Biogeochemistry (2011) 102:223–238
DOI 10.1007/s10533-010-9436-3

Snow depth, soil freezing and nitrogen cycling in a northern hardwood forest landscape

Peter M. Groffman · Janet P. Hardy · Samuel Fashu-Kanu · Charles T. Driscoll · Natalie L. Cleavitt · Timothy J. Fahey · Melany C. Fisk

Received: 1 December 2009 / Accepted: 21 March 2010 /

Abstract Increases in soil freezing associated with decreases in snow cover have been identified as a significant disturbance to nitrogen (N) cycling in northern hardwood forests. We created a range of soil freezing intensity through snow manipulation experiments along an elevation gradient at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains, NH USA in order to improve understanding of the factors regulating freeze effects on nitrate (NO_3^-) leaching, nitrous oxide (N_2O) flux, potential and in situ net N mineralization and nitrification, microbial biomass carbon (C) and N content and respiration, and denitrification. While the snow manipulation treatment produced deep and persistent soil freezing at all sites, effects on hydrologic and gaseous losses of N were less than expected and less than values observed in previous studies at the HBEF. There was no relationship between frost depth, frost heaving and NO_3^- leaching, and a weak relationship between frost depth and winter N_2O flux. There was a significant positive relationship between dissolved organic carbon (DOC) and NO_3^- concentrations in treatment plots but not in reference plots, suggesting that the snow manipulation treatment mobilized available C, which may have stimulated retention of N and prevented treatment effects on N losses. While the results support the hypothesis that climate change resulting in less snow and more soil freezing will increase N losses from northern hardwood forests, they also suggest that ecosystem response to soil freezing disturbance is affected by multiple factors that must be reconciled in future research.

Keywords Climate change · Dissolved organic matter · Methane · Microbial biomass · Nitrate · Nitrous oxide

P. M. Groffman (✉)
Cary Institute of Ecosystem Studies, Box AB,
Millbrook, NY 12545, USA
e-mail: groffmanp@caryinstitute.org

J. P. Hardy
U.S. Army, Cold Regions Research and Engineering
Laboratory, Hanover, NH 03755, USA

S. Fashu-Kanu · C. T. Driscoll
Department of Civil and Environmental Engineering,
Syracuse University, Syracuse, NY 13244, USA

N. L. Cleavitt · T. J. Fahey
Department of Natural Resources, Cornell University,
Ithaca, NY 14853, USA

M. C. Fisk
Department of Zoology, Miami University, Oxford,
OH 45056, USA

Introduction

While much climate change research has focused on the effects of long term changes in temperature on ecosystem processes during the growing season, there has been increasing recognition of the importance of indirect effects and changes during winter (Campbell et al. 2005). In northeastern North America, winter temperatures increased more than summer temperatures (1.2 vs. 0.7°C) during the twentieth century (Hayhoe et al. 2007). An important indirect effect of winter climate change is a reduction in the depth and duration of snowpack, which can lead to increases in soil freezing, with consequent effects on plant, soil and microbial processes and nutrient losses (Brooks and Williams 1999; Groffman et al. 2001a; Williams et al. 1996). These changes are challenging to study due to the complex nature of snowpack/soil freezing dynamics and the difficulty of measuring response variables without disturbing the physical, chemical and biological character of soil (Henry 2007).

There is great uncertainty about the biogeochemical effects of winter climate change (Campbell et al. 2009). In the northeastern US, watershed NO_3^- export increased at several sites following extensive soil freezing in the early 1990s (Judd et al. 2007; Mitchell et al. 1996), and plot studies in several locations have shown that decreases in snowpack lead to soil freezing and increases in nutrient losses (Fitzhugh et al. 2001; Matzner and Borken 2008). While freezing can cause fine root mortality (Cleavitt et al. 2008; Tierney et al. 2001), alter processes such as litter decomposition (Christenson et al. 2010), and increase soil NO_3^- pools (Groffman et al. 2001b) and gaseous losses (Groffman et al. 2006b), these plot-scale effects of soil freezing do not necessarily translate to higher NO_3^- export at the watershed scale (Kaste et al. 2008). For example, while plot studies at the HBEF in the White Mountains of New Hampshire, USA showed strong effects of soil freezing on hydrologic and gaseous N_2O losses (Fitzhugh et al. 2001; Groffman et al. 2006b) we were unable to identify the effects of soil freezing in analysis of long-term (40 year) records of N export from experimental watersheds at the HBEF (Fitzhugh et al. 2003).

The lack of consistent connection between plot- and watershed-scale responses to soil freezing suggests that processing of N is subject to complex controls that do not respond equally under all circumstances (Frei et al. 2005; Henry 2007; Henry 2008; Kaste et al. 2008; Matzner and Borken 2008). In our previous plot studies at the HBEF, soil temperatures never decreased below −4°C (Hardy et al. 2001), yet we observed a doubling of overwinter root mortality (Tierney et al. 2001), raising questions about soil freezing effects on physical disruption of the soil environment and relationships between soil freezing intensity and effects on nutrient cycling. Plots dominated by sugar maple (*Acer saccharum)* had more dramatic responses to freezing than plots dominated by yellow birch (*Betula alleghaniensis),* but the reasons for this difference were not obvious (Fitzhugh et al. 2001).

The present research follows up on questions raised in the first set of snow manipulation studies at the HBEF. Our approach was to exploit the elevation gradient at the HBEF to create a range of soil freezing intensity that would induce variable responses in different components of the system (frost heaving, root damage, N and C cycle processes) that would improve our understanding of the factors regulating freeze effects on N losses from the northern hardwood forest. Paired reference and snow manipulation plots were established at two low elevation south facing locations and at two high elevation north facing locations. Effects of these treatments on soil frost, temperature, moisture and structure were reported by Steinweg et al. (2008), and effects on roots were described by Cleavitt et al. (2008). Groffman et al. (2009) presented data on soil frost, moisture and C and N cycle processes in the reference plots used for this study. In this paper, our objectives were to evaluate the effects of variation in soil freezing intensity on hydrologic and gaseous losses of N and C and on the microbial biomass and activity parameters (potential and in situ net N mineralization and nitrification, microbial biomass C and N content and respiration, and denitrification) that regulate these losses.

Methods

Experimental design

The HBEF is located in the White Mountain National Forest, New Hampshire USA (43°56′ N, 71°45′ W) and is dominated by northern hardwood forest vegetation (Schwarz et al. 2003). Soils are 75–100 cm deep, acidic (pH ~4.0 in the surface horizons) Spodosols developed from unsorted basal tills, with occasional deeper profiles (Soil Survey Staff 2006).

Our experimental design consisted of eight 10 m by 10 m plots (Table 1); two each (one reference, one

Table 1 Description of study sites located at the Hubbard Brook Experimental Forest, NH USA

Site	Plot	Elevation (m)	Aspect (deg)	Slope (%)	Organic horizon depth (cm)	Stoniness[a] (%)	Tree species in order of dominance[b]
Low elevation (lower)	Reference	380	105	9	5–8	15	Beal, Acpe, Fagr, Piru
	Treatment		90	10	6–8	15	Beal, Acsa, Acpe, Fagr, Piru
Low elevation (upper)	Reference	480	190	35	11–19	36	Acsa, Fram, Beal, Fagr
	Treatment		180	50	4–9	20	Acsa, Beal, Fagr
High elevation (east)	Reference	790	350	20	3–10	5	Beal, Acsa, Piru
	Treatment		360	20	6–10	0	Beal, Acsa, Piru, Fagr
High elevation (west)	Reference	755	352	12	3–14	5	Beal, Acsa, Fagr, Acpe, Piru
	Treatment		360	9	4–8	5	Acsa, Beal, Piru

Adapted from Cleavitt et al. (2008)

[a] Quantified by a surface survey with a probe that assessed the presence of rock within 1 cm of the soil surface (Cleavitt et al. 2008)

[b] Beal = *Betula alleghaniensis,* Britt., Acpe = *Acer pensylvanicum* L., Fagr = *Fagus grandifolia* Ehrh., Piru = *Picea rubens* Sarg., Acsa = *Acer saccharum* Marsh., Fram = *Fraxinus americana* L.

treatment) located at four sites chosen to maximize variation in soil freezing intensity. Two low elevation, south-facing sites approximately 350 m apart were located at the HBEF valley bottom (380 and 480 m), and two high elevation north-facing sites approximately 100 m apart were located on Mt. Kineo (755 and 790 m). The valley bottom and Mt. Kineo plots were approximately 8 km apart. Each plot was split into three distinct subplots for repeated sampling. Minor amounts of understory vegetation (all saplings less than 2 cm diameter) were removed from both treatment and reference plots to facilitate snow removal by shoveling. Herbaceous growth was not clipped. Loose sticks that were raised more than 5 cm off the forest floor were removed. Other woody debris that might interfere with shoveling were also removed. Treatment plots were kept snow-free from late November through January in the winters of 2002/2003 and 2003/2004, while the reference plots accumulated snow at ambient rates. After each snowfall, shovels were used to clear the treatment plots of snow. A few cm of compacted snow was left on the ground to protect the forest floor from shovel damage. There was very little spatial or temporal variation in the amount of compacted snow left on the plots.

