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Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest

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Abstract. Overwinter and snowmelt processes are thought to be critical to controllers of nitrogen (N) cycling and retention in northern forests. However, there have been few measurements of basic N cycle processes (e.g. mineralization, nitrification, denitrification) during winter and little analysis of the influence of winter climate on growing season N dynamics. In this study, we manipulated snow cover to assess the effects of soil freezing on *in situ* rates of N mineralization, nitrification and soil respiration, denitrification (intact core, C_2H_2 – based method), microbial biomass C and N content and potential net N mineralization and nitrification in two sugar maple and two yellow birch stands with reference and snow manipulation treatment plots over a two year period at the Hubbard Brook Experimental Forest, New Hampshire, U.S.A. The snow manipulation treatment, which simulated the late development of snowpack as may occur in a warmer climate, induced mild (temperatures > -5 °C) soil freezing that lasted until snowmelt. The treatment caused significant increases in soil nitrate (NO_3^-) concentrations in sugar maple stands, but did not affect mineralization, nitrification, denitrification or microbial biomass, and had no significant effects in yellow birch stands. Annual N mineralization and nitrification rates varied significantly from year to year. Net mineralization increased from $\sim$12.0 g N m^{-2} y^{-1} in 1998 to $\sim$22 g N m^{-2} y^{-1} in 1999 and nitrification increased from $\sim$8 g N m^{-2} y^{-1} in 1998 to $\sim$13 g N m^{-2} y^{-1} in 1999. Denitrification rates ranged from 0 to 0.65 g N m^{-2} y^{-1}. Our results suggest that mild soil freezing must increase soil NO_3^- levels by physical disruption of the soil ecosystem and not by direct stimulation of mineralization and nitrification. Physical disruption can increase fine root mortality, reduce plant N uptake and reduce competition for inorganic N, allowing soil NO_3^- levels to increase even with no increase in net mineralization or nitrification.

Introduction

Overwinter processes are critical controllers of drainage loss of elements from temperate forest ecosystems (Rascher et al. 1987; Likens & Bormann 1995; Stottlemyer & Toczydlowski 1996, 1999; Brooks et al. 1999). Despite this importance, there have been few measurements of biogeochemical processes during winter, and very few studies of the factors that influence spatial and temporal variation in these processes. Previous studies have demonstrated that root and microbial processes are surprisingly active in cold (0–5 °C) and even frozen soils and that a significant portion (20–50%) of ecosystem C and N cycling and soil-atmosphere trace gas fluxes can occur during winter (Vogt et al. 1986; Coxson & Parkinson 1987; Taylor & Jones 1990; Sommerfeld et al. 1993; Clein & Schimel 1995; Melloh & Crill 1995; Hobbie & Chapin 1996; Brooks et al. 1997; Alm et al. 1999). Overwinter processes appear to be highly variable, subject to perturbation (e.g. climate change), and to strongly influence nutrient losses during the subsequent growing season (Mitchell et al. 1996; Brooks et al. 1998; Williams et al. 1998; Groffman et al. 1999; Hardy et al. 1999).

The mechanisms of N retention in forested watersheds have been an important focus of research in ecosystem ecology. There is great current interest in the ability of forest ecosystems to continue to retain N despite high rates of atmospheric deposition and/or fertilization (Aber et al. 1989; Foster et al. 1997; Fenn et al. 1998; Lovett et al. 2000; Magill et al. 2000). Overwinter and snowmelt processes may be critical to high rates of N retention in northern forests (Mitchell et al. 1996; Murdoch et al. 1998; Brooks et al. 1999). However, there have been very few measurements of basic N cycle processes (e.g. mineralization, nitrification, denitrification) during winter and very little analysis of the influence of winter climate on growing season N dynamics.

In recent years, there has been an increasing focus on the importance of forest canopy tree species composition and litter quality as factors controlling N mineralization and nitrification rates in temperate forest soils (Pastor et al. 1984; Zak et al. 1989; Hart et al. 1997; Finzi et al. 1998; Lovett & Rueth 1999; Lawrence et al. 2000). Variation in tree species may also influence patterns of N retention in response to perturbations such as increased N deposition, drought, ice damage and soil freezing, but very few studies have addressed these responses (Lovett et al. 2000). Soil organic matter quality, which is strongly influenced by tree species composition, has been shown to affect the nature and extent of soil microbial response to freeze/thaw and dry/rewet perturbations (Schimel & Clein 1996).

The Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire, U.S.A. (*www.hbrook.sr.unh.edu*) has been the site of numerous biogeochemical studies. Much of the work at HBEF has focused on whole ecosystem element budgets of northern hardwood forest watersheds. While the importance of snowpack dynamics and winter climate on these budgets has been noted (Likens & Bormann 1995), there have been few detailed studies or experimental manipulations focused on overwinter processes at this site. Moreover, there have been relatively few intensive studies of *in situ* N cycle processes (e.g. mineralization, nitrification, denitrification) at the HBEF (Likens et al. 1969; Bormann et al. 1977; Melillo 1977; Federer 1983; Duggin et al. 1991; Christ et al. 1995; Bohlen et al. 2001). Interest in these processes in the soils at HBEF has increased in recent years as rates of atmospheric deposition have increased, forest biomass has ceased aggrading and stream nitrate (NO_3^-) exports have decreased (Likens et al. 1996; Aber & Driscoll 1997).

In this study, we utilized a snow cover manipulation to assess the effects of soil freezing on *in situ* rates of N mineralization, nitrification and soil respiration, intact core-based measurements of denitrification and seasonal levels of microbial biomass C and N content and potential net N mineralization and nitrification in two sugar maple and two yellow birch stands at the HBEF, over two years. The snow manipulation treatment, which was designed to simulate the late development of snowpack as may occur in a warmer climate, and its effects on fine root dynamics, soil solution chemistry and trace gas fluxes are described elsewhere (Groffman et al. 1999; Neilsen et al. 2001; Hardy et al. this issue; Fitzhugh et al. this issue; Tierney et al. this issue). The specific objectives of this study were to (1) evaluate the effects of snow manipulation on winter and summer soil N dynamics and (2) describe seasonal and annual patterns of net N mineralization, nitrification, soil respiration and denitrification in soils beneath two of the dominant canopy tree species at HBEF.

Methods

Experimental design

The HBEF is located in the White Mountain National Forest in New Hampshire, U.S.A. (43°56′ N, 71°45′ W). Vegetation at the HBEF is dominated by American beech (*Fagus grandiflora*), sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghanieusis*). The forest was selectively cut in the 1880’s and 1910’s, 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 unsorted basal tills.

The experimental design for the snow manipulation study consisted of 8, 10 × 10-m plots. The plots were located within four stands, two of each species (80% dominance by the target species), with one snow reduction (treatment) and one reference plot in each stand. In the fall and winter of 1996, we cleared minor amounts of understory vegetation from all (both treatment and reference) plots (to facilitate shoveling). We then installed soil solution samplers (zero tension lysimeters), thermistors for soil temperature monitoring, soil water content monitors (time domain reflectometers), soil atmosphere sampling probes, minirhizotron access tubes and trace gas flux measurement chambers as described elsewhere (Groffman et al. 1999; Hardy et al. this issue; Fitzhugh et al. this issue; Tierney et al. this issue). All plots were equipped with dataloggers to allow for continuous monitoring of soil moisture and temperature. All instruments were installed in fall 1996 to allow for any installation-related disturbance effects to subside before the treatment was introduced in fall/winter of 1997.

From late November until early February in the winters of 1997/1998 and 1998/1999 the treatment plots were kept snow-free to simulate a reduced snowfall, while the reference plots accumulated snow at ambient rates. As soon as practical after each snowfall, shovels were used to clear the treatment plots of the new snow. We allowed a few cm of snow to compact on the ground to protect plot cables and the forest floor from shovel damage, and to increase the albedo of the forest floor to aid in soil freezing.

