogeochemistry models (Brooks and Williams, 1999). This combination would allow for evaluation of the importance of freeze–thaw events to ecosystem water quality, atmospheric chemistry, nutrient cycling, and soil carbon (C) storage functions under different climatic conditions.

In this study, we evaluated the effects of freeze–thaw events on soil respiration, N mineralization, nitrification, and nitrous oxide (N_2O) production in soils from a northern hardwood-dominated forest in New Hampshire, USA. Soils collected from nearly monospecific stands of two important species in this forest (sugar maple, yellow birch) were incubated under laboratory conditions. The work reported here is part of a larger, field snow manipulation study that aims to evaluate the effect of reduced snow cover, as might occur with global warming, on C and N cycling in the northern hardwood forest (Groffman et al., 1999). The specific objectives of the study reported here were (i) to compare the effects of severe (−13°C) versus mild (−3°C) freezing on soil N cycling and (ii) to determine if canopy tree species (sugar maple versus yellow birch) is a controller of the change in soil C and N cycling in response to freeze events. The −3°C treatment was similar to temperatures observed in our field plots during the two mild winters that our snow manipulation treatment was applied (Groffman et al., 1999). The −13°C treatment was based on data from the literature on temperatures known to cause root and microbial mortality (Edwards and Cresser, 1992; Sakai and Larcher, 1987). We hypothesized that (i) mild freezing would have little or no effect on C and N cycling processes in soil and (ii) that severe freezing effects would be more marked in sugar maple

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Table 1. Forest floor depth, soil organic matter concentration, annual N mineralization, annual nitrification and summer respiration rate (June/July) in two sugar maple and two yellow birch dominated plots at the Hubbard Brook Experimental Forest, NH, in 1998. Values are the mean (with standard error) of two sugar maple and two yellow birch plots.

Variable	Units	Sugar maple	Yellow birch
Forest floor depth	cm	6.1 (0.4)	6.3 (1.2)
Organic matter (%)	%		
O_a horizon		51.2 (12)	49.9 (8.9)
B_s horizon		6.2 (1.0)	6.2 (1.3)
N mineralization†	g N m^{-2} y^{-1}	6.9 (0.9)	8.2 (0.1)
Nitrification†	g N m^{-2} y^{-1}	3.8 (2.0)	2.9 (1.5)
Respiration†	μmol m^{-2} s^{-1}	2.6 (0.1)	3.6 (0.1)

† Data from Groffman et al. (in press).

than yellow birch because sugar maple has been shown to support high rates of N cycling in soil relative other species in the northern hardwood forest (Pastor et al., 1984; Finzi et al., 1998; Lovett and Rueth, 1999).

MATERIALS AND METHODS

Site Description

This research was conducted at the Hubbard Brook Experimental Forest (HBEF), a Long-Term Ecological Research site in the White Mountains of central New Hampshire, USA, (43° 56′ N, 71° 45′ W) (www.hbrook.sr.unh.edu; verified July 10, 2001). Vegetation at HBEF is dominated by American beech (*Fagus grandifolia* Ehrh.), sugar maple, and yellow birch. The forest was selectively cut in the 1880s and 1910s and some of the older stands were damaged by a hurricane in 1938. Soils are shallow (75–100 cm), acidic (pH 3.9) Typic Haplorthods developed from basal tills. This site is located in a region where soil freezing is currently rare because of heavy snowfall, but which may be affected by reduced snowfall caused by global climate change (Mitchell et al., 1996).

Sample Collection and Initial Handling

Four sampling areas were chosen, two dominated (>80%) by sugar maple and two dominated by yellow birch (Table 1). Two 10- by 10-m plots were established in each area, and in early June 1997 (soil temperature ~10°C), two small pits were excavated within each plot and soils were collected from three horizons: the O_e, which consists of partially decomposed organic material; the O_a, which consists primarily of decomposed organic matter with some mineral soil; and the A, which contains mineral soil mixed with some organic material. Samples from the two pits were combined.

Each sample was sorted by hand, and large debris (>4 mm) was removed. All data are expressed on an oven dried soil basis (75°C). Seven grams of field moist soil from each sample were shaken with 30 mL of 2 *M* KCl for 1 h and then filtered to extract ammonium (NH_4^+) and nitrate (NO_3^-). The filtrate was analyzed with a flow injection autoanalyzer (Perstorp 3000, Alpkem, Wilsonville, OR) using the salicylate–hypochlorite method for NH_4^+ and cadmium reduction for NO_3^-.