Plot instrumentation

Plot installations were similar to those described by Hardy et al. (2001) with soil and air temperatures measured using Campbell Scientific CR 10x data loggers with thermistor probes (one per plot per depth) placed horizontally at 10 cm intervals to 50 cm depth, plus an aboveground probe. Temperature readings were recorded every minute and hourly averages were stored. Volumetric soil water content (unfrozen) was measured with duplicate Campbell CS615 time domain reflectometry (TDR) probes installed horizontally in the Oa (approximately 0.05 m depth) and Bs (approximately 0.15 m depth) horizons. Snow depth was measured with a metal meter stick at approximately 2 week intervals at between 10 and 100 randomly selected locations per plot. Frost depths were measured at approximately weekly intervals in two frost tubes (Ricard et al. 1976) in each plot. Frost heave in all plots was measured at the time of maximum frost depth by level survey, as described in Cleavitt et al. (2008).

Duplicate soil solution samplers (zero tension lysimeters) of the design described by Johnson et al. (2000) were installed in fall 2000 and spring 2001 at the same depths as the TDR probes. Lysimeter samples were collected at monthly intervals and stored at 4°C prior to analysis. Nitrate was analyzed by ion chromatography (Tabatabai and Dick 1983) and dissolved organic C (DOC) was measured by detection of CO_2 following persulfate digestion and ultraviolet enhanced oxidation (McDowell et al. 1987). Soil solution fluxes were determined by multiplying measured concentrations by the volume of water collected and summing over the interval.

Chamber bases (287 mm diameter, 5 cm depth, three per plot) were installed in spring 2001 in each plot to measure soil:atmosphere fluxes of CO_2, N_2O and CH_4, using the design described by Bowden et al. (1991). The area enclosed by the chamber bases was kept free of vegetation. When plots were snow-free, fluxes were measured monthly by placing chamber tops (40-mm high) on the bases and removing gas samples (9 ml) at 0, 10, 20 and 30 min from gas sampling ports in the center of the chamber top by syringe. When plots were covered with snow, plastic chambers (4 per plot, approximately 5 l) fitted with septa were inserted 5 cm into the snowpack just prior to measurement. These chambers were placed in each corner of the plot to avoid disturbing snow on the plot while sampling (Groffman et al. 2006b). No effort was made to conduct sampling in specific plots at specific times during the day and the order of sampling plots varied from month to month. Samples were transferred to 9 ml evacuated (−26 mm Hg vacuum) glass vials and stored at room temperature prior to analysis by gas chromatography (GC). N_2O and CO_2 were analyzed on a Shimadzu GC-14 GC with electron capture (N_2O) and thermal conductivity (carbon dioxide, CO_2) detectors. CH_4 was analyzed on a Shimadzu GC-8a GC with a flame ionization detector. Standards, blanks and spike check samples were processed the same as field samples. Fluxes were calculated from the linear rate of change in gas concentration, the chamber internal volume and soil surface area. Flux rate calculations were not corrected for actual in situ temperature and pressure. Single points were removed from regressions if they were more than six times higher or lower than the other three values or if they contradicted a clear trend in the other three points. This procedure prevented inclusion of high flux rates based on non-significant regressions. Non-significant regressions were used in flux calculations to avoid biasing the statistical distribution of rates by setting all non-significant regressions to zero.

Microbial biomass and activity measurements

In situ net N mineralization and nitrification were measured using an intact core method (Robertson et al. 1999). In this method, two intact cores (2 cm diameter × 25 cm depth) were removed from each of the 3 sampling subplots in each plot (6 cores total per plot). This sampling interval encompassed the organic horizons and ~10 cm of the mineral soil (E and/or Bs horizons). One core from each subplot was returned to the laboratory for extraction (2 N KCl) of inorganic N (ammonium (NH_4^+) and NO_3^-) and the other was returned to the location from which it was taken for in situ incubation in a plexiglass tube loosely covered at both ends with aluminum foil. During the growing season (April–November), cores were incubated for approximately 4 weeks and there was one overwinter (December–March) incubation. Inorganic N was quantified colorometrically using a Lachat Quikchem 8100 flow injection analyzer. Net N mineralization was calculated from the accumulation of total inorganic N over the course of the incubation. Net nitrification was calculated from the accumulation of NO_3^- during the incubation. Rates were expressed on an areal basis (g N m^{-2}) using bulk density values from Bohlen et al. (2001). Annual rates were produced by summing monthly and overwinter plot means to produce $n = 2$ true replicates for each elevation/treatment combination. Each annual mean is thus the sum of results from approximately eight incubations, each based on six cores (three initial, three incubated).

Separate forest floor and mineral soil samples (distinct from the in situ N mineralization and nitrification samples described above) were removed from each of the 3 sampling subplots in each plot (3 samples total per plot) in April and June of 2003 and 2004 and stored at 4°C for less than 1 week. Samples were hand sorted and mixed and held at field moisture for analyses. Gravimetric soil moisture content was determined by drying at 60°C for 48 h (McInnes et al. 1994). Soil organic matter content was determined by loss on ignition at 450°C for 4 h (Nelson and Sommers 1996). Amounts of inorganic N were determined as described above.

Microbial respiration and potential net N mineralization and nitrification were quantified by measuring CO_2 and inorganic N production during 10 day incubations at field moisture content in the laboratory (~20°C). CO_2 and inorganic N were quantified as described above.

Microbial biomass C and N content were measured using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976). 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. Potential denitrification (denitrification enzyme activity, DEA) was measured using the short-term anaerobic assay developed by Smith and Tiedje (1979) as described by Groffman et al. (1999).

In addition to the sampling on the snow manipulation study plots, microbial biomass and activity were measured each year as part of the long-term monitoring program at the HBEF. Samples have been taken each year in July from three soil horizons (Oie, Oa, Bs) at four elevations in the Bear Brook watershed, a reference area located just west of the biogeochemical reference watershed (W6) at the HBEF since 1994 (Bohlen et al. 2001; Fiorentino et al. 2003; Groffman et al. 2006a). These data provide temporal context for the previous (1997–1999) and current (2002–2004) snow manipulation studies.

Statistical analysis

Treatment effects were evaluated using analysis of variance (ANOVA) of site means to avoid concerns about pseudoreplication that could arise if within-plot replicates were included. Repeated measures ANOVA was used to evaluate treatment effects on trace gas, soil solution and microbial biomass and activity measurements. Statistical relationships between variables were explored with Pearson product moment correlations. The Statistical Analysis System (SAS 1988) was used for all analyses.