From fall 1997–fall 1999, we sampled lysimeters, measured trace gas fluxes and collected video images for roots at weekly to monthly intervals. Thermistors measured soil temperatures every 0.1 m to a depth of 0.5 m and snow temperatures every 0.2 m to a height of 0.8 m. We measured snow and soil temperatures every 5 minutes and data were stored as hourly averages on a datalogger. Every two weeks, we made manual measurements of snow depth variability (n = 100), snow density and snow water equivalence at two sites. Results of these measurements are reported elsewhere in this issue.

In situ *net N mineralization and nitrification*

Net N mineralization and nitrification were measured using an *in situ* intact core method (Robertson et al. 1999). During the growing season, 10, 2-cm diameter intact cores were removed from each plot. Five of the cores were returned to the laboratory for extraction (2 N KCl) of inorganic N (NH_4^+ and NO_3^-) and five were returned to the plot for *in situ* incubation. Cores were incubated for approximately 4 weeks before harvesting and extraction. Inorganic N was quantified colorometrically using a Perstorp^{TM} 3000 flow injection analyzer. Net N mineralization rates were calculated as the accumulation of total inorganic N over the course of the incubation. Net nitrification rates were calculated as the accumulation of NO_3^- over the course of the incubation. Values were converted to an areal basis (g N m^{-2}) using forest floor depth and bulk density values and mineral soil (to 10 cm) density values from Bohlen et al. (2001).

For overwinter incubation, approximately 25 cores were collected in late November. Five of these cores were returned to the laboratory for immediate extraction and 20 were left to incubate *in situ*. Five cores were then harvested at approximately 4 wk intervals (January, February, March, April). Rates of N mineralization and nitrification were calculated from the month-to-month accumulation of total inorganic N and NO_3^- in the incubated cores. The November samples served as the 'initial' extractions for all overwinter months (December through March) due to the difficulty of sampling frozen soil (Stottlemyer & Toczydlowski 1996).

Soil respiration

Soil respiration rates were measured *in situ* using the Li-Cor^{TM} 6400 portable soil CO_2 flux measurement system. Permanent chamber bases (10 cm diameter, 10 per plot) were placed in all plots in spring 1998. Rates were measured at approximately monthly intervals from June through November 1998, and from April through July 1999.

Denitrification

All cores that were collected for *in situ* N mineralization and nitrification analyses were also assayed for denitrification using an acetylene (C_2H_2)-based intact core method (Groffman et al. 1999). Cores were brought back to the laboratory, amended with 10 kPa of acetylene and incubated at laboratory temperature for 6 hr. Gas samples were taken at 2 and 6 hr and stored in evacuated glass tubes until analysis for nitrous oxide (N_2O) concentrations by electron capture gas chromatography. Denitrification values from overwinter cores are not reported because the laboratory incubation likely created artifactual results due to the extreme difference between laboratory and *in situ* temperatures during winter. Annual estimates of denitrification were produced by extrapolating mean daily rates, assuming 274 days of activity per year.

Microbial biomass C and N, and potential net N mineralization and nitrification

Microbial biomass C and N content were measured using the chloroform-fumigation incubation method (Jenkinson & Powlson 1976), twice in 1998 (April, July) and three times in 1999 (April, July, October). Samples were fumigated to lyse microbial cells, inoculated with fresh soil and incubated for 10 days at 25 °C at field moisture content. Carbon dioxide and NH_4^+ released during the incubation were assumed to be directly proportional to the amount of C and N in the microbial biomass of the original sample. Carbon dioxide (CO_2) was measured by thermal conductivity gas chromatography and NH_4^+ was quantified colorometrically after KCl extraction as described above. A proportionality constant ($k_c = 0.41$) was used to calculate biomass C from the CO_2 produced during the incubation. No proportionality constant was used for biomass N. Biomass N was equivalent to the flush of inorganic N produced following fumigation and incubation.

Statistical analysis

Overall treatment and vegetation effects were assessed with two-way repeated measures analysis of variance (with interactions) of plot means from each sampling date with treatment and vegetation as main effects. Seasonal values were computed by taking the mean of all sampling dates within a season (December–March = winter, April–June = spring, July–September = summer, October–November = fall) and multiplying by either 120 (winter) or 81.67 days (spring, summer, fall). Annual values were computed by summing the seasonal values. Seasonal and annual values were analyzed by two-way analysis of variance (with interactions) without repeated measures. The Statistical Analysis System (SAS 1989) was used for all analyses.

Results

Rates of net N mineralization and nitrification were highest in summer, and in the forest floor (Figure 1). There were very few significant differences in seasonal or annual rates of net N mineralization and nitrification between treatment and reference plots (Table 1). On an annual basis, rates of mineralization and nitrification were always higher in treatment plots than reference plots, in both the forest floor and mineral soil (Table 1), but these differences were not statistically significant. Rates of nitrification were consistently higher (but not significantly) in treatment plots in spring (April–June), but effects in other seasons were more varaible (Table 1).

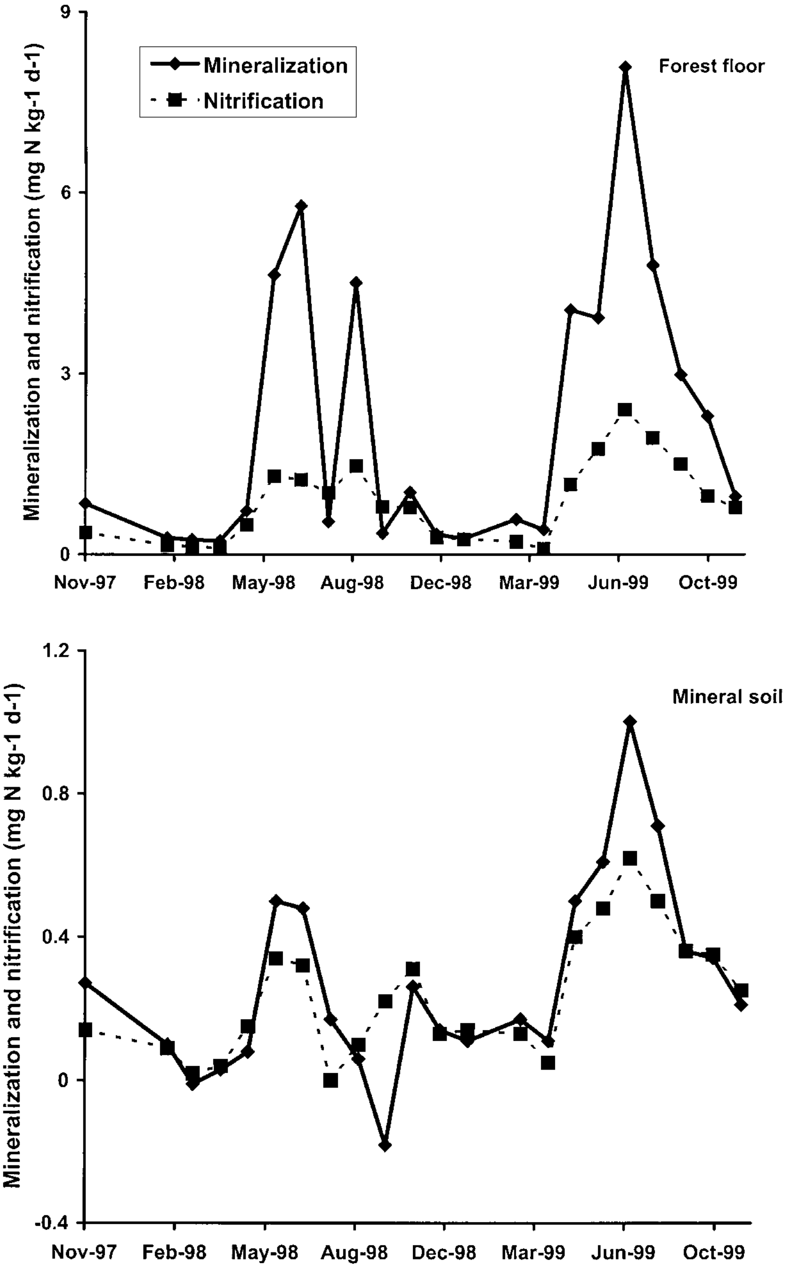

Figure 1. Temporal patterns of *in situ* net mineralization and nitrification in the forest floor (top panel) and mineral soil (bottom panel) from fall 1997–fall 1999. Values are the mean of two sugar maple and two yellow birch sands with reference and treatment plots (n = 8 for each point).