Experimental Treatments

A factorial design with tree species (maple and birch), soil horizons (O_e, O_a, A), and freezing (−13°C and laboratory temperature controls) was established. In addition, a separate group of O_a horizon samples from both species was treated at −3°C. Each group contained eight samples: two sample collection areas × two plots × two laboratory replicates.

Samples were placed in 200-mL-capacity incubation vessels (microlysimeters) with glass fiber filters having a 1.0-μm nominal pore size and treated with 100 mL of micronutrient solution (4.0 m*M* $CaCl_2$, 2.0 m*M* KH_2PO_4, 1.0 m*M* K_2SO_4 and $MgSO_4$, 2.5 μ*M* H_3BO_3, 2.0 μ*M* $MnSO_4$ and $ZnSO_4$, and 0.5 μ*M* $CuSO_4$ and Na_2MoO_4) (Nadelhoffer, 1990). The solution was allowed to remain in contact with the soil for 1 h and was then drained and analyzed for NH_4^+ and NO_3^- as described above. All samples were thus at approximately field capacity moisture during subsequent treatments and incubations. The microlysimeters were then placed in sealed quart-size mason jars for 24 h, after which, two 9-mL samples of the headspace gas were collected with a syringe through a septum in the mason jar lid. The headspace samples were analyzed for carbon dioxide (CO_2) and N_2O with a Tracor 540 gas chromatograph (Tremetrics, Austin, TX) with thermal conductivity and electron capture detectors (Holland et al., 1999).

After gas sampling, the microlysimeters were removed from the mason jars, covered with plastic wrap and placed under treatment conditions for 10 d. The freeze treatment samples were placed in freezers with temperatures of −13°C (range of −15 to −11°C) or −3°C (range of −5 to −1°C), and control samples remained in the laboratory (range of 20 to 25°C). After treatment, all samples were held at laboratory temperature. Leaching and headspace measurements as described above were then repeated once a week for 3 wk, starting immediately after treatment. Finally, a second KCl extraction, as described above, was performed on all incubated samples.

Calculations and Statistical Analysis

Total respiration was determined by extrapolating individual CO_2 evolution measurements over the interval between sampling times and then calculating production for the entire length of the experiment. To calculate total net nitrogen mineralization, NO_3^- and NH_4^+ leached or extracted from soil was summed, and to calculate total net nitrification, leached or extracted NO_3^- was summed.

For CO_2, N_2O, leachable NH_4^+ and leachable NO_3^-, a two-way analysis of variance was conducted for each sampling date, with species, treatment, and species × treatment interaction as the main effects. As there were no significant interaction effects, a Fisher's least significant difference test was run a posteriori to determine specific differences between the three treatments.

For comparison of total respiration, net mineralization, and net nitrification at three different temperatures within the O_a horizon, a two-way analysis of variance with species and treatment as main effects was conducted. As there were no significant interaction effects, a Fisher's least significant difference test was run a posteriori to determine specific differences between the three treatments.

For comparison of total respiration, net mineralization and net nitrification in the −13°C and control treatments in the three different soil horizons, a three-way analysis of variance was conducted, with species, treatment, and horizon as main effects. There were also tests for species × treatment, species × horizon, and treatment × horizon interactions, some of which were significant.

RESULTS

Freezing had a significant effect on both CO_2 and N_2O evolution, but the effect was much more marked for CO_2 (Fig. 1). Immediately following thawing, CO_2 evolution increased from 57.2 to 331 mg C kg^{-1} d^{-1} in the −13°C treatment and from 56.6 to 131 mg C kg^{-1}