Results

The snow removal treatment caused deep (Fig. 1; Table 2) and persistent (Cleavitt et al. 2008; Steinweg et al. 2008) soil freezing in all plots. Although air temperatures were ~2°C colder at high elevation (Groffman et al. 2009), differences in maximum soil frost depth between elevations were only weakly significant ($P < 0.07$). Frost depth in the reference plots was more sensitive to climate variation, with significantly ($P < 0.01$) deeper frost in the warmer low elevation plots than the high elevation plots, and in the low-snow winter of 2003/2004 ($P < 0.02$). Increased soil freezing in the low elevation reference plots resulted from less snowpack early in the season at low elevation and is discussed elsewhere (Groffman et al. 2009). There was less snow in the winter of 2003/2004 than in the winter of 2002/2003; 77 cm of snow were recorded at the high elevation plots on January 7, 2004 while 48 cm of snow were recorded at the same location on January 7, 2003. Winter temperatures were near average (−4.7°C) in both years; −5.8°C in 2002/2003 and −4.5°C in 2003/

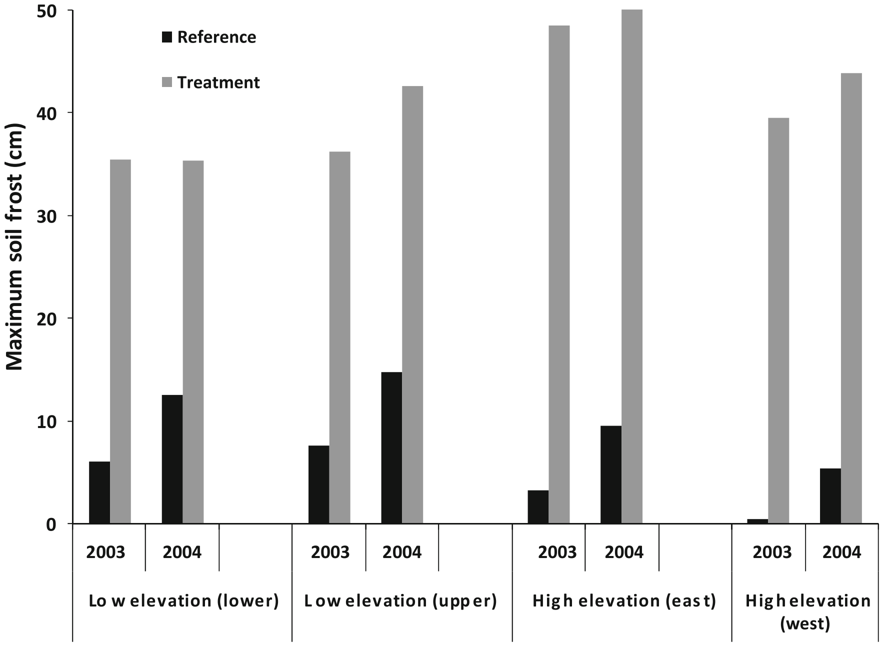

Fig. 1 Maximum soil frost depth during the winters of 2002/2003 and 2003/2004 in snow removal treatment and reference plots at two low elevation and two high elevation sites at the Hubbard Brook Experimental Forest

Table 2 Treatment effects on soil frost, root vitality, soil heaving (absolute values as well as treatment differences), spring (when treatment effects were expected to be most marked) soil solution NO_3^- concentrations and summer and winter N_2O flux

Site	Year	Soil frost (mm)	Root vitality absorbance (g^{-1})	Frost heave in treatment plots (mm)	Frost heave difference[a] (mm)	Spring (March–May) soil solution NO_3^- (μmol l^{-1})	Summer N_2O flux (ng N cm^{-2} h^{-1})	Winter N_2O flux (ng N cm^{-2} h^{-1})
Low elevation (lower)	2003	29.5	−1.3	21	ND	3.75	−2.817	0.881
	2004	22.8	−4.8	27	14	−1.34	0.711	0.487
Low elevation (upper)	2003	28.7	−5.0	7	ND	73.51	−0.055	0.245
	2004	27.9	1.5	−7	12	14.69	0.469	0.669
High elevation (east)	2003	45.3	−0.7	30	ND	3.43	0.875	2.656
	2004	40.6	−2.1	12	5	14.3	−0.178	1.382
High elevation (west)	2003	39.1	−3.1	39	ND	25.81	0.824	0.029
	2004	38.5	−2.4	12	32	68.25	−0.136	0.832

Values are the mean difference between treatment and reference plots at four sites, i.e., mean treatment minus mean reference

[a] Differences in frost heave between treatment and reference plots were only measured in 2004

2004. Frost heaving showed no pattern with either elevation or year (Table 2).

Precipitation was measured at several locations within the HBEF (www.hubbardbrook.org). At the gage (#1) nearest the low elevation sites, annual precipitation was 1309 mm, 1639 mm and 1329 mm in 2002, 2003 and 2004, respectively. Precipitation at the gage (#13) nearest the high elevation sites was 1326, 1704 and 1365 mm in 2002, 2003 and 2004, respectively. The volume of leachate collected in lysimeters was not affected by the snow removal treatment (Table 3), nor was gravimetric soil moisture measured in spring and summer (Table 4), suggesting that the treatment did not alter soil water availability in these plots.

Concentrations and loads of NO_3^- and DOC were elevated in the treatment plots, but the differences were not consistently significant (Figs. 2, 3; Table 3). During spring, when treatment effects were expected to be most marked, there was no treatment effect on NO_3^- concentrations (Fig. 2). Treatment effects were most marked for DOC, in the Bs horizon (Fig. 3; Table 3). Concentrations and fluxes of NO_3^- in soil solutions were higher in the dormant season than in the growing season, and higher in the Oa horizon than the Bs horizon (Table 3). For DOC, there were no consistent seasonal differences in concentration, and variation with soil depth (i.e. from the Oa to the Bs horizons), was less marked than for NO_3^- (Table 3). There was a significant correlation between DOC and NO_3^- concentrations in soil solutions in treatment plots ($r = 0.28$, $P < 0.0001$), but not in reference plots ($r = 0.00$, $P < 0.9491$). There was no relationship between frost depth or heaving and either soil solution NO_3^- or DOC concentration or flux.

While the treatment increased NO_3^- and DOC concentrations in lysimeters during the dormant season, it did not cause marked increases above pre-treatment concentrations in an analysis over all plots (Figs. 2, 3), and the differences were not consistently significant. However, treatment effects differed markedly by site, i.e. the differences between treatment and reference plots in spring (March–May) lysimeter NO_3^- concentrations were markedly larger in two of the plots (the low elevation (upper) and high elevation (west) sites) (Table 2). Sugar maple was the dominant overstory tree in the treatment plots at both these sites (Table 1), while yellow birch was dominant in the other two sites. These sites also had the largest differences in organic horizon depth and tree species dominance between reference and treatment plots.

Soil atmosphere fluxes of CO_2 (Fig. 4a) and CH_4 (Fig. 4c) showed marked seasonal patterns (highest production of CO_2 and uptake of CH_4 in summer), with no treatment effect. N_2O showed no seasonal pattern (Fig. 4b), and was higher in treatment plots than reference plots during winter (December–March), but the difference was only significant in 2004 (Fig. 5). Winter N_2O flux was higher in

Table 3 Volume of soil solution collected in zero-tension lysimeters, the concentration of NO_3^- and dissolved organic carbon (DOC) in soil solution, and the soil solution flux of NO_3^- and DOC during the growing and dormant seasons

	Growing season		Dormant season	
	Reference	Treatment	Reference	Treatment
Oa horizon				
Volume (L)	2.0 (0.1)	2.1 (0.7)	2.2 (0.5)	2.3 (0.8)
NO_3^- (μmol L^{-1})	6.5 (1.2)	6.8 (1.0)	25 (8)	30 (10)
NO_3^- flux (μmol $period^{-1}$)	13.6 (2.8)	14.5 (2.2)	52.3 (16.6)	77.7 (29.3)
DOC (μmol L^{-1})	484 (75)	474 (68)	342 (62)	462 (110)
DOC flux (μmol $period^{-1}$)	808 (73)	1,025 (160)	752 (132)	1106 (256)
Bs horizon				
Volume (L)	2.1 (0.1)	2.2 (0.6)	2.3 (0.6)	2.3 (0.7)
NO_3^- (μmol L^{-1})	6.0 (0.9)	6.8 (1.1)	13.7 (1.8)	16.3 (3.8)
NO_3^- flux (μmol $period^{-1}$)	12.0 (2.1)	14.4 (2.3)	32 (4.2)	38 (8.4)
DOC (μmol L^{-1})	227 (17)*	347 (22)	167 (11)*	381 (96)
DOC flux (μmol $period^{-1}$)	475 (44)*	752 (48)	388 (30)*	881 (213)

