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Net nitrogen mineralization and leaching in response to warming and nitrogen deposition in a temperate old field: the importance of winter temperature

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Abstract While climate warming can increase plant N availability over the growing season by increasing rates of N mineralization, increased N mineralization over winter at a time when plant roots are largely inactive, coupled with an increased frequency of soil freeze–thaw cycles, may increase soil N leaching losses. We examined changes in soil net N mineralization and N leaching in response to warming and N addition ($6 \, \mathrm{g \, m^{-2} \, year^{-1}}$) in a factorial experiment conducted in a temperate old field. We used two warming treatments, year-round and winter-only warming, to isolate the effects of winter warming on soil N dynamics from the year-round warming effects. We estimated net N mineralization using in situ soil cores with resin bags placed at the bottom to catch throughput, and we measured N leaching using lysimeters located below the plant rooting zone at a depth of 50 cm. There were minor effects of warming on changes in soil extractable N and resin N in the soil cores over winter. Nevertheless, the overall effects of both warming and N addition on net N mineralization (the sum of changes in soil extractable N and resin N) were not significant over this period. Likewise, there were no significant treatment effects on the concentration of N in leachate collected below the plant rooting zone. However, in response to winter warming, net N mineralization over summer was approximately double that of both the ambient and year-round warming treatments. This result demonstrates a potentially large and unexpected effect of winter warming on soil N availability in this old field system.

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Introduction

In addition to the direct effects of climate warming on ecosystem productivity, warming may alter productivity indirectly by influencing soil N dynamics (Rustad et al. 2001). Increased rates of N mineralization driven by climate warming may increase plant N availability, which, coupled with an extended growing season, could stimulate plant growth in N-limited systems, provided water is not limiting (Sierra 1997; De Valpine and Harte 2001). However, many studies of N mineralization in the context of warming have been restricted to the plant growing season, whereas seasonal variation in interactions between soil N dynamics and warming may complicate the overall effects of warming on plant N availability (Sturm et al. 2005; Aerts et al. 2006). Microbial activity and N cycling continue at temperatures below freezing (Mikan et al. 2002), and winter climate may play a critical role in the retention or loss of N and other biologically important nutrients (Schimel et al. 2004; Kielland et al. 2006). For example, warming over winter, combined with microbial C limitation at this time, can increase N mineralization rates (Giblin et al. 1991; Miller et al. 2007), whereas plant N uptake largely diminishes over winter due to root dormancy and cold temperatures (Clarkson et al. 1992; Henry and Jefferies 2003). Therefore, in the absence of substantial N immobilization by soil microorganisms over winter, the increased soluble N that accumulates in soil as a result of winter climate warming may be lost as leachate or as trace gases during spring thaw (Hobbie and Chapin 1996; Matzner and Borken 2008). Similarly, warming may result in an increased frequency of

soil freeze–thaw cycles over winter (Kattenberg et al. 1996; Isard and Schaetzl 1998), and these freeze–thaw cycles can promote the physical disruption of soil aggregates, and the lysis of microbial cells and fine roots, leading to increased soluble N concentrations in soil followed by N leaching losses (Schimel and Clein 1996; Tierney et al. 2001).

Many studies of soil N dynamics over winter have been conducted in arctic or alpine regions (e.g., Clein and Schimel 1995; Larsen et al. 2002; Grogan and Jonasson 2003; Edwards et al. 2006), which are projected to experience a large degree of climate warming over the next century relative to other systems (IPCC 2007). In contrast, the over-winter N dynamics of northern temperate systems have received less attention (Campbell et al. 2005). Although temperate systems are expected to experience less severe warming over winter than arctic systems, many northern temperate ecosystems experience prolonged periods of freezing temperatures over winter, and these systems may be particularly vulnerable to an increased frequency of freeze–thaw cycles with warming since their soils remain close to the freezing point over much of winter (Henry 2008). These systems are also experiencing increasingly high rates of atmospheric N deposition (Galloway et al. 2004; Sickles and Shadwick 2007), and the extent to which these systems can retain this added N under future climate scenarios has important implications for primary productivity and plant species composition (Tilman and Downing 1994; Vitousek et al. 1997).

In this study, we examined the interactive effects of climate warming and N deposition on net N mineralization and N leaching in a temperate old field. We applied warming treatments either year-round or exclusively during the winter using overhead infrared heaters, and these treatments were crossed with N addition treatments (6 g m^{-2} year^{-1}) in a factorial design. While we applied the all-year warming treatment to approximate the projected warming scenario for our region over the coming decades, we designed the winter-only warming treatment to isolate the effects of winter warming on soil N dynamics from the year-round warming effects. We hypothesized that warming would increase net N mineralization over all seasons, leading to increased N leaching over winter. We also predicted that the net effects of winter warming and increased N deposition would be additive, such that N mineralization and leaching would be greatest in the plots experiencing both treatments.