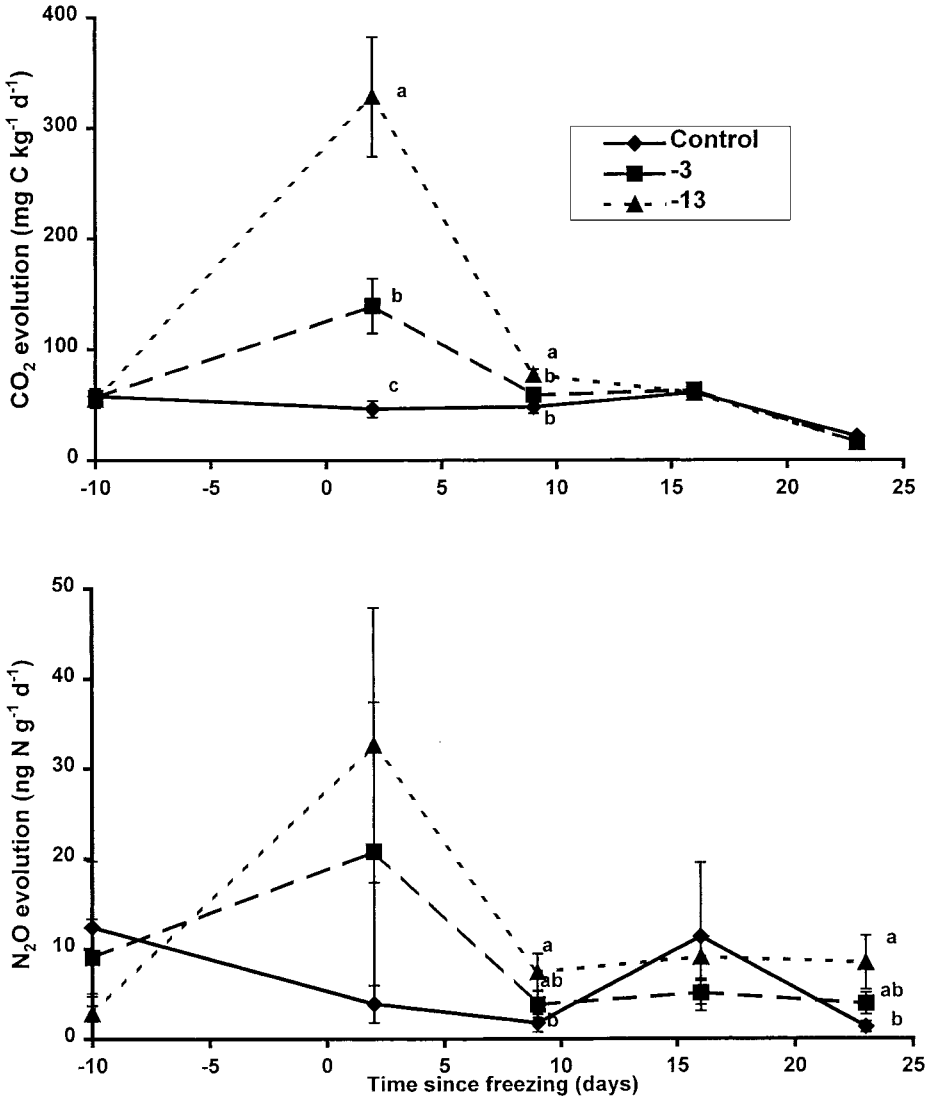

Fig. 1. Evolution of CO_2 (top panel) and N_2O (bottom panel) from Oa horizon soils subjected to −3 and −13°C treatments. Values are means of samples from maple and birch stands combined, with standard error. Points with different superscripts are significantly different at $P < 0.05$.

d^{-1} in the −3°C treatment (Fig. 1a, all differences $P < 0.05$). Evolution of N_2O increased immediately following treatment, from 3.1 to 28.9 μg N kg^{-1} d^{-1} in the −13°C treatment and from 9.1 to 20.8 μg N kg^{-1} d^{-1} in the −3°C treatment (Fig. 1b). However, because of high variability between samples, neither of these increases was statistically significant. A significant treatment effect on N_2O was not observed until 1 wk following the treatment, at which time the N_2O evolution rate of the −13°C treatment (6.7 μg N kg^{-1} d^{-1}), was significantly ($P < 0.05$) higher than that of the control (1.8 μg N kg^{-1} d^{-1}).

There were significant species effects on both CO_2 and N_2O evolution, but the effects were much more marked for N_2O. There was a small, but statistically significant ($P < 0.05$) species effect on CO_2 production at the start of the experiment, with rates of 45 mg C kg^{-1} d^{-1} in the maple samples compared to 67 mg C g^{-1} d^{-1} in the birch samples (Fig. 2a). Evolution of N_2O was consistently higher from maple than birch samples (Fig. 2b).