Values are mean (standard error, $n = 24$–32) over sample dates[a] with complete lysimeter collections from March 2003–May 2004

[a] Growing season dates with complete lysimeter collections (>1,000 ml of solution in all lysimeters) included August, September, October and November 2003. Dormant season dates include March and December 2003 and April and May 2004

* These differences between treatments were statistically significant ($P < 0.01$) in an ANOVA comparing all data from all treatment and reference plots, but were not significant in a repeated measures ANOVA due to marked site variation in treatment response with elevation, i.e. there were significant ($P < 0.01$) increases in DOC concentration and load at the high elevation sites but not at the low elevation sites

Table 4 Soil microbial biomass C and N, respiration, potential net N mineralization and nitrification, NO_3^-, NH_4^+, denitrification potential and gravimetric moisture in the forest floor (Oa, Oe, Oi horizons) and mineral soil (Bs horizon) of four reference and four treatment plots sampled in Spring and Summer of 2003 and 2004

	Forest floor		Mineral soil	
	Reference	Treatment	Reference	Treatment
Microbial biomass C (mg C kg^{-1})	3327 (381)	3112 (298)	1008 (106)	931 (71)
Microbial biomass N (mg N kg^{-1})	430 (41)	416 (48)	166 (28)*	97 (11)
Microbial respiration (mg C kg^{-1} d^{-1})	277 (36)	249 (25)	65 (16)	47 (5)
Potential net N mineralization (mg N kg^{-1} d^{-1})	9.5 (1.0)	8.9 (1.1)	1.8 (0.5)	1.0 (0.3)
Potential net nitrification (mg N kg^{-1} d^{-1})	2.7 (0.5)	3.4 (0.6)	1.3 (0.4)	0.8 (0.2)
Soil NO_3^- (mg N kg^{-1})	3.8 (0.9)	6.4 (1.3)	2.0 (0.5)	1.8 (0.4)
Soil NH_4^+ (mg N kg^{-1})	53 (8)	56 (8)	6.2 (1.0)	9.5 (1.6)
Denitrification potential (μg N kg^{-1} h^{-1})	1272 (399)	1201 (309)	281 (57)	224 (34)
Soil moisture (%, w/w)	62 (2)	61 (2)	39 (3)	39 (3)

Values are mean (standard error, $n = 16$)

* Indicates significant difference between treatment and reference plots at $P < 0.10$

treatment than reference plots at all sites, in both years (Table 2). There was a significant correlation between the treatment effects on soil frost and winter N_2O flux, i.e. plots with the greatest difference in soil frost between treatment and reference plots had the greatest difference in N_2O flux between treatment and reference plots (Table 2, $r = 0.61$, $P < 0.10$). There was no relationship between treatment effects on winter soil temperature and effects on winter N_2O flux (data not presented). Treatment effects on summer N_2O flux

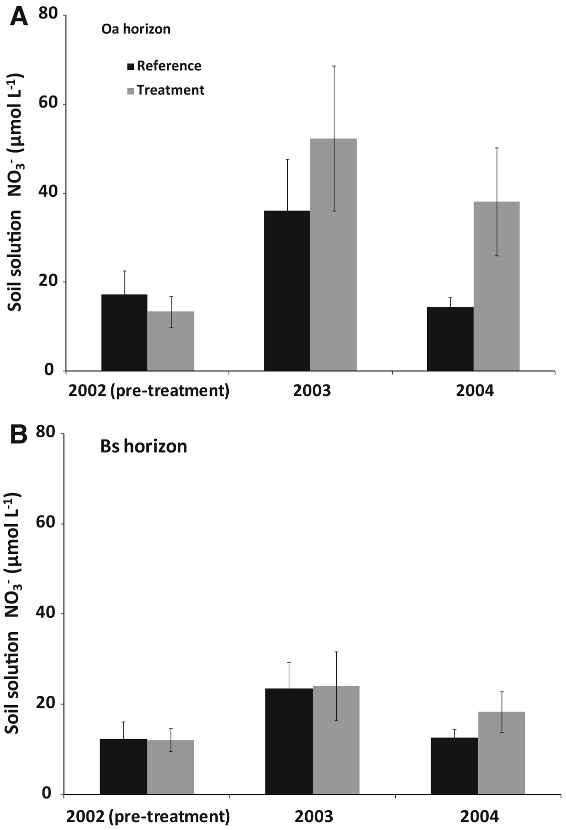

Fig. 2 Concentrations of NO_3^- in water collected in lysimeters beneath the Oa (**a**) and Bs horizons (**b**) in snow removal treatment and reference plots during spring (March–May) before (2002) and after (2003 and 2004) treatment. Values are means (with standard error, $n = 24$–40) of all samples collected in spring from four treatment and four reference plots

were inconsistent, i.e. variable in sign, but could be quite large, e.g., lower low elevation plot in summer 2003. Annual N_2O fluxes ranged from 4 to 78 mg N m^{-2} y^{-1} and averaged 24 mg N m^{-2} y^{-1} in reference plots and 63 mg N m^{-2} y^{-1} in treatment plots from 2002 to 2004. Several dates in fall and spring showing net N_2O consumption may have reduced both treatment effects and annual emission estimates.

Microbial biomass C and N content and respiration, potential net N mineralization and nitrification, soil NH_4^+ and NO_3^- pools and denitrification potential showed few responses to the snow removal treatment in either the forest floor or mineral soil (Table 4).

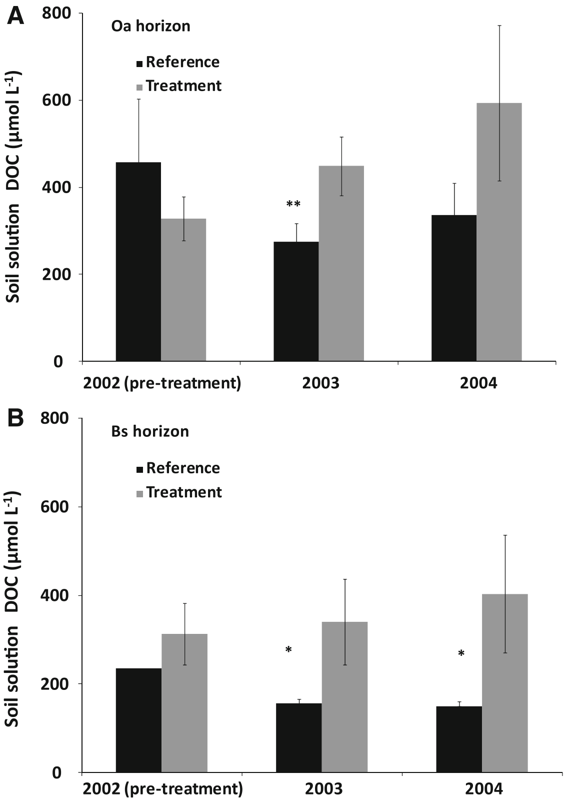

Fig. 3 Concentrations of dissolved organic carbon (DOC) in water collected in lysimeters beneath the Oa (**a**) and Bs horizons (**b**) in snow removal treatment and reference plots during spring (March–May) before (2002) and after (2003 and 2004) treatment. Values are means (with standard error, $n = 24$–40) of all samples collected in spring from four treatment and four reference plots. *, ** Indicates significant difference between treatments at $P < 0.05$ and 0.10 respectively

In situ net N mineralization and nitrification did not respond to the snow removal treatment in any of the sites (Table 5). In an analysis over all sites and treatments, in situ rates were higher in 2003/2004 than in 2002/2003 and at high elevation than low elevation. In an analysis of variance over all treatments, mineralization was higher ($P < 0.05$) at high elevation in 2003/2004 but not in 2002/2003. Nitrification was higher at high elevation in both 2002/2003 ($P < 0.05$) and 2003/2004 ($P < 0.01$).

Potential net N mineralization and denitrification potential were lower during the summers of 2003 and 2004 than during the summers of 1998 and 1999, when previous snow manipulation studies were done at the HBEF (Fig. 6). Microbial respiration was low in 2003 and high in 2004 compared to values measured in the late 1990s.