Materials and methods

Site description

We conducted our field research in a former agricultural field at the Agriculture Canada Southern Crop Protection and Food Research Centre, in London, Ontario (43°01′46″N, 81°12′52″W). The site has not been ploughed, fertilized, or mowed for over 25 years, and was planted prior to that time with rotations of wheat, corn and soybean. The soil is classified as well to imperfect drained silt loam glacial till (Hagerty and Kingston 1992), and mean pH, measured over the top 15 cm using a 2:1 ratio of deionized water to soil, is 7.6 (SE 0.1) (Bell, in review). The mean annual air temperature for the site is 7.5°C, with a low monthly mean of −6.3°C (January) and a high monthly mean of 20.5°C (July), and a mean annual precipitation of 818 mm (Canadian Climate Normals 1971–2000; Environment Canada, National Climate Data and Information Archive). The vegetation at the site is dominated by the perennial grasses *Poa pratensis* L. and *Bromus inermis* Leyss., while the forb *Cirsium arvense* L. and the legume *Lotus corniculatus* L. are also common, but patchy. The forbs *Asclepias syriaca* L., *Aster ericoides* L. and *Solidago altissima* L. are also present at lower density.

Design of main warming and N addition experiment

The field manipulations consisted of three warming treatments (warming all year, warming only in winter, and no warming) crossed with two N treatments (added N and N control) organized in a randomized block split-plot design, with warming administered at the plot level and N additions assigned to two spatially separated circular subplots within each plot (Fig. 1; $n = 10$ for each of the six treatment combinations). Each of the 60 subplots was 1.13 m in diameter and extended outwards by an additional 10 cm into a buffer zone that also received the experimental treatments. Plots
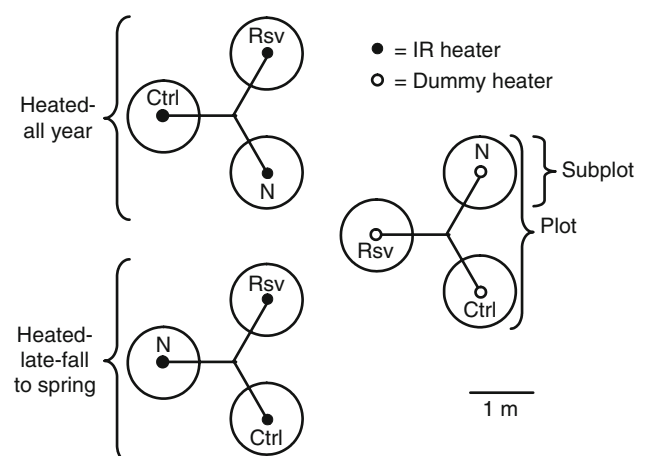
Fig. 1 Overhead scale drawing of one of ten of the experimental blocks. Plots receive either year-round warming, winter-only warming or experience ambient temperatures (dummy heaters). Subplots within each plot are randomly assigned to either an N control (*Ctrl*), N addition (*N*) or to plots reserved for later treatments (*Rsv*; not included in this study)

were heated by single 150-W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, Calif.), suspended 50 cm above the plot centers and operated continuously at full intensity. These heaters simulate solar heating without giving off photosynthetically active radiation (Harte et al. 1995). In the N addition plots, we added a pulse of aqueous NH_4NO_3 in late March at a rate of $2 \text{ g m}^{-2} \text{ year}^{-1}$, and in late May we added additional NH_4NO_3 in the form of slow-release pellets (Florikan ESA, Sarasota, Fla.) at a rate of $4 \text{ g m}^{-2} \text{ year}^{-1}$. These addition rates were designed to approximate projected increases in atmospheric deposition in this region by the year 2050 (Galloway et al. 2004), whereas current rates of total N deposition for this region are closer to $1 \text{ g m}^{-2} \text{ year}^{-1}$ (Sickles and Shadwick 2007). N control plots received the same amount of water as the N addition plots in the spring (equivalent to <2 mm of rain), but no added N. We began warming the plots in late November 2006, and we monitored the microclimatic effects on the soil using 107-BAM-L temperature probes at 1-cm depth and CS616-L time domain reflectometry probes (for estimating volumetric water content) located at 0- to 15-cm depth (both probes from Campbell Scientific Canada, Edmonton, AB, Canada). All soil temperature data were recorded year-round, on an hourly basis. Starting in fall 2007, we assessed snow cover twice daily using overhead images of the plots obtained with two CC640 digital cameras (Campbell Scientific Canada) fixed on tripod masts. We conducted winter warming treatments from 20 November 2006 to 11 April 2007, and again from 15 November 2007 to 7 April 2008. Both winters were approximately 2°C warmer than the 1971–2000 climate normals and the second winter featured anomalously high precipitation (Table 1). The summer growing season documented in this study was close to the normal mean temperature but unusually dry (Table 1). The temperature of the soil at 1-cm depth in the warmed plots was raised by between 1 and 4°C relative to ambient plots over the snow-free season, whereas warmed plots sometimes reached colder temperatures than ambient plots (Fig. 2) when the snow was melted in the former but the latter remained protected by snow. Therefore, warming increased the temporal variability of soil temperatures over both winters but not over the summer (comparison of SDs presented in Table 1).