The freeze treatments significantly affected concentrations of leachable NH_4^+ and NO_3^- (Fig. 3). The −13°C treatment caused an increase in leachable NH_4^+ in soils from the O_a horizon, but this effect was not significant ($P < 0.01$) until 1 wk after treatment ended, when the −13°C samples averaged 76.9 mg N kg^{-1}, while the control samples averaged only 39.1 mg N kg^{-1} (Fig. 3a). The −3°C samples, with average leachable NH_4^+ of 42.5 mg N kg^{-1}, were not significantly different from the controls. In contrast to NH_4^+, leachable NO_3^- in O_a horizon soils decreased ($P < 0.10$) immediately after freezing to −13°C, with average leachable NO_3^- of 2.1 mg N kg−1 in the −13°C samples and 4.6 mg N kg^{-1} in the controls (Fig. 3b). The −3°C treatment, with an average of 2.2 mg N kg^{-1}, was not significantly different from either of the other groups.

Leachate from birch soils consistently had higher ($P < 0.01$ or 0.05) concentrations of NH_4^+ (Fig. 4a) and lower ($P < 0.01$) concentrations of NO_3^- (Fig. 4b) than maple soils. The O_e horizon had higher respiration than the O_a ($P < 0.01$) and A ($P < 0.05$) horizons (Table 2).

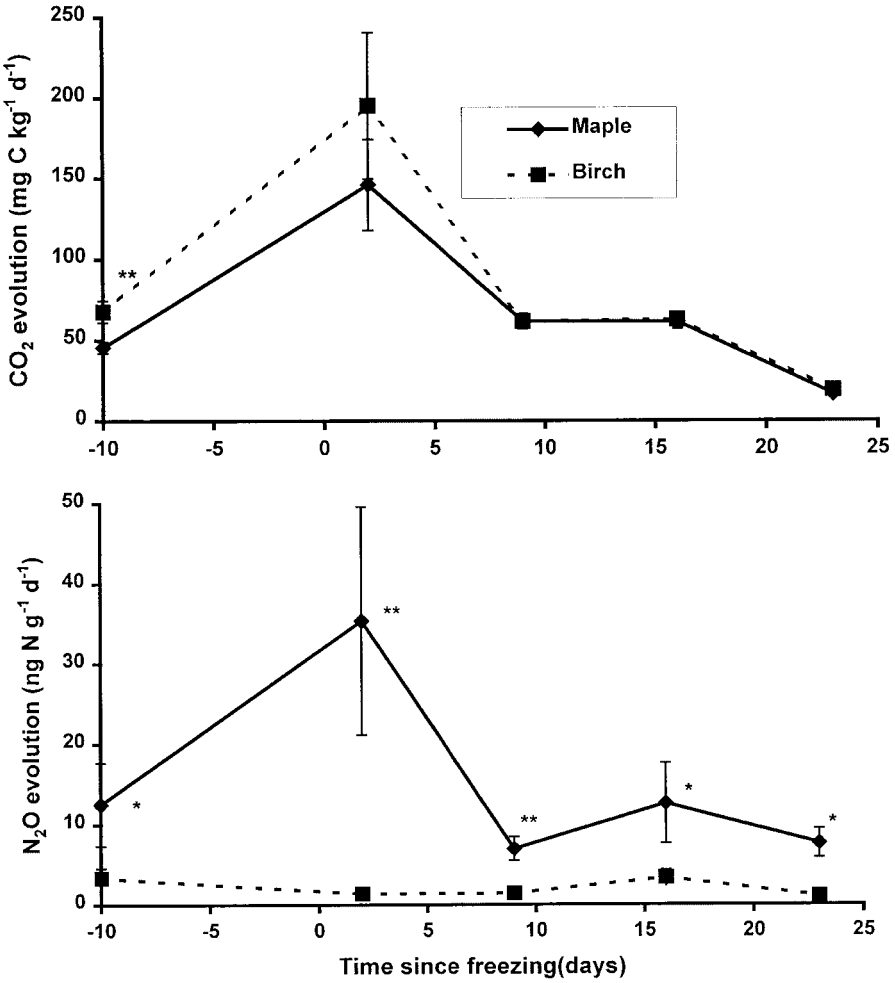

Fig. 2. Evolution of CO_2 (top panel) and N_2O (bottom panel) from O_a horizon soils from maple and birch stands. Values are means of samples from all treatments combined, with standard error. Asterisks indicate points that are significantly different at $P < 0.01$ (**) or $P < 0.05$ (*).