Table 1. Seasonal and annual *in situ* net N mineralization and nitrification rates in forest floor and mineral soil of reference and treatment plots in 1997/1998 and 1998/1999. Seasonal values were produced by computing a mean seasonal rate from 2–4 sample dates in each season and then multiplying by either by 120 (winter) or 81.67 (spring, summer, fall) days. Values are mean (standard error) for two sugar maple and two yellow birch stands with treatment and reference plots (n = 4). Annual values are the sum of the seasonal values. *, **, *** indicate significant differences between treatments or years at $p < 0.01$, 0.05, 0.10 respectively

Horizon	Year/season	Net N mineralization		Net nitrification	
		Reference	Treatment	Reference	Treatment
		g N m^{-2} y^{-1} or season			
Forest floor	1997/1998	7.1 (1.0)	8.9 (1.7)	3.0 (0.9)	3.5 (0.4)
	Winter	0.8 (0.3)	0.5 (0.3)	0.3 (0.1)	0.1 (0.1)
	Spring	1.8 (0.3)***	3.8 (1.2)	0.7 (0.2)	1.2 (0.3)
	Summer	3.7 (0.4)	4.0 (1.0)	1.3 (0.4)	1.3 (0.2)
	Fall	0.8 (0.2)	0.7 (0.3)	0.8 (0.3)	0.9 (0.1)
Mineral soil	1997/1998	4.8 (1.1)	4.3 (1.8)	4.1 (1.6)	4.8 (0.6)
	Winter	0.6 (0.8)	1.7 (0.7)	0.5 (0.3)	0.6 (0.3)
	Spring	1.5 (0.5)	2.1 (0.5)	1.2 (0.5)	1.8 (0.3)
	Summer	1.6 (0.4)	1.2 (1.5)	0.9 (0.3)	0.9 (0.5)
	Fall	1.2 (0.1)	−0.7 (0.9)	1.5 (0.6)	1.7 (0.5)
Forest floor	1998/1999	12.2 (1.9)	12.0 (0.7)	4.4 (0.9)	5.0 (0.9)
	Winter	0.7 (0.2)	0.7 (0.1)	0.3 (0.1)	0.2 (0.1)
	Spring	4.2 (0.8)	4.1 (0.7)	1.3 (0.3)	1.7 (0.2)
	Summer	5.8 (0.7)	5.7 (0.1)	1.9 (0.4)	2.2 (0.5)
	Fall	1.6 (0.4)	1.6 (0.0)	1.0 (0.3)	0.9 (0.2)
Mineral soil	1998/1999	9.7 (2.6)	11.1 (0.8)	7.8 (2.2)	8.5 (1.7)
	Winter	1.2 (0.5)	1.3 (0.5)	0.7 (0.3)	0.8 (0.3)
	Spring	2.5 (1.5)	4.2 (0.3)	2.4 (0.8)	2.9 (0.6)
	Summer	4.6 (0.6)	3.7 (0.3)	3.2 (0.7)	2.8 (0.6)
	Fall	1.4 (0.5)	1.9 (0.3)	1.6 (0.5)	2.0 (0.5)
Whole profile	1997/1998	11.9 (1.7)*	13.2 (2.6)*	7.1 (2.3)**	8.4 (0.9)**
Whole profile	1998/1999	21.9 (3.4)	23.1 (1.0)	12.2 (2.9)	13.5 (2.5)

Yellow birch had higher rates of N mineralization than sugar maple, but only in 1999 (Figure 2). There were no significant species effects on nitrification (Figure 2).

Mineralization and nitrification rates were higher ($p < 0.05$ or 0.01) in 1998/1999 than in 1997/1998 in both the forest floor and mineral soil

Table 2. Annual denitrification rates in 1997/1998 and 1998/1999. Values are derived from the means of 7 sample dates in 1998 and 10 sample dates in 1999 for two sugar maple and two yellow birch stands with treatment and reference plots. Mean rates were extrapolated to an annual value, assuming 274 days of activity per year

Year	Sugar maple		Yellow birch	
	Reference	Treatment	Reference	Treatment
	$g\ N\ m^{-2}\ y^{-1}$			
1998	0.14 (0.04)	0.53 (0.34)	0.14 (0.04)	0.09 (0.04)
1999	0.65 (0.37)	0.12 (0.13)	−0.59 (0.43)	0.69 (0.36)

(Table 1). Between 7 and 15% of annual whole soil profile mineralization and nitrification occurred during winter (December–March). Between 40 and 64% of total soil profile mineralization activity took place in the forest floor.

The treatment increased soil NO_3^- concentrations in both the forest floor (Figure 3) and mineral soil (Figure 4), but the overall effect was only significant ($p < 0.10$) in the forest floor of the sugar maple stands. Soil NO_3^- concentrations were higher ($p < 0.01$) in sugar maple than yellow birch soils, but the overall effect was only significant for treated plots, in the forest floor. Soil NH_4^+ and total inorganic N (NO_3^- plus NH_4^+) concentrations were not affected by treatment or vegetation type.

Soil respiration rates were not affected by the snow manipulation treatment (data not presented). However, rates were higher ($p < 0.01$) in yellow birch than sugar maple, in both 1998 and 1999 (Figure 5(a)). Respiration rates were higher ($p < 0.01$) in June and July of 1999 than in the same months in 1998 (Figure 5(b)).

There were no significant treatment, vegetation or seasonal effects on rates of denitrification in 1998 (Table 2). In 1999, there was a significant ($p < 0.05$) treatment effect within the yellow birch stands and a significant ($p < 0.05$) vegetation effect within the control treatment. These differences were due to negative denitrification rates in yellow birch stands during summer, which were likely due to a methodological artifact (Groffman et al. 1999). Denitrification rates ranged from 0–0.65 g N m^{-2} y^{-1}. Due to lack of seasonal effects, only annual means are presented in Table 2.

Microbial biomass C and N concentrations and potential net N mineralization and nitrification rates were not affected by treatment (Table 3) or species (Table 4) in any season. Potential net nitrification rates were always higher in sugar maple than yellow birch and potential net N mineralization