We calculated plant aboveground and belowground N pools for fall 2007 (Table 2) using measurements of percent N in root and shoot tissue from Turner and Henry (2009) and plant biomass estimates from Hutchison and Henry (in review). For the latter, aboveground plant biomass was estimated non-destructively on 7 August and 18 October from one 10-cm × 10-cm quadrat in each of the 60 subplots. The number of shoots within each quadrat was recorded, and shoot height was converted to mass using allometric

Table 1 Mean air temperature and total precipitation over both the 2006–2007 and 2007–2008 winters (1 November–31 March) and the plant growing season in 2007 (1 April–31 October) for the experimental site relative to 1971–2000 climate normals (Environment Canada, National Climate Data and Information Archive)

|	1971–2000	2007	2008
Mean winter air temperature (°C)	−2.8	−0.9	−0.4
Total winter precipitation (mm)	394	364	499
Mean winter soil temperature (°C)[a]			
Ambient plots	n.a.	2.6 (2.8)	1.6 (2.2)
Warmed plots	n.a.	2.9 (3.2)	1.7 (2.5)
Mean growing season air temperature (°C)	15.4	15.9	n.a.
Total growing season precipitation (mm)	399	286	n.a.
Mean growing season soil temperature (°C)[a]			
Ambient plots	n.a.	16.0 (5.6)	n.a.
Warmed plots	n.a.	16.8 (5.7)	n.a.

[a] Mean soil temperature data (SD of hourly means *in parentheses* to provide a relative measure of temporal variability) were obtained from probes at 1-cm depth in each plot

equations derived from shoots located outside of the plots. Belowground plant biomass was measured directly from roots recovered from a 2-cm-diameter × 15-cm-deep soil core collected from each subplot on 6 November. Soil microbial N pools were obtained from Bell et al. (in review), who used the chloroform fumigation extraction technique on subsamples of 15-cm-deep soil cores collected from 27 to 31 August.

Net N mineralization

We used intact in situ soil cores, encased in 4.3-cm-diameter × 15-cm-deep PVC tubes, to quantify changes in net N mineralization in response to the warming and N treatments. Based on the method of DiStefano and Gholz (1986), we placed one resin bag on top of each core to deionize deposition from above and placed two resin bags on the bottom, with the upper one capturing ions leaching from the soil core and the lower one deionizing water entering the tube from below. In each resin bag, we combined approximately 2.5 g of a sulfonic acid-based cation resin (HCR-W2, H^+ form) with 2.5 g of a trimethylbenzyl ammonium-based anion resin (21 K, Cl^- form) (Dow Chemical, Calgary, Alberta). We based these combinations on wet exchange capacities (1.3 Eq l^{-1} anion to 1.8 Eq l^{-1} cation) and activated the resins by soaking them in 5 M NaCl overnight (Szillery et al. 2006).

We placed one soil core in each of the 60 plots from 22 November 2006 to 18 April 2007 to quantify net N mineralization over the first winter (Fig. 2a). We incubated additional
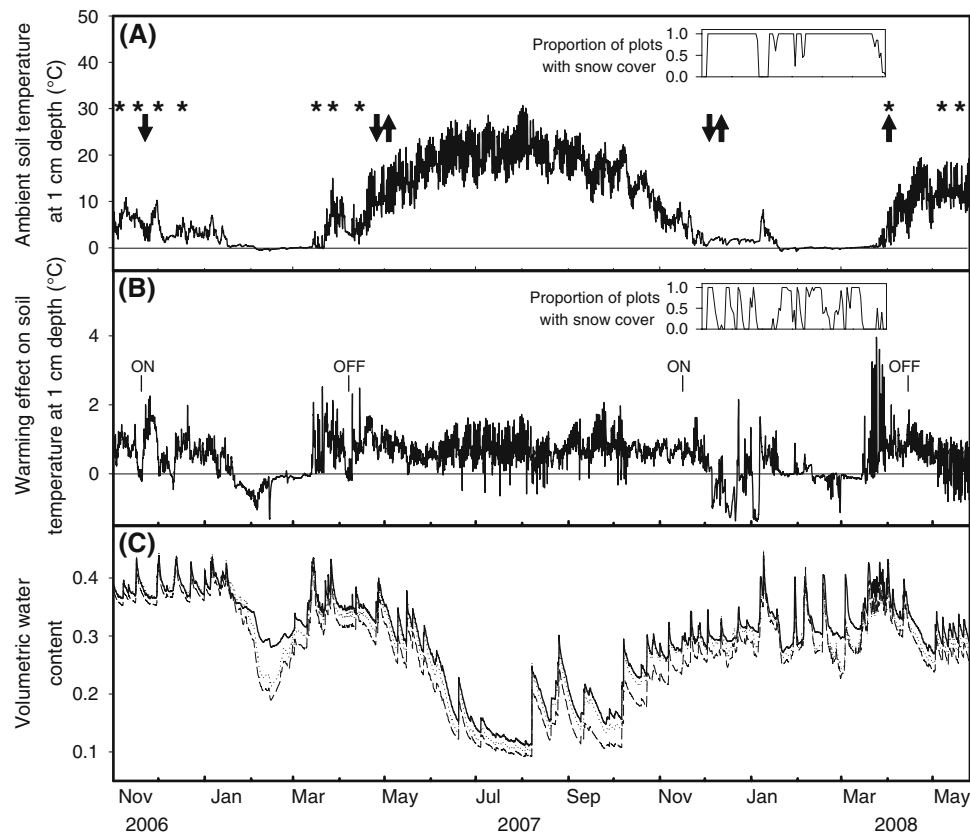
Fig. 2 **a** ambient soil temperatures at 1-cm depth and **b** the effect of year-round warming on soil temperature at 1-cm depth relative to ambient soil temperatures, both pooled over the N treatments ($n = 20$). *Insets* display the proportion of plots with snow cover under **a** ambient air temperature and **b** warming (data not available for the first winter, although periods of snow cover in ambient plots are evident when diurnal soil temperature fluctuations are greatly reduced). The *vertical arrows* display the timing of net N mineralization core insertion and removal from the plots. Lysimeter sampling dates are indicated by an *asterisk*. The activation of the winter-warmed plots (*ON* and *OFF*), and temperatures in the winter-warmed plots followed those of the year-round warmed plots when active and the ambient plots when turned off. **c** The volumetric water content from 0- to 15-cm depth in ambient plots (*solid line*), winter-warmed plots (*dotted line*) and year-round warmed plots (*dashed line*), pooled over N treatments ($n = 20$)