Respiration increased significantly ($P < 0.05$) with freezing to −13°C in the O_a and A horizons, but did not change significantly with freezing in the O_e horizon. Respiration increased with freezing to −3°C, but not significantly. These effects occurred in both species.

Similar to respiration, N mineralization varied by horizon, again with the highest rate in the O_e horizon and the lowest in the A (Table 3). In general, mineralization decreased with freezing to −13°C in the O_e, increased in the O_a and did not change in the A horizon, but these patterns were not universally significant. Mineralization showed almost no response to freezing to −3°C.

Horizon and treatment differences were not as strong for nitrification as they were for respiration and mineralization. The O_e and A horizons differed significantly in both species, but the O_a horizon differed from the A only in the maple soils (Table 4). Nitrification decreased somewhat after freezing for all horizons, temperatures

Table 2. Cumulative respiration over 33 d in soils from three horizons from maple and birch stands in control, −3 and −13°C treatments. All values are mean ± standard error.

Horizon	Treatment	Maple		Birch	
			mg C kg^{-1}		mg C kg^{-1}
O_e	Control	a x†	10 473 ± 1 255	a x	17 414 ± 2 046
	−13	x	10 213 ± 1 598	x	17 501 ± 1 514
O_a	Control	b x	1 497 ± 97	b x	1 734 ± 256
	−3	x	2 120 ± 230	x	2 866 ± 401
	−13	y	3 882 ± 258	y	5 063 ± 1 220
A	Control	c x	313 ± 22	c x	436 ± 49
	−13	y	508 ± 36	y	391 ± 74

† Letters a, b, and c indicate significant ($P < 0.05$) differences between control values for the different horizons within a species ($P < 0.05$). Letters x, y and z indicate significant ($P < 0.05$) differences between treatments within a single horizon within a species.

Table 3. Cumulative nitrogen mineralization over 33 d in soils from three horizons from maple and birch stands in control, −3 and −13°C treatments. All values are mean ± standard error.

Horizon	Treatment	Maple		Birch	
			mg N kg^{-1}		mg N kg^{-1}
O_e	Control	a x†	844 ± 96	a x	1027 ± 58
	−13	x	667 ± 71	y	832 ± 38
O_a	Control	b x	161 ± 20	b x	232 ± 27
	−3	x	147 ± 18	x	232 ± 23
	−13	y	223 ± 12	x	310 ± 42
A	Control	c x	34 ± 4	c x	41 ± 5
	−3	y	48 ± 2	x	42 ± 4

† Letters a, b, and c indicate significant ($P < 0.05$) differences between control values for the different horizons with a species ($P < 0.05$). Letters x, y and z indicate significant ($P < 0.05$) differences between treatments within a single horizon within a species.