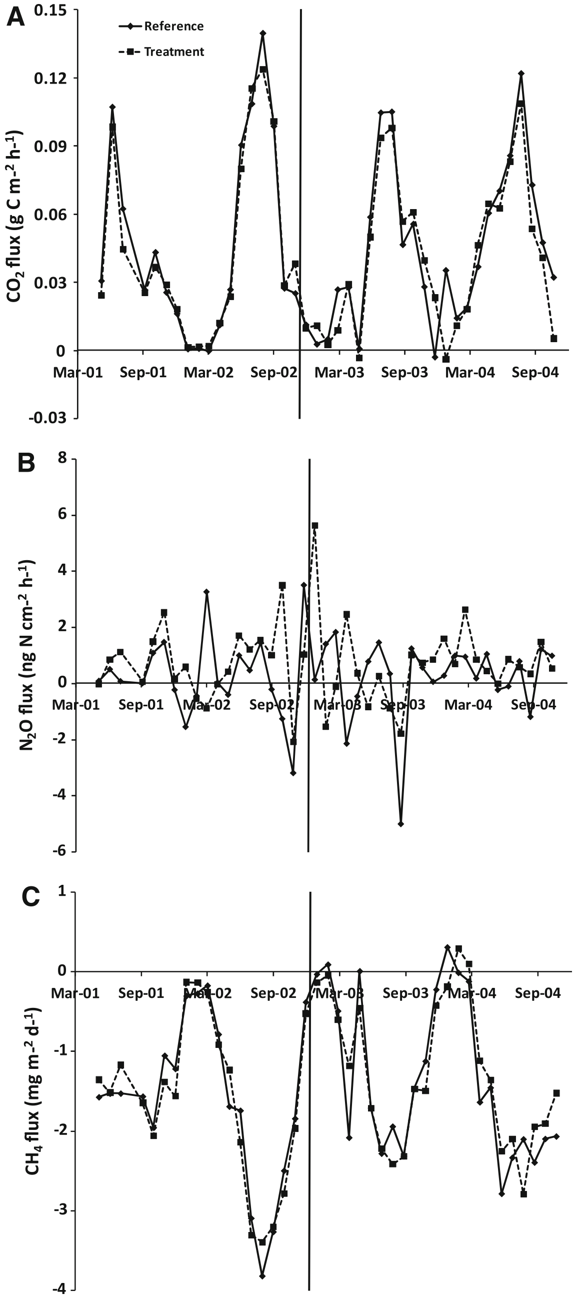

Fig. 4 Soil:atmosphere fluxes of CO_2 (**a**), N_2O (**b**) and CH_4 (**c**) from Spring 2001 through Fall 2004. Values are means of two low elevation and two high elevation plots. *Vertical line* shows the separation between pre- and post-treatment data

Discussion

This study was designed to follow up on questions raised in the first set of snow manipulation studies at the HBEF. Our approach was to exploit the elevation gradient at the HBEF to create a range of soil freezing intensity that would induce variable responses in different components of the system (frost heaving, root damage, N and C cycle processes) that would improve our understanding of the factors regulating freeze effects on N losses from the northern hardwood forest.

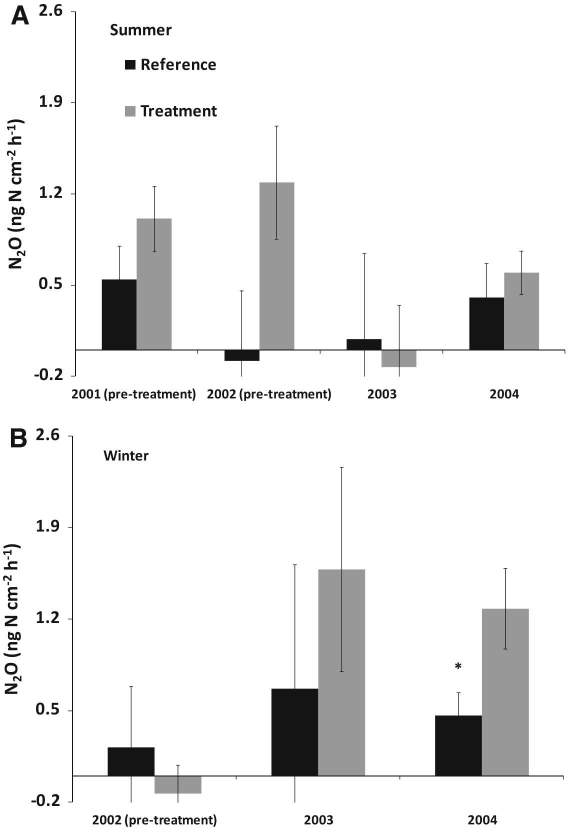

Fig. 5 Soil:atmosphere fluxes of N_2O during summer (**a**) and winter (**b**) in snow removal treatment and reference plots before (2001, 2002) and after (2003, 2004) treatment. Values are means (with standard error, $n = 24$–30) of all flux measurements from four treatment and four reference plots. * Indicates significant difference at $P < 0.10$

Variable responses to soil freezing

While the snow manipulation treatment produced deep and persistent soil freezing at all four sites, effects on hydrologic and gaseous losses of N were less than expected and less than has been observed in previous studies at the HBEF. There was no relationship between freeze intensity, as indicated by

Table 5 In situ net N mineralization and nitrification rates in reference and treatment plots at two low elevation and two high elevation sites in 2002/2003 and 2003/2004

Site	Net N mineralization		Net nitrification	
	Reference ($g\ N\ m^{-2}\ y^{-1}$)	Treatment ($g\ N\ m^{-2}\ y^{-1}$)	Reference ($g\ N\ m^{-2}\ y^{-1}$)	Treatment ($g\ N\ m^{-2}\ y^{-1}$)
2002/2003				
Low elevation				
Lower	3.7	4.1	0.2	0.4
Upper	11.2	10.7	3.0	4.2
High elevation				
East	10.4	12.2	5.2	6.1
West	11.3	10.1	5.4	5.5
2003/2004				
Low elevation				
Lower	7.1	6.8	0.5	0.1
Upper	13.0	14.6	5.2	5.2
High elevation				
East	19.1	17.1	9.8	10.9
West	18.7	14.6	9.6	10.5

maximum soil frost depth and heaving, and NO_3^- leaching, and only a weak relationship between frost depth and winter N_2O flux. In our previous studies, the treatment induced marked increases in NO_3^- leaching, with concentrations of NO_3^- in soil solution exceeding 350 μmol l^{-1} in treatment plots in sugar maple stands during the growing season following treatment (Fitzhugh et al. 2001). Here, NO_3^- concentrations were less than 100 μmol l^{-1}, and treatment effects were inconsistent. In our previous study, annual fluxes of N_2O ranged from 40 to over 200 mg N m^{-2} y^{-1}, with consistent treatment effects (Groffman et al. 2006b), while in this study, annual N_2O flux ranged from 4 to 78 mg N m^{-2} y^{-1} with few treatment effects. Several dates in fall and spring showing net N_2O consumption, as has been occasionally observed in other forest studies (Goldberg and Gebauer 2009; Kellman and Kavanaugh 2008), may have reduced both treatment effects and annual emission estimates.

It is possible that the differences between the present and past studies were caused by simple spatial variation in factors such as forest floor depth among the plots chosen for manipulation, or by the fact that the reference plots all experienced some degree of soil freezing. In our previous studies, reference plots had little or no soil freezing, while in this study soil freezing was present on most reference plots, in both years. However, there were still significant differences in soil freezing between the treatment and reference plots in this study, and treatment effects on biogeochemical processes were much less marked than in our previous studies. As noted above, in our previous studies, soil freezing induced much higher concentrations of NO_3^- and N_2O that what was observed in any of the plots in the current study.