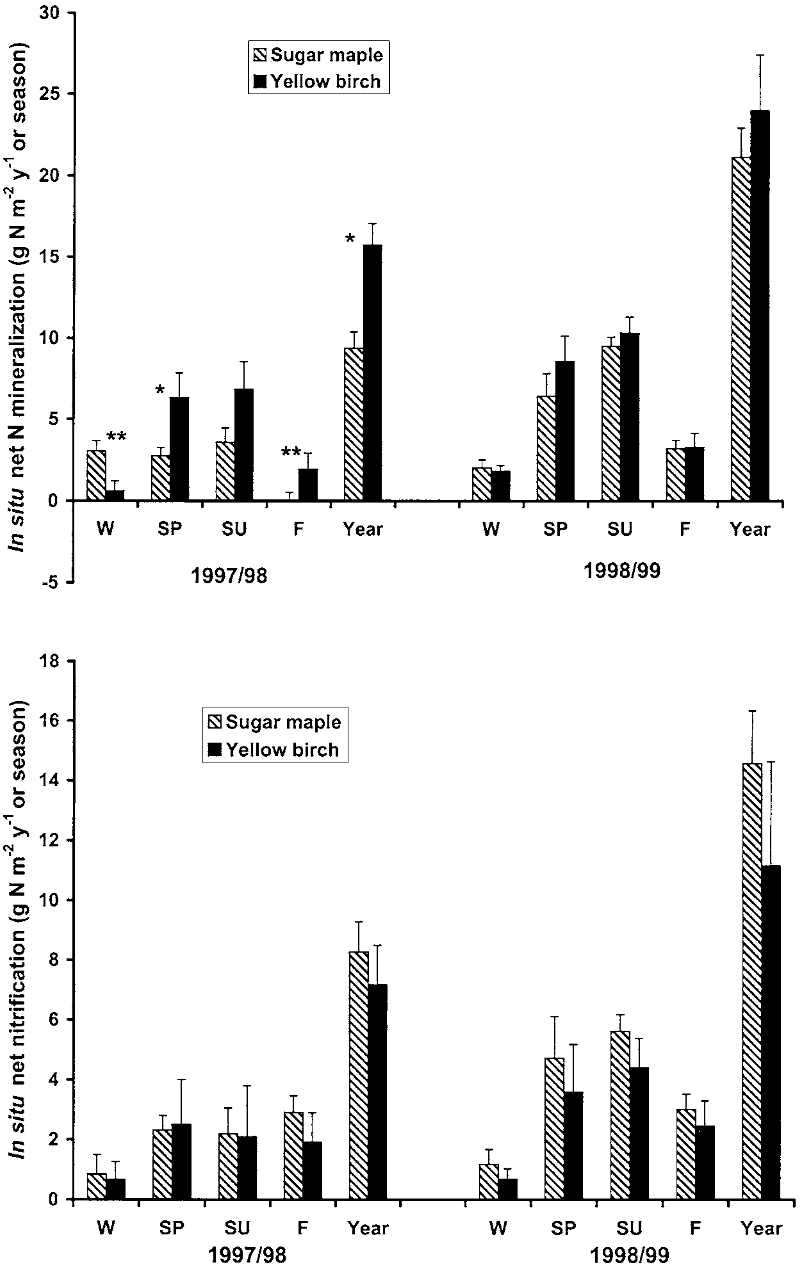

Figure 2. Seasonal and annual *in situ* net N mineralization (top panel) and nitrification (bottom panel) in the forest floor and mineral soil (0–10 cm) of sugar maple and yellow birch stands from fall 1997 to fall 1999. Values are the mean (with standard error) of two reference and two treatment plots per date ($n = 4$ four each point). *, ** indicates a significant difference between vegetation types at $p < 0.05$ and 0.10 respectively.

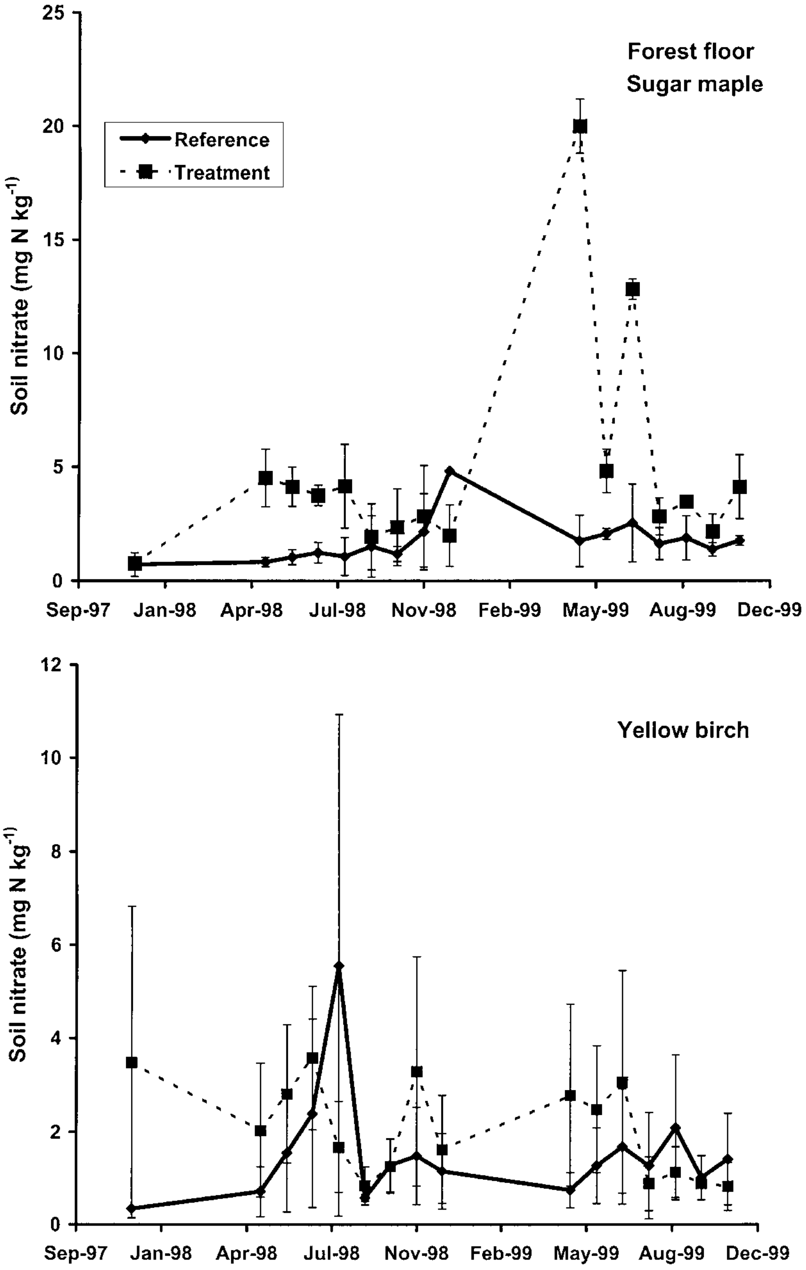

Figure 3. Soil nitrate in the forest floor of reference and treatment plots in sugar maple (top panel) and yellow birch (bottom panel) stands from fall 1997–fall 1999. Values are the mean (with standard error) of two reference and two treatment plots per date (n = 4 for each point).