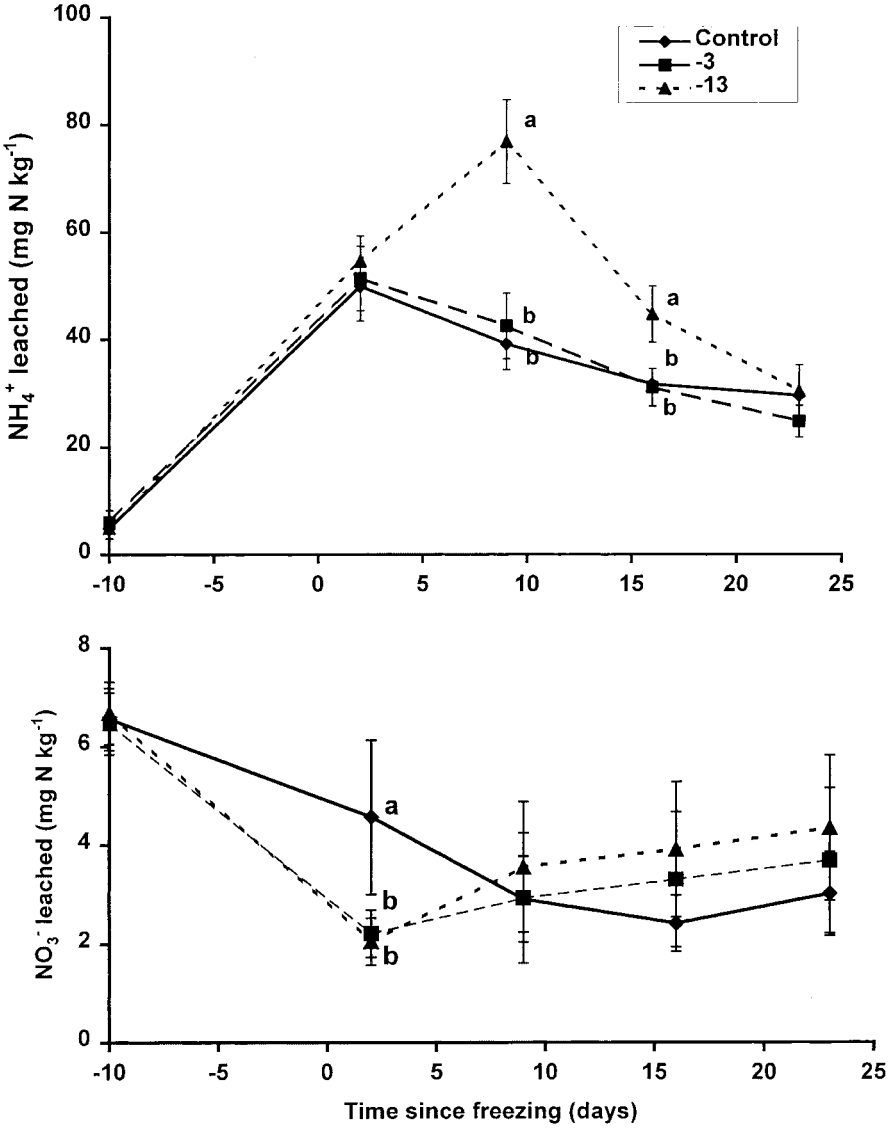

Fig. 3. Leaching of NH^+_4 (top panel) and NO^-_3 (bottom panel) from O_a horizon soils subjected to –3 and –13°C treatments. Values are means of samples from maple and birch stands combined, with standard error. Points with different superscripts are significantly different at $P < 0.05$.

and species. However, this effect was only significant ($P < 0.10$) in the O_e horizon from the maple plots.

DISCUSSION

Respiration increased immediately after freezing in soils of both tree species, suggesting that freezing increased C availability and microbial activity as expected (Edwards and Cresser, 1992). The pulse of respiration was brief (less than 7 d, Fig. 1), but total respiration over the 33-d experiment was increased by freezing in the O_a and A horizons (Table 2).

The freeze-induced increase in C availability may have been associated with microbial mortality, as has been observed in several other studies (Skogland et al., 1988; Winter et al., 1994; Schimel and Clein, 1996; Ryan et al., 2000). However, in our field studies, we did not observe any decrease in microbial biomass in soils that remained frozen (−1.0 to −4.0°C) for more than 8 wk (Groffman et al., in press). In our field studies, microbial biomass was approximately 2200 mg C kg^{-1} in treatment and reference plots of both sugar maple and yellow birch. It is likely that freeze effects on C availability other than microbial mortality, e.g., release of C occluded in soil aggregates, played a role in the increase

Table 4. Cumulative nitrification over 33 d in soils from three horizons from maple and birch stands in control, −3 and −13°C treatments. All values are mean ± standard error.

Horizon	Treatment	Maple		Birch	
		— mg N kg^{-1} —		— mg N kg^{-1} —	
O_e	Control	a x†	39 ± 9	a x	17 ± 6
	−13	y	21 ± 3	x	10 ± 3
O_a	Control	a x	28 ± 8	ab x	8 ± 2
	−3	x	28 ± 7	x	7 ± 2
	−13	x	29 ± 7	x	5 ± 2
A	Control	b x	5 ± 1	b x	1.9 ± 0.4
	−13	x	3 ± 1	x	1.4 ± 0.2

† Letters a, b, and c indicate significant ($P < 0.05$) differences between control values for the different horizons with a species ($P < 0.05$). Letters x, y and z indicate significant ($P < 0.10$) differences between treatments within a single horizon within a species.