Table 2 Mean plant aboveground and belowground N pools in fall [from Turner and Henry (2009) and Hutchison and Henry (in review)] and microbial N pools in late summer (from Bell et al., in review) in response to warming and N addition treatments (SEs *in parentheses*)

N addition	Warming	Aboveground plant N (g m^{-2})	Belowground plant N (g m^{-2})	Soil microbial N (g m^{-2})
No	No	8.3 (1.1)	0.39 (0.07)	7.7 (0.5)
No	Winter only	9.5 (2.0)	0.36 (0.05)	7.9 (0.7)
No	Year-round	8.3 (2.2)	0.48 (0.07)	7.4 (0.3)
Yes	No	12.9 (3.3)	0.40 (0.08)	10.6 (2.3)
Yes	Winter only	13.2 (2.4)	0.41 (0.07)	15.3 (3.6)
Yes	Year-round	11.4 (2.4)	0.33 (0.07)	9.7 (1.0)

cores in each plot from 28 April 2007 to 16 November 2007, and from 22 November 2007 to 31 March 2008 to quantify net N mineralization over the plant growing season and second winter, respectively. The mean air temperature and total precipitation over each of these intervals is detailed in Table 1. Following incubation in the field, we mixed the soil in each core and extracted a 15-g subsample in 75 ml of 2 M KCl for 1 h, then filtered the extracts through pre-leached Whatman no. 1 filter paper and froze

them. We also extracted N from samples collected from each plot prior to incubation to quantify initial extractable N concentrations. We analyzed the extracts colorimetrically for NO_3^- (EPA method 353.2, detection limit of 1.5 μM) and NH_4^+ (EPA method 350.1, detection limit of 0.7 μM) using a SmartChem 140 discrete auto-analyzer (Westco Scientific Instruments, Brookfield, Conn.). We discarded both outer resin bags and extracted the inner-bottom resin bag in 2 M KCl for 30 min. We used the post-incubation

extractable NH_4^+ and NO_3^- from the soil core and inner-bottom resin bag minus the pre-incubation extractable NH_4^+ and NO_3^- from the soil to estimate net N mineralization (Hanselman, et al. 2004).

Soil N leachate: lysimeter sampling

We installed polytetrafluoroethylene-quartz suction lysimeters (Prenart Equipment, Frederiksberg, Denmark) in June 2006 in the center of each plot at a depth of 50 cm to measure N leaching losses below the rooting zone (sample collection dates indicated in Fig. 2a; soils were too dry for leachate collection over the summer and fall in 2007). We analyzed the lysimeter samples colorimetrically for NO_3^- and NH_4^+ using the methods described above. We estimated total soluble N by persulfate oxidation (Cabrera and Beare 1993) followed by analysis for NO_3^-, then estimated total soluble organic N by subtracting NH_4^+ and NO_3^- from the total soluble N.