Several recent studies have also found variable N loss responses to snow manipulation and soil freezing. In a watershed-scale field experiment in Norway, Kaste et al. (2008) observed that experimentally-increased soil frost had relatively little effect on concentrations and fluxes of NO_3^- and NH_4^+ and that increased mobilization of inorganic N was associated with higher soil temperatures caused by either naturally heavy snow pack or experimental insulation. Hentschel et al. (2009) conducted a snow manipulation field experiment in Germany and found no effect on in situ net N mineralization during spring, but observed an increase in NO_3^- leaching during late summer, a temporal pattern similar to that reported by Fitzhugh et al. (2001). Joseph and Henry (2008) found that freeze/thaw events led to a doubling of NO_3^- leaching from intact soil–plant mesocosms but that warming pulses had no effect on

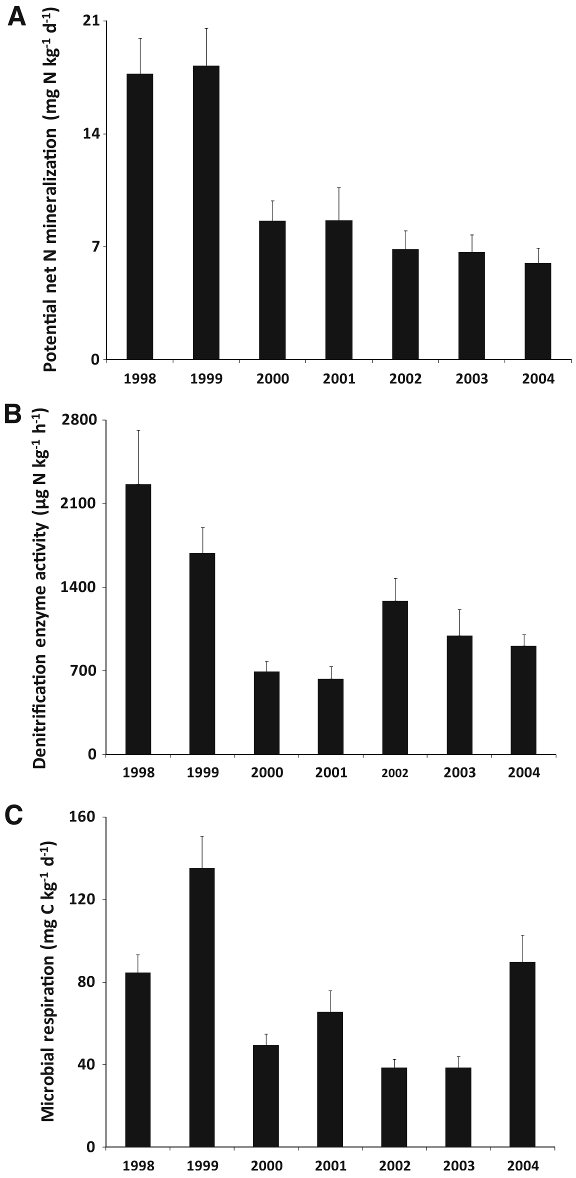

Fig. 6 Summer potential net N mineralization (**a**), denitrification (**b**) and microbial respiration (**c**) in the Bear Brook reference sampling area at the HBEF from 1998 to 2004. Values are means (with standard error, $n = 60$) of samples taken from three horizons (Oie, Oa, Bs) at four elevations in July of each year

leaching. These varying results raise questions about the importance of soil freezing disturbance to N losses as climate changes in northern hardwood forests, and more fundamental questions about the biogeochemical factors regulating response to this disturbance within and between sites.

Coupling between C and N as a regulator of freeze response

One factor that may underlie variable responses to snow manipulation and soil freezing is the coupling between C and N dynamics. If freezing mobilized available C, it could have stimulated microbial immobilization and/or denitrification of inorganic N (Goodale et al. 2005; Hafner and Groffman 2005; Hafner et al. 2005; Sobczak et al. 2003) that was also mobilized by freezing, preventing a N loss treatment response. Previous Hubbard Brook snow manipulation studies showed significant treatment effects on N losses, but no treatment effect on DOC concentration or flux (Fitzhugh et al. 2001). However, concentrations and fluxes of DOC were higher in yellow birch stands, which had smaller and more variable N loss response to treatment, than sugar maple stands. In the current study, we observed significant treatment effects on DOC concentrations and fluxes, and there was a significant positive relationship between DOC and NO_3^- concentrations in treatment plots but not in reference plots. This relationship suggests that the snow manipulation treatment did indeed mobilize available C, and this may have stimulated retention of inorganic N that was also mobilized by the treatment, preventing treatment effects on N cycling and NO_3^- loss. Our results are somewhat puzzling in that if DOC was a strong controller of N losses, we might expect to see a negative relationship between DOC and NO_3^- concentrations. However, here we are comparing treatment responses in two sets of studies; our previous study where there was no evidence of DOC mobilization, and the current study where there was evidence of DOC mobilization and much less marked effects on N cycling and loss. We also might expect to see a DOC-induced increase in microbial N and/or denitrification in the treatment plots but we did not, likely because the amount of N immobilized and/or denitrified was small relative to the pool size of microbial N and the variability and sensitivity of our microbial biomass and denitrification measurements.

Previous studies have reported highly variable responses of DOC to soil freezing. Laboratory experiments with intact Norwegian heathland soil cores found that prolonged (8 weeks) soil frost increased DOC, dissolved organic N (DON) and NH_4^+

concentrations and decreased NO_3^- concentrations (Austnes and Vestgarden 2008; Vestgarden and Austnes 2009), consistent with the hypothesis that mobilization of DOC can prevent NO_3^- responses to soil freezing. Field studies in Norway also noted increases in DOC, DON and NH_4^+ in response to mild freezing, with no increase in NO_3^- (Austnes et al. 2008; Kaste et al. 2008). In laboratory experiments with German forest soils, there was an increase in DOC in response to freezing of organic soil horizons at temperatures of −8 and −13°C, but not at −3°C, a much more common field temperature (Hentschel et al. 2008); no response was observed in field experiments (Hentschel et al. 2009). There is a clear need for further research on the factors regulating DOC response to soil freezing disturbance and on how this response regulates NO_3^- loss following this disturbance.

Vegetation composition as a regulator of freeze response

Consistent with our previous study, the snow manipulation treatment had marked effects on stands dominated by sugar maple compared to those dominated by other species. Root damage (Cleavitt et al. 2008) and NO_3^- leaching were most strongly affected by treatment in the low elevation (upper) and high elevation (west) sites that were dominated by sugar maple (Table 2). In this study, the dominance by sugar maple was much more subtle than in the previous study which involved pure stands of sugar maple and yellow birch, yet the effects of sugar maple were still evident. Many studies have found that sugar maple stands support high rates of soil N cycling and loss following disturbance (Lovett and Mitchell 2004). Episodes of sugar maple decline in eastern North America have been linked to soil freezing events (Auclair et al. 1996; Boutin and Robitaille 1995).

Mechanistically, susceptibility to N loss following disturbance in sugar maple stands appears to be driven by low soil C:N (Lovett et al. 2002). In a review of soil freezing studies, Matzner and Borken (2008) concluded that freeze–thaw events are more likely to increase net N mineralization in agricultural than forest soils, due to the generally lower soil C:N in agricultural soils. In contrast, Vestgarden and Austnes (2009) found that vegetation type was a strong controller of variation in NO_3^- and DOC response to freezing in montane areas in Norway, but the differences were not strongly linked to variation in soil C:N. Vegetation influence on soil freezing response is likely influenced by several factors including nitrification, NO_3^- immobilization, denitrification, available C and root mortality and recovery.

Annual variation in C and N dynamics as a regulator of freeze response

There is increasing realization of the importance of variation in annual C and N cycling in forests due to interactions between climate and plant and microbial processes (Yuan et al. 2009). Key interactions include the effects of climate on decomposition in one season which can, in turn, influence C availability, N dynamics and responses to disturbance such as soil freezing during subsequent seasons (Knapp and Seastedt 1986). While these interactions are relatively easy to study in grasslands or agricultural systems, they are more difficult to discern in forests where pools of detritus are large and small changes in large fluxes can have significant biogeochemical effects (e.g., a 10% reduction in plant uptake can lead to a doubling or tripling of watershed N export (Likens and Bormann 1995)).