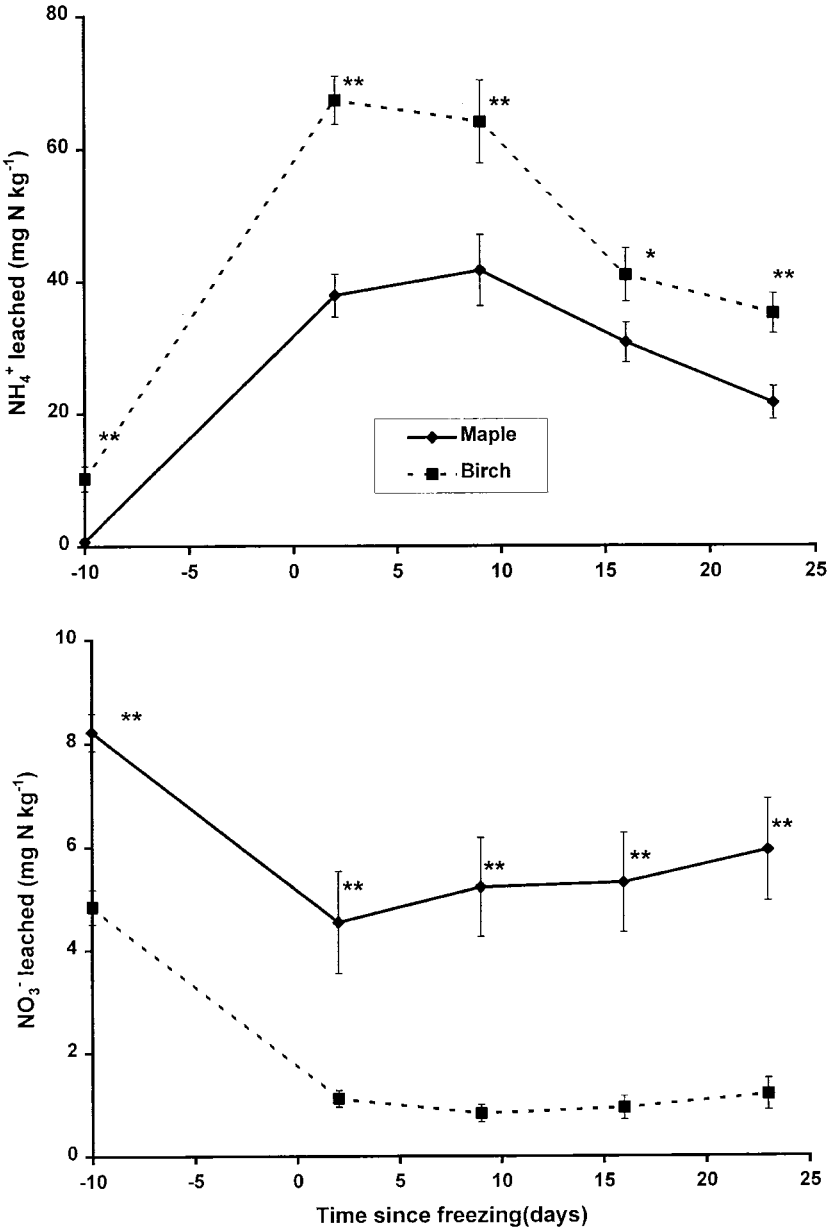

Fig. 4. Leaching of NH_4^+ (top panel) and NO_3^- (bottom panel) from O_a horizon soils from maple and beech stands. Values are means of samples from all treatments combined, with standard error. Asterisks indicate points that are significantly different at $P < 0.01$ (**) or $P < 0.05$ (*).

in respiration that we observed, especially in the −3°C treatment.

It is interesting to note that respiration was not elevated in the O_e horizon, perhaps because the amount of available carbon in this horizon was already so high that the release of carbon following freezing did not significantly increase microbial activity. It is also possible that microbes in this horizon are more adapted to freezing stress because this surface horizon likely freezes more frequently than the deeper horizons. It is important to note that the total mass of O_e horizon soil is small compared with that of the other horizons, so the importance of this horizon is minimal to overall soil respiration (Huntington et al., 1988; Bohlen et al., 2001). The O_a horizon contributes the most to the total respiration of the soil profile because it combines a relatively high respiration rate with a large total mass. Thus, it is likely that total soil profile respiration increases after severe freezing (−13°C) in these northern hardwood forest soils.