Statistical analyses

For analyzing treatment effects on changes in soil extractable inorganic N and resin extractable inorganic N, and the sum of the two (net N mineralization) over each sampling interval we used a split-plot ANOVA that included warming as a fixed between-plot factor, N addition as a fixed within-plot factor, and the interaction term for their treatment combination. We also included block number as a random factor in the ANOVA model. Error terms for significance tests were as specified in Kirk (1995), and we ran the analysis using the REML method of the Fit model platform in JMP 4.0 (SAS Institute). For winter 2007, we pooled both warming treatments since warming started in late fall 2006, and the N factor was removed from the ANOVA model since N addition did not commence until spring 2007. For the lysimeter data, we modified the above model to account for repeated measures of the same lysimeter within each of the three main sampling periods by adding date as a fixed factor, along with all treatment interactions with date, and by adding subplot as a random factor. For each analysis, we log-transformed the dependent variables to improve normality and homogeneity of variances. We used Tukey's honest significant difference tests to resolve differences among dates following a significant ANOVA result ($P < 0.05$).

Results

Soil N mineralization cores and resin bags

Overall, treatment effects on both changes in soil extractable inorganic N (Fig. 3a) and resin extractable inorganic N (Fig. 3b) from the soil mineralization cores varied both seasonally and among years. Warming significantly reduced increases in soil extractable inorganic N over winter in 2007 ($P_{warm} = 0.049$; Fig. 3a, i), whereas only winter warming significantly decreased (under N control) or increased (under added N) soil extractable inorganic N over winter 2008 ($P_{warm \times N} = 0.033$; Fig. 3a, iii). Soil extractable inorganic N also increased significantly over the plant growing season in response to the winter warming treatment in 2007 ($P_{warm} < 0.001$; Fig. 3a, ii). The relative contributions of NO_3^- and NH_4^+ to changes in soil extractable inorganic N also varied seasonally (Table 3). The accumulation of NH_4^+ was responsible for the overall increase in soil extractable inorganic N over winter in 2007 (Fig. 3a, i), and there was also a small but significant effect of warming on initial extractable inorganic N (Table 3). NH_4^+ also accumulated in the soil in general over winter in 2008. However, decreases in NO_3^- and large increases in NH_4^+ over the incubation period were responsible, respectively, for the significant decrease (under N control) and increase (under N addition) in soil extractable inorganic N in response to winter warming in Fig. 3a (Table 3). The increases in soil extractable inorganic N over the plant growing season in winter-warmed plots (Fig. 3a, ii) were explained primarily by increased NO_3^- (Table 3).

Inorganic N extracted from the inner resin bags positioned at the bottoms of the soil mineralization cores was substantially higher over the plant growing season than in the two winters (Fig. 3b). Winter warming approximately doubled the inorganic N captured by the resin bags over the growing season ($P_{warm} = 0.018$; Fig. 3b, ii). While there were no significant warming effects over winter 2007 (Fig. 3b, i), warming over winter 2008 increased resin extractable inorganic N significantly ($P_{warm} = 0.004$; Fig. 3b, iii). NO_3^- accounted for 94, 99 and 86% of the total inorganic extracted from the resins incubated over winter 2007, the plant growing season 2007 and winter 2008, respectively (data not shown).

When changes in soil extractable inorganic N and resin extractable inorganic N were combined to obtain estimates of net N mineralization (e.g., the sum of a given bar in Fig. 3a with the bar directly below it in Fig. 3b), there were no significant treatment effects over winter 2007 ($P_{warm} = 0.17$) or winter 2008 ($P_{warm} = 0.11$, $P_N = 0.32$ and $P_{warm \times N} = 0.17$). However, net N mineralization in the winter-warmed plots was significantly higher than in the other treatments over the plant growing season in 2007 ($P_{warm} = 0.007$).

Soil lysimeter N

There were no significant treatment effects on NO_3^-, NH_4^+, soluble organic N or total soluble N collected from soil lysimeters in fall 2006, spring 2007 or spring 2008. However, there were significant differences in total soluble N