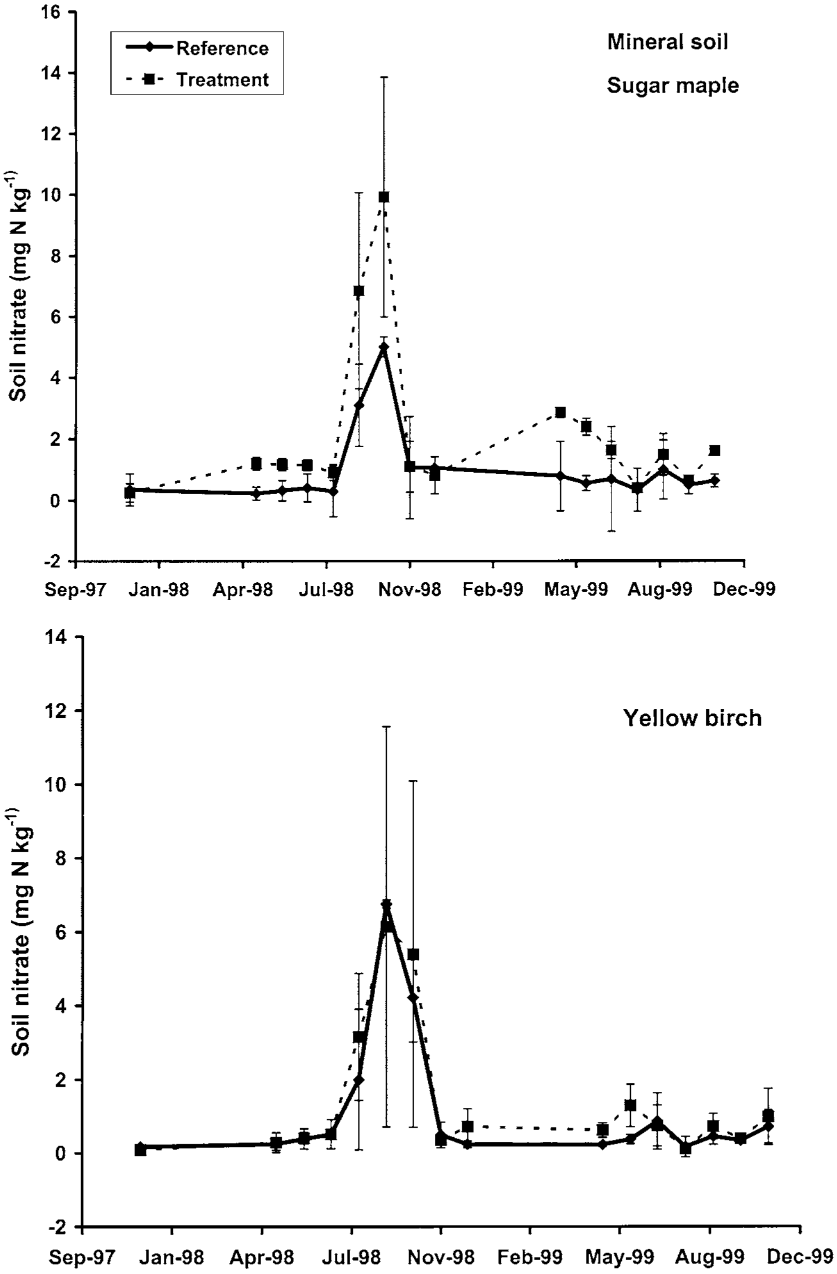

Figure 4. Soil nitrate in the mineral soil (0–10 cm) of reference and treatment plots in sugar maple (top panel) and yellow birch (bottom panel) stands from fall 1997–fall 1999. Values are the mean (with standard error) of two reference and two treatment plots per date (n = 4 for each point).

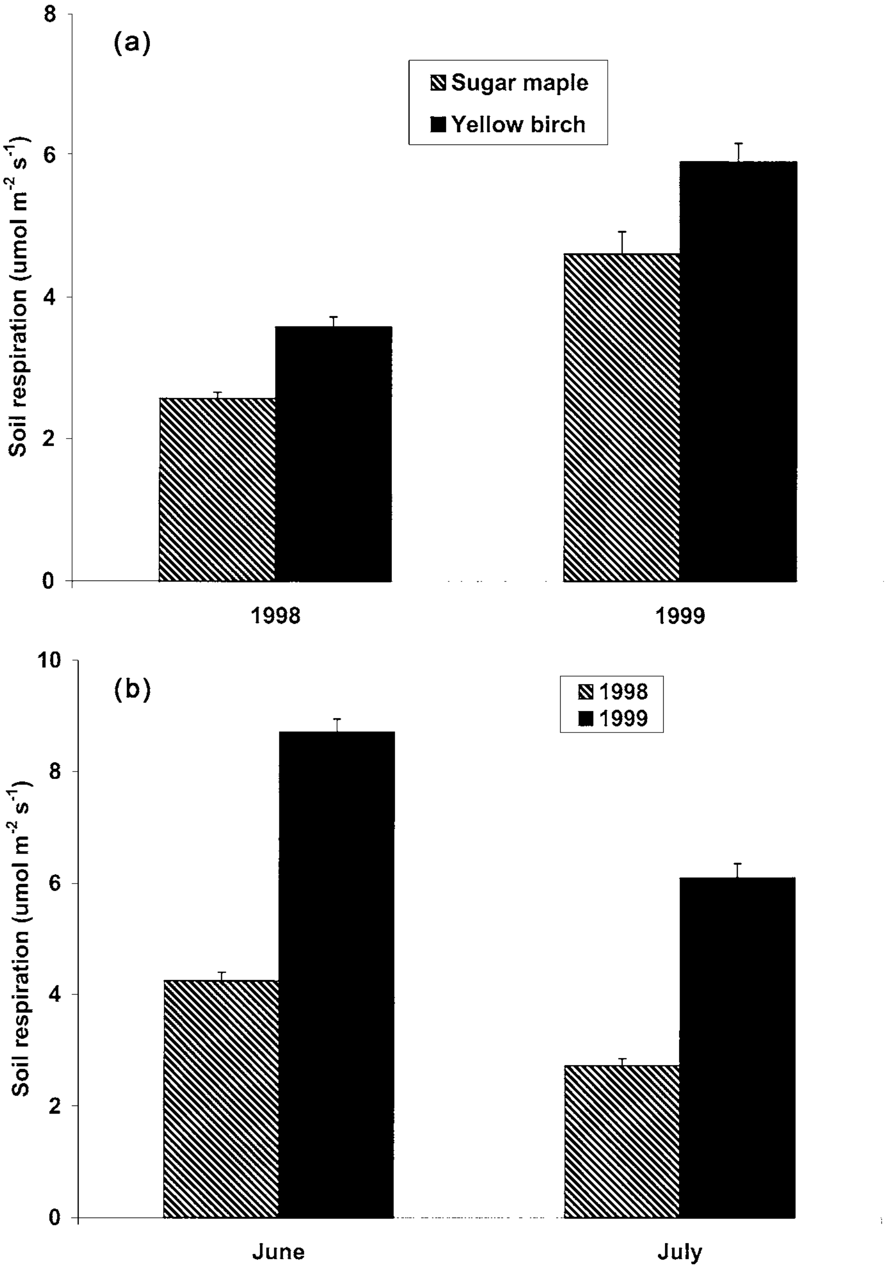

Figure 5. In situ soil respiration rates. (a): Mean values (with standard error) of reference and treatment plots in two sugar maple and two yellow birch sites sampled at 10 dates between June 1998 and July 1999. (b): Main values (with standard error) of all eight plots (two sugar maple and two yellow birch stands with reference and treatment plots) comparing sample dates in June and July of 1998 and 1999.

Table 3. Microbial biomass C and N in reference and treatment plots in two sugar maple and two yellow birch stands in 1998 and 1999. Values are the mean (with standard error) of four reference and four treatment plots sampled at two dates (April, July) in 1998 and three dates (April, July, October) in 1999

	1998		1999	
	Reference	Treatment	Reference	Treatment
Microbial biomass C ($mg\ C\ kg^{-1}$)	2359 (386)	2376 (387)	2162 (281)	2171 (273)
Microbial biomass N ($mg\ N\ kg^{-1}$)	304 (48)	341 (57)	251 (36)	244 (36)
Potential net N mineralization ($mg\ N\ kg^{-1}\ d^{-1}$)	12.5 (2.9)	13.9 (3.2)	5.1 (1.0)	5.1 (1.0)
Potential net nitrification ($mg\ N\ kg^{-1}\ d^{-1}$)	4.2 (0.9)	4.9 (0.9)	2.9 (0.6)	2.9 (0.5)

Table 4. Microbial biomass C and N in sugar maple and yellow birch stands in 1998 and 1999. Values are the mean (with standard error) of both treatment and reference plots for two stands for each vegetation type sampled at two dates (April, July) in 1998 and three dates (April, July, October) in 1999. Different superscripts indicate statistically significant ($p < 0.05$) differences between vegetation types

	1998		1999	
	Sugar maple	Yellow birch	Sugar maple	Yellow birch
Microbial biomass C ($mg\ C\ kg^{-1}$)	2616 (440)	2135 (321)	2329 (282)	2004 (268)
Microbial biomass N ($mg\ N\ kg^{-1}$)	274 (38)	370 (61)	245 (35)	250 (37)
Potential net N mineralization ($mg\ N\ kg^{-1}\ d^{-1}$)	10.6 (2.3)	15.8 (3.6)	4.9 (0.9)	5.3 (1.1)
Potential net nitrification ($mg\ N\ kg^{-1}\ d^{-1}$)	5.3 (1.0)	3.7 (0.8)	3.8 (0.6)^{a}	2.0 (0.4)^{b}

showed an opposite pattern, but these trends were rarely significant (Table 4). Microbial biomass C and N concentration and potential net N mineralization and nitrification did not vary significantly with season (data not shown) but were higher in 1998 than in 1999.