At the HBEF, long-term monitoring of ecosystem C and N dynamics and response to disturbance illustrates the importance of annual variation as a regulator of ecosystem response to disturbances such as soil freezing. Data from several long-term monitoring efforts suggest that the present snow manipulation study was done during a period of relatively low N cycling and moderate C availability compared with our earlier snow manipulation studies, which would decrease the likelihood of high N losses following freezing disturbance. Low N cycling is suggested by low potential net N mineralization and denitrification in the summers of 2003 and 2004 compared to the summers of 1998 and 1999 (Fig. 6). Moderate C availability is illustrated by comparing potential microbial respiration in the summers of 2003 and 2004 with the summers of 1998 and 1999 (Fig. 6). Stream and soil solution NO_3^- concentrations were elevated at the HBEF in the summers of 1998 and 1999 due to ice storm damage to the forest canopy in January 1998 (Houlton et al. 2003), although damage was minor in our snow manipulation plots. Years with exceptionally warm temperatures (1998 and 1999, Dittman et al. 2007) and severe summer drought (2002) also have occurred during the interval of our experiments and could have influenced N and C availability. Watershed outputs of NO_3^- have been declining over the past 20 years, suggesting a possible trend towards decreasing N availability (Goodale et al. 2003; Judd et al. 2007).

Comparison of rates of in situ N mineralization and nitrification, which were measured using the same methods in both sets of snow manipulation studies at the HBEF, suggests that differences in inorganic N production do not account for the difference between the two sets of studies. In situ rates were similar in both studies; mineralization ranged from 11.9 to 23.1 g N m^{-2} y^{-1} in the 1997–1999 studies and from 3.7 to 19.1 g N m^{-2} y^{-1} in the 2002–2004 studies. Nitrification ranged from 7.1 to 13.5 g N m^{-2} y^{-1} in 1997–1999 and from 0.2 to 10.9 g N m^{-2} y^{-1} in 2002–2004. These data do show however that annual variation in inorganic N production can be significant. For example, mineralization was almost twice as high in 1999 than in 1998; a difference of almost 10 g N m^{-2} y^{-1}, and NO_3^- production by nitrification was also higher in 1999 by a difference of almost 5 g N m^{-2} y^{-1}. In the current study, both mineralization and nitrification were higher in 2004 than in 2003 (Table 5), with nearly 5 g N m^{-2} y^{-1} more NO_3^- production in 2004 than in 2003. Further research is necessary to understand the factors causing annual variation in mineralization and nitrification processes and controlling the fate of the inorganic N that they produce.

Other processes important in determining forest response to freeze disturbance that could have varied between our previous and current snow manipulation studies are consumption of inorganic N by denitrification, heterotrophic microbes and plant uptake. Trends in denitrification potential (Fig. 6) suggest that increases in denitrification do not account for the smaller response to treatment in the current studies as denitrification potential was lower in the summers of 2003 and 2004 than it was in the summers of 1998 and 1999. N_2O (one of the products of denitrification) flux was also lower in the current studies than in the previous studies. Microbial biomass C and N content were also similar between the two sets of studies, and there was no response to treatment in either study. Microbial biomass rarely responds to soil freezing treatments in ecosystems that naturally have significant amounts of snow and below freezing temperatures (Matzner and Borken 2008).

The major sink for inorganic N, and the key regulator of N loss response to disturbance at the HBEF is plant uptake (Bormann and Likens 1979; Dittman et al. 2007; Houlton et al. 2003). Soil freezing resulted in significant root mortality in both sets of snow manipulation studies (Cleavitt et al. 2008; Tierney et al. 2001), and decreases in plant uptake associated with this mortality are likely responsible for increases in N losses associated with soil freezing. A key uncertainty is the nature and extent of root recovery from freeze disturbance. Root production was higher in treatment plots during summer in both sets of studies, but was assessed using different methods, so it is not clear if differences in root recovery contributed to the difference in response to disturbance between studies. The importance of root production is evident in the current study, where production was higher in 2004 than 2003 (Cleavitt et al. 2008). Increased production and associated plant uptake in 2004 likely limited leaching losses, despite significantly higher rates of in situ mineralization and nitrification during that year. Root regrowth was particularly high in the sugar maple-dominated plots that exhibited marked increases in NO_3^- leaching, further illustrating the likely importance of plant uptake as a regulator of N loss response to freeze disturbance.

Conclusions

Results from this second set of snow manipulation studies at the HBEF generally support the hypothesis that climate change resulting in less snow and more soil freezing will increase N losses from northern hardwood forests. However, while we observed increases in NO_3^- leaching and N_2O flux in treatment plots, effects were not as large as expected nor as those observed in our previous snow manipulation studies, and there was no relationship between N losses and the intensity of soil freezing. Ecosystem response to soil freezing disturbance appears to be affected by DOC mobilization, plant community composition, root mortality and regrowth, and poorly understood multi-year patterns in ecosystem C and N processes. Reconciling these factors will be necessary to improve understanding of the effects of disturbances such as soil freezing on the structure and function of the northern hardwood forest. This reconciliation will be difficult as climate and atmospheric chemistry in the region are constantly changing and will require a coordinated effort of long-term monitoring, experimental manipulations and synthetic modeling.

Acknowledgments We thank Lisa Martel for excellent field, laboratory and data analysis work. This research was supported by U.S. National Science Foundation Grants DEB 98-10221 (Hubbard Brook Long Term Ecological Research) and DEB 00-75387 (Ecosystem Studies). This research was conducted at the Hubbard Brook Experimental Forest, which is operated and maintained by the USDA Forest Service, Northern Research Station, Newtown Square, PA. This paper is a contribution to the Hubbard Brook Ecosystem Study.

References

Auclair AND, Lill JT, Revenga C (1996) The role of climate variability and global warming in the dieback of northern hardwoods. Water Air Soil Pollut 91:163–186

Austnes K, Vestgarden LS (2008) Prolonged frost increases release of C and N from a montane heathland soil in southern Norway. Soil Biol Biochem 40:2540–2546

Austnes K, Kaste O, Vestgarden LS, Mulder J (2008) Manipulation of snow in small headwater catchments at Storgama, Norway: effects on leaching of total organic carbon and total organic nitrogen. Ambio 37:38–47

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

Bormann FH, Likens GE (1979) Pattern and process in a forested ecosystem. Springer-Verlag, New York

Boutin R, Robitaille G (1995) Increased soil nitrate loss under mature sugar maple trees affected by experimentally-induced deep frost. Can J For Res 25:588–602

Bowden RD, Melillo JM, Steudler PA, Aber JD (1991) Effects of nitrogen additions on annual nitrous-oxide fluxes from temperate forest soils in the northeastern United States. J Geophys Res Atm 96:9321–9328

Brooks PD, Williams MW (1999) Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. Hydrol Process 13:2177–2190

Campbell JL, Mitchell MJ, Groffman PM, Christenson LM, Hardy JP (2005) Winter in northeastern North America: a critical period for ecological processes. Front Ecol Environ 3:314–322

Campbell JL, Rustad LE, Boyer EW, Christopher SF, Driscoll CT, Fernandez IJ, Groffman PM, Houle D, Kiekbusch J, Magill AH, Mitchell MJ, Ollinger SV (2009) Consequences of climate change for biogeochemical cycling in forests of northeastern North America. Can J For Res 39:264–284

Christenson LM, Mitchell MJ, Groffman PM, Lovett GM (2010) Winter climate change implications for decomposition in Northeastern forests: comparisons of sugar maple litter to herbivore fecal inputs. Glob Change Biol. doi: 10.1111/j.1365-2486.2009.02115.x

Cleavitt NL, Fahey TJ, Groffman PM, Hardy JP, Henry KS, Driscoll CT (2008) Effects of soil freezing on fine roots in a northern hardwood forest. Can J For Res 38:82–91

Dittman JA, Driscoll CT, Groffman PM, Fahey TJ (2007) Dynamics of nitrogen and dissolved organic carbon at the Hubbard Brook Experimental Forest. Ecology 88: 1153–1166

Fiorentino I, Fahey TJ, Groffman PM, Driscoll CT, Eagar C, Siccama TG (2003) Initial responses of phosphorus biogeochemistry to calcium addition in a northern hardwood forest ecosystem. Can J For Res 33:1864–1873

Fitzhugh RD, Driscoll CT, Groffman PM, Tierney GL, Fahey TJ, Hardy JP (2001) Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. Biogeochemistry 56:215–238