Fig. 3 **a** changes in soil extractable inorganic N and **b** resin-captured inorganic N from soil N mineralization cores incubated over winter 2007 (*i*; 22 November–18 April, prior to N addition), the plant growing season in 2007 (*ii*; 28 April–16 November) and winter 2008 (*iii*; 22 November–31 March). Means and SEs are presented, with statistically significant ANOVA results displayed in each panel (*n* = 60). Significant differences between group means within each panel (Tukey's honest significant difference; HSD) are denoted by different *lower-case letters*, with *horizontal bars* denoting pooled data
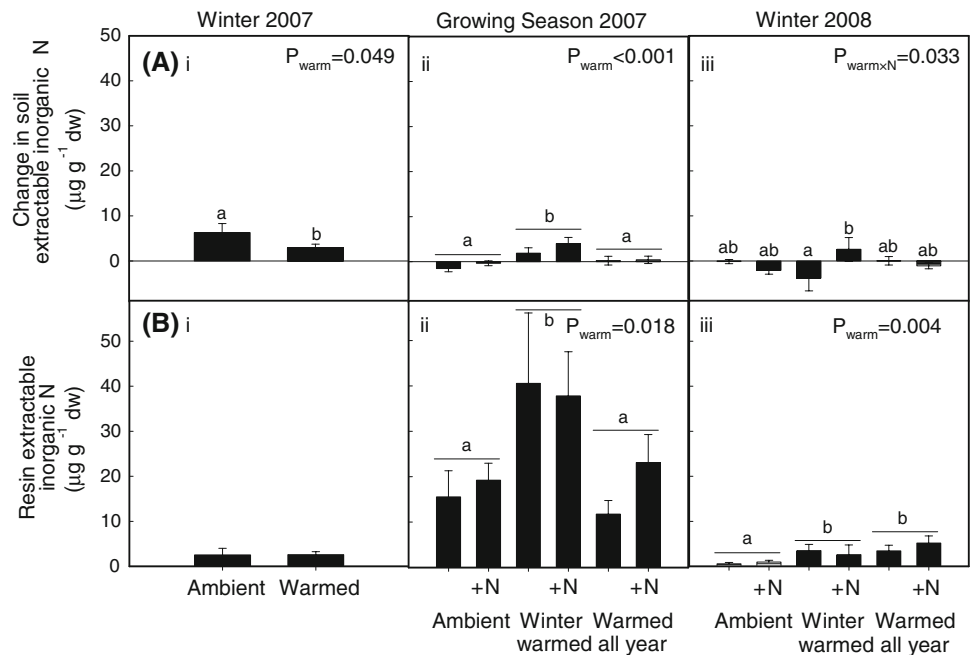
Table 3 Extractable NO_3^- and NH_4^+ from soil mineralization cores both pre-incubation (*Initial*) and post-incubation (*Final*), expressed per unit of soil dry weight (*d.w.*)

Treatment		n	Initial NO_3^- ($\mu g\ g^{-1}$ d.w.)	Final NO_3^- ($\mu g\ g^{-1}$ d.w.)	Initial NH_4^+ ($\mu g\ g^{-1}$ d.w.)	Final NH_4^+ ($\mu g\ g^{-1}$ d.w.)
Warming	N					
Winter (2007)						
Ambient	Pooled	20	0.56 (0.05) a	0.78 (0.09)	0.78 (0.09) a	6.87 (2.01)
Warmed	Pooled	40	0.87 (0.11) b	1.27 (0.33)	1.13 (0.09) b	3.59 (0.78)
Growing season (2007)						
Ambient	Pooled	20	0.75 (0.05)	2.14 (0.69) a	1.63 (0.28) a	0.49 (0.07)
Winter-warmed	Pooled	20	0.66 (0.04)	4.58 (0.90) b	1.67 (0.16) ab	0.71 (0.22)
Warmed all year	Pooled	20	0.80 (0.08)	1.55 (0.23) a	2.20 (0.29) b	0.52 (0.10)
Winter (2008)						
Ambient	Control	10	2.11 (0.61)	0.89 (0.09) a	0.59 (0.18)	1.65 (0.59) ab
	N added	10	3.26 (0.91)	0.93 (0.09) a	0.61 (0.25)	0.88 (0.15) a
Winter-warmed	Control	10	6.10 (2.52)	1.75 (0.63) b	0.55 (0.08)	1.09 (0.21) ab
	N added	10	3.78 (1.07)	3.01 (1.22) b	0.52 (0.12)	3.89 (1.07) b
Warmed all year	Control	10	1.83 (0.57)	1.13 (0.25) ab	0.67 (0.16)	1.40 (0.47) ab
	N added	10	2.84 (0.68)	1.13 (0.18) ab	1.10 (0.24)	1.73 (0.38) ab

Cores were incubated over winter 2007 (22 November–18 April, prior to N addition), growing season 2007 (28 April–16 November) and winter 2008 (22 November–31 March)

Different *lower-case letters* indicate significant differences among treatments within a date (Tukey's honest significant difference test, $P < 0.05$). Data were pooled across N treatments (*Pooled*) when differences among treatments were not significant. SEs for each mean are provided *in parentheses*

among seasons and among sampling dates within seasons (Fig. 4). Total soluble N increased across the first three sampling dates in fall 2007, explained primarily by increased soluble organic N and NH_4^+ (Fig. 4, i). It decreased across sampling dates in spring 2007, explained primarily by decreased NO_3^- (Fig. 4, ii). Total soluble N concentrations were also lower in spring 2008 than in spring 2007 (Fig. 4, ii, iii).