Discussion

Snow manipulation effects

The snow manipulation treatment produced mild, but persistent soil freezing in both 1997/1998 and 1998/1999. Soils froze to at least 10 cm depth by mid January and stayed frozen through mid March, but soil temperatures seldom decreased below −4 °C (Hardy et al. this issue). The treatment reduced infiltration and soil moisture by approximately 15% during snowmelt but had no effect on these variables during the growing season (Hardy et al. this issue). The treatment significantly increased fine root mortality in spring and increased fine root production during summer in both years (Tierney et al. this issue). There were marked increases in soil solution losses of inorganic N and P, dissolved organic C and N and base cations and increases in the acidity of drainage water in treatment plots (Fitzhugh et al. this issue; Fitzhugh 2000).

Treatment effects on soil N cycle processes were complex and surprising in several ways. We originally hypothesized that soil freezing would result in root and microbial mortality, which would lead to increases in hydrologic and gaseous N losses as the dead root and microbial cells were decomposed, mineralized and nitrified. However, soil temperatures in the treatment plots, which seldom decreased below −4 °C, were less severe than those shown to directly cause root or microbial mortality in short-term laboratory experiments (Sakai & Larcher 1987; Edwards & Cresser 1992). The treatment did increase fine root mortality, perhaps due to the duration of the freeze, the cycles of freezing and thawing, or via physical disturbance due to ice expansion, formation of ice lenses and/or capillary water movement associated with frost heaving. The N release associated with fine root mortality (Tierney et al. this issue) was a significant fraction of the hydrologic N loss reported by Fitzhugh et al. (this issue) at some sites. However, the fact that we did not observe an increase in N mineralization rates in the treatment plots suggests that root mortality was not the only source of N loss from the treatment. Other processes, such as reduced N uptake by fine roots and physical disturbance of root-soil-microbial interactions, likely led to reduced competition for inorganic N and the increases in soil NO_3^- concentrations and NO_3^- leaching that we observed. Mild freezing appears to disrupt the normally tight coupling between mineralization, immobilization and plant uptake (Pawluk 1988; Miller & Jastrow 1990). This coupling is considered to be an important controller of N losses from forest ecosystems (Bormann & Likens 1979; Mullen et al. 1998).

Our results have implications for assessments of the function of forest ecosystems under changing climatic conditions. Mild freeze events are likely to become more common if snowpacks develop later and melt earlier, as is predicted to occur as the climate warms due to increased atmospheric CO_2 levels (Cooley 1990). These effects are distinct from the more dramatic root and microbial mortality-induced freeze effects that we originally set out to study. However, mild freeze events are more likely to be increasingly common than severe freeze events in a warmer world.

Mild freeze effects may be highly variable and mechanistically complex. In contrast to our results, Boutin and Robitaille (1994) found that superficial frost (induced by a one week snow removal treatment) had no effect on NO_3^- leaching, while deep frost (induced by full season snow removal) caused extensive tree mortality and large increases in NO_3^- leaching. It is possible that their superficial frost treatment was too mild to cause physical disruption of soil-root-microbial interactions.

While we did not observe any treatment effects on microbial biomass, Brooks et al. (1997) and Lipson et al. (2000) found that microbial biomass was highly variable, and functioned as an important N 'buffer' during snowmelt. They observed greater biomass at sites with deep continuous snow cover where soils remained thawed, and lower biomass at sites with shallow discontinuous snow cover that had frozen soils. We did not sample frequently enough during snowmelt to detect dynamic temporal changes in microbial biomass as these investigators did. Also, temperatures in their studies were lower (-6 to -10 °C), possibly low enough to cause microbial mortality.

Similar to microbial biomass, rapidly changing conditions during snowmelt may have contributed to our inability to detect a treatment effect on rates of denitrification. Nyborg et al. (1997) found very high rates of denitrification (over 20 kg N ha^{-1}) in a 10-day period just around snowmelt. Future studies of freeze events should consider very high frequency (e.g. daily or twice a week) sampling for both microbial biomass and denitrification during snowmelt and/or soil thaw.

Our results suggest that forest species composition will be an important regulator of the response of forest ecosystems to increased freeze frequency. Sugar maple treatment plots had higher soil NO_3^- concentrations and higher rates of potential net nitrification than yellow birch treatment plots. This contrasting response was likely linked to differences in internal N dynamics between these species, driven either by inherent differences in the degradation rates of litter or by differences in mycorrhizal associations (yellow birch is an ectomycorrhizal species, while sugar maple is endomycorrhizal). The fact that soil respiration rates were higher in yellow birch than sugar maple suggests that soil C flux and therefore immobilization and/or root uptake pressure were higher under yellow birch. Our results are consistent with recent studies that suggest that natural or anthropogenic factors that influence species composition will be critical determinants of ecosystem response to environmental change (Tilman 1998; Lovett & Rueth 1999; Nielsen et al. submitted).

In a more basic sense, our study raises questions about the importance of freeze frequency as a regulator of the nature and extent of N cycling and loss in forest ecosystems. We suggest that freeze frequency should be considered in large-scale functional evaluations of ecosystems, much like the distinctions that are made between deciduous and evergreen forests or between temperate and tropical forests. Forests with soils that freeze frequently, e.g. those in areas south of our site with much less snow cover, may have inherently different patterns of element cycling, storage and loss than those that do not.

The importance of overwinter activity

Winter activity proved to be less important than we originally hypothesized. Given that snow covered soils generally do not freeze, and previous studies suggesting that a high percentage (20–50%) of C and N cycling occur overwinter (see citations in Introduction), we were surprised that only between 5 and 15% of annual mineralization and nitrification activity took place during winter. Our results are similar to those obtained by Hill and Shackleton (1989), Boone (1992), Stottlemyer and Toczydlowski (1996) and Lamontagne (1998), who also found very low rates of N mineralization and/or nitrification during winter in temperate forest ecosystems.

In contrast to our results, Brooks et al. (1996) found that overwinter N mineralization in alpine tundra at Niwot Ridge, Colorado U.S.A. was greater than growing season activity (2–6 g N m^{-2} in winter vs. 0.9–1.2 g N m^{-2} in summer). Williams et al. (1998) found that denitrification N gas loss was greater than annual atmospheric input at Niwot Ridge. It is important to note that summer activity at Niwot Ridge is likely limited by low soil moisture and that annual rates of N cycle possesses there are low relative to HBEF.

Similar to Brooks et al. (1996), DeVito et al. (1999), working in maple/beech/poplar stands in Ontario, Canada more similar to HBEF than Niwot Ridge, found 49% of annual mineralization and 23% of annual nitrification took place overwinter. They suggest that methodological differences (e.g. leaving overwinter cores in the ground all winter) may lead to underestimation of rates. There is a clear need for more research, with better methods, to determine the factors controlling variation in overwinter activity in forest ecosystems.

Annual fluxes and variability

This paper presents an extensive data set on N mineralization, nitrification and denitrification for the HBEF, which has been the site of numerous whole-watershed N analyses. Annual rates of N mineralization in 1998 (11.9–13.2 g N m^{-2} y^{-1}) were similar to those reported for other, similar forest ecosystems (Melillo 1977; Scott & Binkley 1997; Reich et al. 1997; DeVito et al. 1999; Goodale et al. 2001), but the rates in 1999 (around 23 g N m^{-2} y^{-1}) are high compared to other studies. The fact that our estimates include both the forest floor and the top 10 cm of the mineral soil, while many studies ignore, or include variable amounts of mineral soil, may account for our higher values. In our studies, the mineral soil accounted for from 40 to 60% of the total mineralization and nitrification activity. The distribution of activity within the soil profile that we observed is similar to that reported by Federer (1983) and Fisk and Fahey (1990) for HBEF soils.