Fitzhugh RD, Likens GE, Driscoll CT, Mitchell MJ, Groffman PM, Fahey TJ, Hardy JP (2003) Role of soil freezing events in interannual patterns of stream chemistry at the Hubbard Brook experimental forest, New Hampshire. Environ Sci Technol 37:1575–1580

Frei A, Brown R, Miller JA, Robinson DA (2005) Snow mass over North America: observations and results from the second phase of the atmospheric model intercomparison project. J Hydrometeorol 6:681–695

Goldberg SD, Gebauer G (2009) Drought turns a central European Norway spruce forest soil from an N_2O source to a transient N_2O sink. Glob Change Biol 15: 850–860

Goodale CL, Aber JD, Vitousek PM (2003) An unexpected nitrate decline in New Hampshire streams. Ecosystems 6:75–86

Goodale CL, Aber JD, Vitousek PM, McDowell WH (2005) Long-term decreases in stream nitrate: successional causes unlikely; Possible links to DOC? Ecosystems 8: 334–337

Groffman PM, Holland EA, 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. Oxford University Press, New York, pp 272–288

Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001a) Colder soils in a warmer world: a snow manipulation study in a northern hardwood forest ecosystem. Biogeochemistry 56:135–150

Groffman PM, Driscoll CT, Fahey TJ, Hardy JP, Fitzhugh RD, Tierney GL (2001b) Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. Biogeochemistry 56:191–213

Groffman PM, Fisk MC, Driscoll CT, Likens GE, Fahey TJ, Eagar C, Pardo LH (2006a) Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. Ecosystems 9:1289–1305

Groffman PM, Hardy JP, Driscoll CT, Fahey TJ (2006b) Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. Glob Change Biol 12:1748–1760

Groffman PM, Hardy JP, Fisk MC, Fahey JT, Driscoll CT (2009) Climate variation and soil carbon and nitrogen cycling processes in a northern hardwood forest. Ecosystems 12:927–943

Hafner SD, Groffman PM (2005) Soil nitrogen cycling under litter and coarse woody debris in a mixed forest in New York State. Soil Biol Biochem 37:2159–2162

Hafner SD, Groffman PM, Mitchell MJ (2005) Leaching of dissolved organic carbon, dissolved organic nitrogen, and other solutes from coarse woody debris and litter in a mixed forest in New York State. Biogeochemistry 74:257–282

Hardy JP, Groffman PM, Fitzhugh RD, Henry KS, Welman AT, Demers JD, Fahey TJ, Driscoll CT, Tierney GL, Nolan S (2001) Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. Biogeochemistry 56:151–174

Hayhoe K, Wake C, Huntington T, Luo L, Schwartz M, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, Troy T, Wolfe D (2007) Past and future changes in climate and hydrological indicators in the US Northeast. Clim Dyn 28:381–407

Henry HAL (2007) Soil freeze-thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biol Biochem 39:977–986

Henry HAL (2008) Climate change and soil freezing dynamics: historical trends and projected changes. Clim Change 87:421–434

Hentschel K, Borken W, Matzner E (2008) Leaching losses of nitrogen and dissolved organic matter following repeated freeze/thaw events in a forest soil. J Plant Nutr Soil Sci 171:699–706

Hentschel K, Borken W, Zuber T, Bogner C, Howe B, Matzner E (2009) Effects of soil frost on nitrogen net mineralization, soil solution chemistry and seepage losses in a temperate forest soil. Glob Change Biol 15:825–836

Houlton BZ, Driscoll CT, Fahey TJ, Likens GE, Groffman PM, Bernhardt ES, Buso DC (2003) Nitrogen dynamics in ice storm-damaged forest ecosystems: implications for nitrogen limitation theory. Ecosystems 6:431–443

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

Johnson CE, Driscoll CT, Siccama TG, Likens GE (2000) Element fluxes and landscape position in a northern hardwood forest watershed ecosystem. Ecosystems 3:159–184

Joseph G, Henry HAL (2008) Soil nitrogen leaching losses in response to freeze-thaw cycles and pulsed warming in a temperate old field. Soil Biol Biochem 40:1947–1953

Judd KE, Likens GE, Groffman PM (2007) High nitrate retention during winter in soils of the Hubbard Brook experimental forest. Ecosystems 10:217–225

Kaste O, Austnes K, Vestgarden LS, Wright RF (2008) Manipulation of snow in small headwater catchments at Storgama, Norway: effects on leaching of inorganic nitrogen. Ambio 37:29–37

Kellman L, Kavanaugh K (2008) Nitrous oxide dynamics in managed northern forest soil profiles: is production offset by consumption? Biogeochemistry 90:115–128

Knapp AK, Seastedt TR (1986) Detritus accumulation limits productivity of tallgrass prairie. Bioscience 36:662–668

Likens GE, Bormann FH (1995) Biogeochemistry of a forested ecosystem, 2nd edn. Springer-Verlag, New York

Lovett GM, Mitchell MJ (2004) Sugar maple and nitrogen cycling in the forests of eastern North America. Front Ecol the Environ 2:81–88

Lovett GM, Weathers KC, Arthur MA (2002) Control of nitrogen loss from forested watersheds by soil carbon: nitrogen ratio and tree species composition. Ecosystems 5:712–718

Matzner E, Borken W (2008) Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. Eur J Soil Sci 59:274–284

McDowell WH, Cole JJ, Driscoll CT (1987) Simplified version of the ampoule-persulfate method for determination of dissolved organic carbon. Can J Fish Aquat Sci 44: 214–218

McInnes KJ, Weaver RW, Savage MJ (1994) Soil water potential. In: Weaver RW (ed) Methods of soil analysis, part 2—microbiological and biochemical properties. Soil Science Society of America, Madison, WI, pp 53–58

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

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL (ed) Methods of soil analysis, part 3—chemical methods. Soil Science Society of America, Madison, WI, pp 961–1010

Ricard JA, Tobiasson W, Greatorex A (1976) The field-assembled frost gage [technical note]. CRREL, Hanover, NH

Robertson GP, Wedin D, Groffman PM, Blair JM, Holland EA, Nadelhoffer KA, 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. Oxford University Press, New York, pp 258–271

SAS (1988) SAS/STAT user's guide, release 6.03. Sas Institute Incorporated, Cary, NC

Schwarz PA, Fahey TJ, McCulloch CE (2003) Factors controlling spatial variation of tree species abundance in a forested landscape. Ecology 84:1862–1878

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. Soil Biol Biochem 11: 262–267

Sobczak WV, Findlay S, Dye S (2003) Relationships between DOC bioavailability and nitrate removal in an upland stream: an experimental approach. Biogeochemistry 62: 309–327

Soil Survey Staff (2006) Keys to soil taxonomy, 10th edn. US Department of Agriculture, Natural Resources Conservation Service, Washington, D.C

Steinweg JM, Fisk MC, McAlexander B, Groffman PM, Hardy JP (2008) Experimental snowpack reduction alters organic matter and net N mineralization potential of soil macroaggregates in a northern hardwood forest. Biol Fertil Soils 45:1–10

Tabatabai MA, Dick WA (1983) Simultaneous determination of nitrate, chloride, sulfate, and phosphate in natural waters by ion chromatography. J Environ Qual 12: 209–213

Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. Biogeochemistry 56: 175–190

Vestgarden LS, Austnes K (2009) Effects of freeze-thaw on C and N release from soils below different vegetation in a montane system: a laboratory experiment. Glob Change Biol 15:876–887

Williams MW, Brooks PD, Mosier A, Tonnessen KA (1996) Mineral nitrogen transformations in and under seasonal snow in a high-elevation catchment in the Rocky Mountains, United States. Water Resour Res 32:3161–3171

Yuan W, Luo Y, Richardson AD, Oren R, Luyssaert S, Janssens VA, Ceulemens R, Zhou X, Grunwald T, Aubinet M, Berhofer C, Baldocchi D, Chen J, Dunn AL, DeForest JL, Dragoni D, Goldstein AH, Moors E, Munger JW, Monson RK, Suyker AE, Starr G, Scott RL, Tenhunen J, Verma SB, Vesala T, Wofsy SC (2009) Latitudinal patterns of magnitude and interannual variability in net ecosystem exchange regulated by biological and environmental variables. Glob Change Biol 15:2905–2920