Discussion

Net N mineralization

Overall, net N mineralization over the plant growing season was substantially higher than over winter, and the most striking result of our net N mineralization analyses was the large effect of winter warming relative to ambient temperatures

Fig. 4 Concentrations of NO_3^- (*black bars*), NH_4^+ (*light gray bars*) and organic N (*dark gray bars*) in solution collected from 50-cm-deep soil lysimeters in fall 2006 (*i*), spring 2007 (*ii*) and spring 2008 (*iii*), pooled across all treatments (*n* = 60). *Error bars* denote SE for the mean total soluble N (the sum of NO_3^-, NH_4^+ and organic N), and different *lower-case letters* indicate differences among dates in total soluble N (*P* < 0.05, Tukey's HSD test)
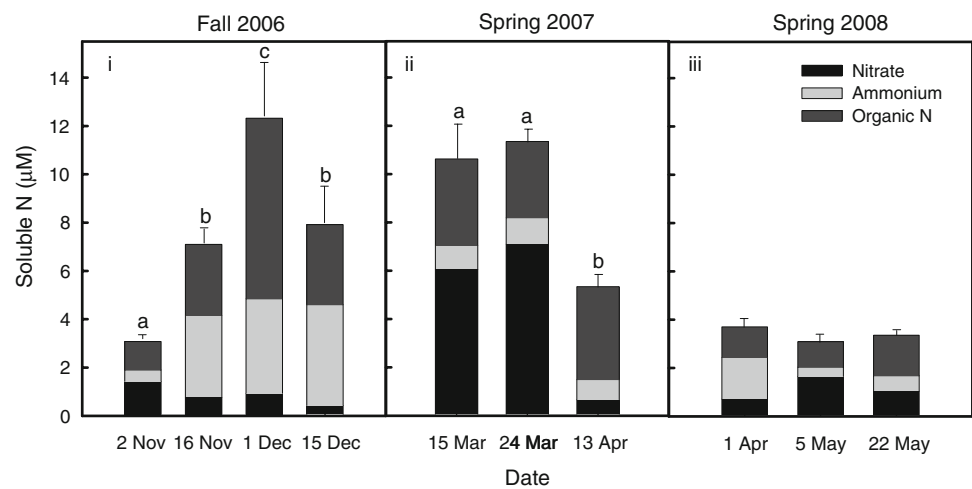
and year-round warming on resin extractable inorganic N over the growing season. This result was unexpected, and could not be explained directly by warming since the soil temperatures in the winter-warmed plots corresponded with those of the year-round warming plots over winter and those of the ambient plots over the plant growing season. Likewise, there were no significant differences among winter-warmed plots and the other plots with respect to plant biomass or species composition in 2007 (Hutchison, in review). Instead, net N mineralization in the winter-warmed plots may have increased as a result of the extended cool period that occurred in these plots following spring melt when their heaters were turned off (e.g., winter-warmed plots thawed early but then, unlike the year-round heated plots, ceased to be warmed). A potential consequence of turning off the heaters in the winter warming treatment was that soil mineralization may have increased after thaw at a time when soils were still too cool for substantial plant N uptake (Henry and Jefferies 2003; Castle et al. 2006). However, we collected soil for the growing season net N mineralization cores in late April, 9 days after the heaters were turned off in the winter-warmed plots, and extractable inorganic N was not elevated in the winter-warmed plots at this time. Likewise, while soil protease activity may increase in response to soil warming in early spring (Rejsek et al. 2008), soil soluble organic N concentrations were not elevated in winter-warmed plots in early spring (Bell et al., in review). Given that the restructuring of microbial communities can accelerate biogeochemical processes (Schimel and Gulledge 1998; Schmidt and Lipson 2004), carryover effects of this prolonged cool period on the soil microbial community in winter-warmed plots might explain increased inorganic N captured by the resin over the growing season.

Plant and microbial N pool data obtained from other studies conducted simultaneously at our site in 2007 (Table 2) were consistent with the increase in resin extractable N in

the winter-warmed plots. This increase was approximately 20 μg N g^{-1} of dry soil (comparison of winter-warmed bars and all others in Fig. 3b, ii), which amounts to approximately 2.8 g m^{-2} of N (based on 200 g dry soil per core, and a core surface area of 0.00145 m^2). Increases of a similar magnitude were observed for the plant aboveground and microbial N pools in winter-warmed plots relative to the N pool sizes in ambient and year-round warmed plots (1.1 and 2.8 g m^{-2} of N respectively, averaged over N addition treatments; Table 2). Therefore, N losses from the soil cores, which did not contain live plant roots and associated microorganisms, appear to have equated to increased plant and microbial N uptake in the surrounding soil.

Contrary to our initial hypothesis, experimental warming did not increase net N mineralization over winter in either 2007 or 2008. However, winter warming did significantly alter both changes in soil extractable inorganic N and resin extractable inorganic N, the two components of the N mineralization estimate. Over winter 2007, decreased temperatures in warmed plots, which occurred as a consequence of accelerated snow melt, coincided with lower increases in soil extractable inorganic N than in ambient temperature plots. This result is consistent with overall decreases in net N mineralization in response to colder soil temperatures observed elsewhere (Emmer and Tietema 1990; Goncalves and Caryle 1994; Schimel et al. 2004). The small but significant increase in initial extractable inorganic N prior to winter 2007 could be attributed to periods of heater testing conducted in the plots in late October and early November prior to the start of the experiment. Over winter 2008, there was no overall effect of warming on changes in soil extractable inorganic N, and unlike winter 2007, there was a significant effect of warming on resin extractable inorganic N. Increased N capture in this inner-bottom resin bag in the warmed plots may be explained by the large effect of warming on freeze–thaw cycle frequency over winter 2008 relative to over winter 2007, as freeze–thaw cycles may

increase N leaching losses due to microbial lysis (Yanai et al. 2004; Larsen et al. 2002) or the disruption of soil aggregates (Six et al. 2004).