The nitrification rates that we measured (from 7–13.5 g N m^{-2} y^{-1}) are high relative to previous work at Hubbard Brook (Melillo 1977) and at other similar forest sites in northeastern North America (Campbell et al. 2000; Goodale et al. 2001). Nitrification as a percentage of N mineralization was approximately 60% of mineralization, which is also high compared to Melillo's (1977) observations from similar forests at HBEF. It is not surprising that nitrification has increased at HBEF from the mid-1970's to the late 1990's as these forests have aged and stopped aggrading (Bormann & Likens 1979).

Our estimates of annual denitrification ranged from negligible, which was expected for these forests (Bowden 1986; Barton et al. 1999), to more than 0.6 g N m^{-2} y^{-1}. These higher rates are significant relative to atmospheric deposition (Lovett & Rueth 1999) and stream NO_3^- export (Likens & Bormann 1995) at HBEF and suggest that denitrification may be a more important flux in these forests than previously thought.

Inter-annual variation in N mineralization and nitrification appears to be important in northern hardwood forest ecosystems and is clearly worthy of further study. Mineralization and nitrification increased in both the forest floor and mineral soil from 1998 to 1999, despite the fact that soils were generally drier during June, July and much of August of 1999. Total soil profile nitrification changed less than mineralization from year to year. Respiration rates were also higher in 1999 than 1998, suggesting a general increase in microbial activity. Other sites at Hubbard Brook also showed high *in situ* N mineralization rates in 1999 compared to 1998 (Houlton et al. submitted), suggesting that the increase was not an artifact caused by plot sampling and/or disturbance. In contrast to *in situ* mineralization and respiration, microbial biomass C and N and potential net N mineralization and nitrification were lower in 1999 than 1998.

There have been few studies that have explored inter-annual variation in N cycle processes in northern hardwood forests. Bohlen et al. (2001) observed significant inter-annual variation in microbial processes in soils at HBEF. Hughes and Fahey (1994) reported significant inter-annual variation in litterfall N at HBEF. In contrast, DeVito et al. (1999) observed very little annual variation in mineralization and nitrification over two years of measurement in beech/maple/poplar stands in Ontario, Canada (approximately 11 g N m^{-2} y^{-1} in both years), with nitrification at 58% of N mineralization. Our data suggest that there are complex internal N dynamics in northern hardwood ecosystems that may cause significant inter-annual variation in ecosystem productivity, delivery of NO_3^- and other ions to streams and soil: atmosphere trace gas fluxes. The factors regulating these dynamics should be explored in future research.

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References

Aber JD, Nadelhoffer KJ, Steudler P & Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. Bio. Sci. 39: 378–386

Aber JD & Driscoll CT (1997) Effects of land use, climate variation and N deposition on N cycling and C storage in northern hardwood forests. Biogeochem. Cycles 11: 639–648

Alm J, Saarnio AJ, Nykanen H, Silvola J & Martikainen PJ (1999) Winter CO_2, CH_4 and N_2O fluxes on some natural and drained boreal peatlands. Biogeochem. 44: 163–186

Barton L, McLay CDA, Schipper LA & Smith CT (1999) Annual denitrification rates in agricultural and forest soils: a review. Aust. J. Soil Res. 37(6): 1073–1093

Bohlen PJ, Groffman PM, Fahey TJ, Driscoll CT & Siccama TG (2001) Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82: 965–978

Boone RD (1992) Influence of sampling date and substrate on nitrogen mineralization: comparison of laboratory-incubation and buried-bag methods for two Massachusetts forest soils. Can. J. For. Res. 22: 1895–1900

Bormann FH, Likens GE & Melillo JM (1977) Nitrogen budget for an aggrading northern hardwood forest ecosystem. Sci. 196: 981–983

Bormann FH & Likens GE (1979) Pattern and Process in a Forested Ecosystem. Springer-Verlag, New York

Boutin R & Robitaille G (1994) Increased soil nitrate losses under mature sugar maple trees affected by experimentally induced deep frost. Can. J. For. Res. 25: 588–602

Bowden WB (1986) Gaseous nitrogen emissions from undisturbed terrestrial ecosystems: An assessment of their impacts on local and global nitrogen budgets. Biogeochem. 2: 249–279

Brooks PD, Williams MW & Schmidt SK (1996) Microbial activity under alpine snwopacks, Niwot Ridge, Colorado. Biogeochem. 32: 93–113

Brooks PD, Schmidt SK & Williams MW (1997) Winter production of CO_2 and N_2O from alpine tundra: environmental controls and relationship to inter-system C and N fluxes. Oecologia 110: 403–413

Brooks PD, Williams MW & Schmidt SK (1998) Inorganic nitrogen and microbial biomass dynamics before and during snowmelt. Biogeochem. 43: 1–15

Brooks PD, Campbell DH, Tonnessen KH & Heuer K (1999) Natural variability in N export from headwater catchments: Snow cover controls on ecosystem N retention. Hydrol. Proc. 14–15: 2191–2201

Campbell JL, Eagar C, McDowell WH & Hornbeck JW (2000) Analysis of nitrogen dynamics in the Lye Brook wilderness area, Vermont, U.S.A. Water, Air, Soil Pollut. 122: 63–75

Christ M, Zhang Y, Likens GE & Driscoll CT (1995) Nitrogen retention capacity of a northern hardwood forest soil under ammonium sulfate additions. Ecol. Appl. 5: 802–812

Clein JS & Schimel JP (1995) Microbial activity of tundra and taiga soils at sub-zero temperatures. Soil Biol. Biochem. 27: 1231–1234

Cooley KR (1990) Effects of CO_2-induced climatic changes on snowpack and streamflow. Hydrol. Sci. 35: 511–522

Coxson DS & Parkinson D (1987) Winter respiratory activity in Aspen woodland forest floor litter and soils. Soil Biol. Biochem. 19: 49–59

DeVito KJ, Westbrook CJ & Schiff SL (1999) Nitrogen mineralization and nitrification in upland and peatland forest soils in two Canadian Shield catchments. Can. J. For. Res. 29: 1793–1804

Duggin JA, Voigt GK & Bormann FH (1991) Autotrophic and heterotrophic nitrification in response to clear-cutting northern hardwood forest. Soil Biol. Biochem. 8: 779–787

Edwards AC & Cresser MS (1992) Freezing and its effect on chemical and biological properties of soil. Adv. Soil Sci. 18: 59–79

Federer CA (1983) Nitrogen mineralization and nitrification: Depth variation in four New England forest soils. Soil Sci. Am. J. 47: 1008–1014

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNutty SG, Ryan DE & Stottlemyer R (1998) Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecol. Appl. 8: 706–733

Finzi AC, van Breemen N & Canham CC (1998) Canopy tree-soil interactions within temperate forests: Species effects on soil carbon and nitrogen. Ecol. Appl. 8: 440–446

Fisk M & Fahey TJ (1990) Nitrification potential in the organic horizon following clearfelling of northern hardwood forests. Soil Biol. Biochem. 22: 277–279

Fitzhugh RD (2000) Canopy tree-soil-microbial interactions in a northern hardwood ecosystem: Effects of soil freezing disturbance on soil solution chemistry and nutrient loss. PhD Dissertation, Syracuse University. 245 pp

Fitzhugh RD, Driscoll CT, Groffman PM, Tierney GL, Fahey TJ & Hardy JP (this issue) Effects of soil freezing disturbance on soil solution nitrogen, phosphorus and carbon chemistry in a northern hardwood ecosystem. Biogeochem. 56: 215–238

Foster DR, Aber JD, Melillo JM, Bowden RD & Bazzaz FA (1997) Forest response to disturbance and anthropogenic stress. Biosci. 47: 437–445

Goodale CL & Aber JD (2001) The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecol. Appl. 11: 253–267

Groffman PM, Hardy JP, Nolan S, Driscoll CT, Fitzhugh RD & Fahey TJ (1999) Snow depth, soil frost and nutrient loss in a northern hardwood forest. Hydrol. Proc. 13: 2275–2286