Nitrogen availability showed a complex response to freezing, with an increase in mineralization (maple only) and no effect on nitrification. Concentrations of leachable NH_4^+ increased, while leachable NO_3^- decreased, following freezing of the O_a horizon at −13°C (Fig. 2). Total mineralization over the 33-d experiment was increased in the O_a and A horizons and decreased in the O_e horizon, but these effects were only significant for

maple (Table 3). Total nitrification was not affected in the O_a and A horizons and was decreased in the O_e horizon (Table 4). These differences in the response of N availability to freezing were likely driven by variation in levels of available C between horizons and species. Total respiration data suggest that there is more available C in the birch soils, and in the O_e horizon, both before and after freezing. Increased available C likely reduces net mineralization of N in birch and O_e horizon soils, resulting in more marked increases in available N following freezing in the maple soils and in the O_a and A horizons. Kaiser et al. (1998) and Schimel and Clein (1996) also found differences in freeze response in soils from different vegetation treatments. They also suggested that these differences were driven by variation in C availability.

The lack of nitrification response to freezing is somewhat surprising. Autotrophic nitrifiers are thought to be quite susceptible to damage and to recover from stress very slowly (Focht and Verstraete, 1977). Conversely, the increase in NH_4^+ following freezing might be expected to increase nitrification. It is possible that nitrification did increase, but denitrification may also have increased, such that a significant portion of the excess NO_3^- produced after freezing was denitrified rather than leached. Although denitrification was not measured in this experiment, the increased production of N_2O upon freezing to −13°C suggests that denitrification may have increased. Several other investigators have suggested that increases in N_2O flux following freezing are due to denitrification (Edwards and Killham, 1986; Christensen and Christensen, 1991; Flessa et al., 1995; Nyborg et al., 1997; Kaiser et al., 1998; Röver et al., 1998), but we have no way to tell if this was the case in our study. The uncertain and puzzling nitrification response is further complicated by the fact that we do not know if our soils were dominated by autotrophic or heterotrophic nitrifiers. Whereas autotrophic nitrifiers are thought be quite susceptible to stress, there are no studies that have addressed the response of heterotrophic nitrifiers to freezing stress.

Freezing stimulated production of N_2O. However, as is commonly the case with this flux, high variability made it difficult to see treatment effects (Brumme et al., 1999; Groffman et al., 2000). Freezing effects on N_2O appeared to persist longer than effects on respiration, i.e., fluxes were still higher in the −13°C treatment after 33 d (Fig. 3). These data are consistent with many previous studies (cited above) that suggest that freezing events are critical controllers of the nature and extent of N_2O losses from soils.

Similar to mineralization and nitrification, there were marked differences between birch and maple in N_2O flux. Maple soils consistently had higher N_2O flux than birch soils (Fig. 4), and a more marked response to freezing as well. These results are consistent with the CO_2 data that suggest that birch soils have higher levels of available C and more conservative N cycling than those beneath maple. Following freezing stress, or even in the absence of stress, mineralized N is more likely to be reimmobilized in the C-rich birch soils than in the maple soils where nitrification, denitrification, and associated N_2O flux are more likely to occur. These results are consistent with other studies showing that maple supports relatively dynamic N cycles compared with other temperate forest canopy tree species, likely because of its high litter quality (i.e., low C:N and lignin:N) (Pastor et al., 1984, Finzi et al., 1998; Lovett and Rueth, 1999).

It is interesting to note that freezing to −3°C had very little effect on C and N cycling in these soils. Microbes appear to be able to resist the stress of mild freezing, likely because of their ability to accumulate solutes, which lowers their freezing point (Edwards and Cresser, 1992; Lipson and Monson 1998; Lipson et al., 2000). These results are in contrast to our (Groffman et al., in press) and other (cited above) field studies that report marked responses in C and N cycling, especially N_2O flux, to mild freeze events. Under field conditions, other effects of freezing, e.g., disruption of soil structure, are likely responsible for stimulation of C and N cycling. It is also possible that in laboratory studies with disturbed soils it may be difficult to see the effects of freezing above the effects of disturbance from sampling handling and preparation.

The results from this study suggest that changes in climate and snow cover that increase the frequency of soil freezing events could increase N and C losses from northern hardwood forest ecosystems, with potential effects on soil fertility and carbon storage, receiving water quality, and atmospheric chemistry. Given that freeze effects vary strongly with species, horizon, and the intensity of freezing, it will be necessary to consider each of these factors in evaluations of climate change effects on northern hardwood forest C and N cycles. These effects and factors should be incorporated into models designed to depict overwinter processes and the effects of climate change on these processes.

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