When samples were collected to establish initial extractable N concentrations for the net N mineralization cores, the lack of increase in extractable inorganic N for samples collected from N addition plots over all of the sampling dates was unexpected. This lack of N enrichment in the soluble N pool in these plots could be attributed either to losses from the system following N addition or to rapid uptake by plants and soil microorganisms. Concentrations of N in leachate collected by the soil lysimeters were not significantly higher in N addition plots than in N control plots, which suggests that the added N was not lost through leaching below the rooting zone. Soil N trace gas emissions, which we did not measure, may also contribute substantially to ecosystem N losses and can be particularly high over winter or in early spring (Christensen and Tiedje 1990; Chang and Hao 2001). Nevertheless, from Table 2, it appears that approximately half of the added N may have been taken up by plants and the other half immobilized by soil microorganisms. Plant productivity did not increase significantly over the 2007 growing season in response to N addition, presumably as a result of water limitation, but N addition did increase aboveground plant productivity over the following, wetter growing season (Hutchison and Henry, in review). However, the ability of the plants and microorganisms to consistently sequester a large proportion of the added N will likely become limited over the longer term when the system becomes N saturated (Aber et al. 1989).

Overall, the severing of roots and their subsequent decomposition in the cores were likely the main limitations of the intact soil core method we used (Hart et al. 1994). This ion exchange resin method of DiStefano and Gholz (1986) has advantages over other soil containment methods in that soil water content is able to fluctuate, and the products of mineralization can be removed from the core, minimizing the feedbacks of inorganic N accumulation on soil processes. Nevertheless, N transformations such as nitrification and denitrification are likely affected by the absence of live roots, and soil moisture would be altered due to a lack of water removal by transpiration and reduced hydraulic coupling of the intact core with the surrounding soil.

Soil N leaching: lysimeter sampling

As discussed above, the result that low concentrations of soluble N were present in lysimeter samples collected at 50-cm depth, even in N addition plots, indicates that the bulk of N deposition in these plots is either intercepted by plant roots, microbes and soil at more shallow depths, or lost to the atmosphere in the form of N trace gases. In addition, contrary to our initial hypothesis, it did not appear

that winter warming enhanced N leaching losses in this system. While it is possible that large but transient pulses of N may have been missed in the intervals between lysimeter sampling events, such pulses are often expected to occur at spring thaw (Lipson et al. 2000; Grogan and Jonasson 2003), and we did not detect an N pulse at this time in either year. Furthermore, soil water penetration to 50-cm depth over the summer and fall 2007 was insufficient for the collection of lysimeter samples.

The seasonal trends in lysimeter soluble N concentrations pooled across all treatments appeared to correspond with plant root activity. For example, decreasing root N uptake in late fall is consistent with higher N leaching losses (Kielland et al. 2006), and the increase in soluble organic N over the fall may be driven by plant senescence and the loss of soluble compounds from plant litter (Aber and Melillo 1980). Similarly, increased root activity upon soil warming in spring (Henry and Jefferies 2003) explains the decrease in soluble N concentration by late spring 2007. Decreased NO_3^- leaching as spring progressed was further consistent with increased plant uptake (Scherer-Lorenzen et al. 2003). Following a winter with high snow fall, soluble NO_3^- concentrations in lysimeter samples were very low throughout spring 2008. Therefore, the low lysimeter N concentrations in spring 2008 likely represent a dilution effect.

Conclusion

Our most intriguing finding was that while the direct effects of winter warming on soil N dynamics were minor in our system, winter warming had important carryover effects on N dynamics over the plant growing season, whereas year-round warming did not. Although the mechanism behind this dramatic winter warming effect remains unclear, this result highlights the potential importance of unusual temperature events (in this case, most likely the extended cool period in early spring caused by the heater shut off in winter-warmed plots) in influencing annual N dynamics. Nevertheless, the implications of this increased net N mineralization over the growing season in winter-warmed plots are unclear, particularly since this value was estimated using in situ soil cores. While the increased resin N may imply increased leaching through the soil column and losses from the system, alternatively, our N pool data suggest that outside of the net N mineralization cores this soluble N may have been captured over the growing season by live plant roots and associated microorganisms, which were absent from the cores. The latter is also consistent with our lysimeter results. However, if changes to soil N dynamics caused by winter warming do not increase ecosystem N losses over the short term because plants have the capacity to take up the excess N, then our results suggest that

warming over winter may amplify soil N losses under conditions of N saturation.

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