Groffman PM, Holland E, Myrold DD, Robertson GP & Zou X (1999) Denitrification. In: Robertson GP, Bledsoe CS, Coleman DC & Sollins P (Eds) Standard Soil Methods for Long Term Ecological Research (pp 272–290). Oxford University Press, New York

Hardy JP, Albert MR & Marsh P (1999) Snow Hydrology: The Integration of Physical, Chemical and Biological Systems. John Wiley and Sons, Ltd., Chichester

Hardy JP, Groffman PM, Fitzhugh RD, Henry KS, Welman TA, Demers JD, Fahey TJ, Driscoll CT, Tierney GL & Nolan S (this issue) Snow depth, soil frost and water dynamics in a northern hardwood forest. Biogeochem. 56: 151–174

Hart SC, Binkley D & Perry DA (1997) Influence of red alder on soil nitrogen transformations in two conifer forests of contrasting productivity. Soil Biol. Biochem. 29: 1111–1123

Hill AR & Shackleton M (1989) Soil N mineralization and nitrification in relation to nitrogen solution chemistry in a small forested watershed. Biogeochem. 8: 167–184

Hobbie SE & Chapin FS (1996) Winter regulation of tundra litter carbon and nitrogen dynamics. Biogeochem. 35: 327–338

Houlton BZ, Driscoll CT, Fahey TJ, Likens GE, Groffman PM, Bernhardt E & Buso D (in preparation) Nitrogen dynamics and loss following a nice storm: Implications for nitrogen limitation to temperate zone forests. Submitted to Ecosystems

Hughes JW & Fahey TJ (1994) Litterfall dynamics and ecosystem recovery during forest development. Forest Ecol. Manag. 63: 181–198

Jenkinson DS & Powlson DS (1976) The effects of biocidal treatments on metabolism in soil V. A method for measuring soil biomass. Soil Biol. Biochem. 8: 209–213

Lamontagne S (1998) Nitrogen mineralization in upland Precambrain Shield catchments: contrasting the role of lichen-covered bedrock and forested areas. Biogeochem. 41: 53–69

Lawrence GB, Lovett GM & Baevsky YH (2000) Atmospheric deposition and watershed nitrogen export along an elevational gradient in the Catskill Mountains, New York. Biogeochem. 50: 21–43

Likens GE, Borman FH & Johnson NM (1969) Nitrification: Importance to nutrient losses from a cutover forested ecosystem. Sci. 163: 1205–1206

Likens GE & Bormann FH (1995) Biogeochemistry of a Forested Ecosystem, Second Edition. Springer-Verlag, New York

Likens GE, Driscoll CT & Buso DC (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. Sci. 272: 244–245

Lipson DA, Schmidt SK, Russel K & Monson K (2000) Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. Soil Biol. Biochem. 32: 441–448

Lovett GM & Rueth H (1999) Potential nitrogen mineralization and nitrification in american beech and sugar maple stands along a nitrogen deposition gradient in the northeastern U.S. Ecol. Appl. 9: 1330–1344

Lovett GM, Weathers KC & Sobczak WV (2000) Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. Ecol. Appl. 10: 73–84

Magill AH, Aber JD, Berntson GM, McDowell WH, Nadelhoffer KJ, Melillo JM & Steudler P (2000) Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems 3: 238–253

Melillo JM (1977) Mineralization of Nitrogen in Northern Hardwood Forest Ecosystems. Ph.D Dissertation, Yale University

Melloh RA & Crill PM (1995) Winter methane dynamics beneath ice and in snow in a temperature poor fen. Hydrol. Proc. 9: 947–956

Miller RM & Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol. Biochem. 22: 579–584

Mitchell MJ, Driscoll CT, Kahl JS, Likens GE, Murdoch PS & Pardo LH (1996) Climate control of nitrate loss from forested watersheds in the northeastern United States. Environ. Sci. Technol. 30: 2609–2612

Mullen RB, Schmidt SK & Jaeger CH III (1998) Nitrogen uptake during snowmelt by the snow buttercup, *Ranunculus adoneus*. Arctic and Alpine Res. 30: 121–125

Murdoch PS, Burns DA & Lawrence GB (1998) Relation of climate change to the acidification of surface waters by nitrogen deposition. Environ. Sci. Technol. 32: 1642–1647

Nielsen CB, Groffman PM, Hamburg SP, Fahey TJ, Driscoll CT & Hardy JP (2001) Freezing effects on carbon and nitrogen cycling in soils from a northern hardwood forest. Soil Sci. Soc. Am. J. 65: in press

Nyborg M, Laidlaw JW, Solberg ED & Malhi SS (1997) Denitrification and nitrous oxide emissions from a Black Chernozemic soil during spring thaw in Alberta. Can. J. Soil. Sci. 77: 153–160

Pastor J, Aber JB, McClaugherty CA & Melillo JM (1984) Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecol. 65: 256–268

Pawluk S (1988) Freeze – thaw effects on granular structure reorganization for soil materials of varying texture and moisture content. Can. J. Soil. Sci. 68: 485–494

Rascher CM, Driscoll CT & Peters NE (1987) Concentration and flux of solutes from snow and forest floor during snowmelt in the West-Central Adirondack region of New York. Biogeochem. 3: 209–224

Reich PB, Grigal DF, Aber JD & Gower ST (1997) Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecol. 78: 335–347

Robertson GP, Wedin D, Groffman PM, Blair JM, Holland EA, Nadelhoffer KJ & Harris D (1999) Soil carbon and nitrogen availability: Nitrogen mineralization, nitrification and carbon turnover. In: Robertson GP, Bledsoe CS, Coleman DC & Sollins P (Eds) Standard Soil Methods for Long Term Ecological Research (pp 258–271). Oxford University Press, New York

Sakai A & Larcher W (1987) Frost Survival of Plants. Springer-Verlag, Berlin

SAS (1989) SAS/STAT User's Guide, Release 6.03. SAS Institute Incorporated. Cary, North Carolina

Schimel JP & Clein JS (1996) Microbial response to freeze-thaw cycles in tundra and taiga soils. Soil Biol. Biochem. 28: 1061–1066

Scott NA & Binkley D (1997) Foliage litter quality and annual net N minrealization: comparison across North American forest sites. Oecologia 11: 151–159

Sommerfeld RA, Mosier AR & Musselman RC (1993) CO_2, CH_4 and N_2O flux through a Wyoming snowpack and implications for global budgets. Nature 361: 140–142

Stottlemyer R & Toczydlowski D (1996) Precipitation, snowpack, stream-water ion chemistry, and flux in a northern Michigan watershed, 1982–1991. Can. J. Fish. Aq. Sci. 53: 2659–2672

Stottlemyer R & Toczydlowski D (1999) Seasonal changes in precipitation, snowpack, snowmelt, soil water and streamwater chemistry, Northern Michigan. Hydrol. Proc. 13: 2215–2232

Taylor BR & Jones HG (1990) Litter decomposition under snow cover in a balsam fir forest, Can. J. Bot. 68: 112–120

Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD & Driscoll CR (this issue) Soil freezing alters fine root dynamics in a northern hardwood forest. Biogeochem. 56: 175–190

Tilman D (1998) Species composition, species diversity, and ecosystem processes: Understanding the impacts of global change. In: Pace ML & Groffman PM (Eds) Successes, Limitations and Frontiers in Ecosystem Science (pp 452–472) Springer-Verlag, New York

Vogt KA, Grier CC & Vogt DJ (1986) Production, turnover and nutrient dynamics of above- and belowground detritus of world forests. Adv. Ecol. Res. 15: 303–377

Williams MW, Brooks PD & Seastedt T (1998) Nitrogen and carbon soil dynamics in response to climate change in a high-elevation ecosystem in the rocky mountains, U.S.A. Arctic & Alpine Res. 30: 26–30

Zak DR, Host GE & Pregitzer KS (1989) Regional variability in nitrogen mineralization, nitrification and overstory biomass in northern Lower Michigan. Can. J. For. Res. 19: 1521–1526