

## Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils

A. R. CONTOSTA,<sup>1</sup>† S. D. FREY,<sup>1</sup> AND A. B. COOPER<sup>2</sup>

<sup>1</sup>Department of Natural Resources & the Environment, University of New Hampshire, Durham, New Hampshire 03824 USA

<sup>2</sup>School of Resource and Environmental Management, Simon Fraser University, Burnaby, British Columbia V5A 1S6 Canada

**Abstract.** Although numerous studies have examined the individual effects of increased temperatures and N deposition on soil biogeochemical cycling, few have considered how these disturbances interact to impact soil C and N dynamics. Likewise, many have not assessed season-specific responses to warming and N inputs despite seasonal variability in soil processes. We studied interactions among season, warming, and N additions on soil respiration and N mineralization at the Soil Warming × Nitrogen Addition Study at the Harvard Forest. Of particular interest were wintertime fluxes of C and N typically excluded from investigations of soils and global change. Soils were warmed to 5°C above ambient, and N was applied at a rate of 5 g m<sup>-2</sup> y<sup>-1</sup>. Soil respiration and N mineralization were sampled over two years between 2007 and 2009 and showed strong seasonal patterns that mirrored changes in soil temperature. Winter fluxes of C and N contributed between 2 and 17% to the total annual flux. Net N mineralization increased in response to the experimental manipulations across all seasons, and was 8% higher in fertilized plots and 83% higher in warmed plots over the duration of the study. Soil respiration showed a more season-specific response. Nitrogen additions enhanced soil respiration by 14%, but this increase was significant only in summer and fall. Likewise, warming increased soil respiration by 44% over the whole study period, but the effect of warming was most pronounced in spring and fall. The only interaction between warming × N additions took place in autumn, when N availability likely diminished the positive effect of warming on soil respiration. Our results suggest that winter measurements of C and N are necessary to accurately describe winter biogeochemical processes. In addition, season-specific responses to the experimental treatments suggest that some components of the belowground community may be more susceptible to warming and N additions than others. Seasonal changes in the abiotic environment may have also interacted with the experimental manipulations to evoke biogeochemical responses at certain times of year.

**Key words:** nitrogen fertilization; nitrogen mineralization; season; soil respiration; soil warming.

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† **E-mail:** alix.contosta@unh.edu

### INTRODUCTION

Mean global temperature is projected to increase between 0.3 and 6.4°C by the year 2100 (IPCC 2007). Over the same period, global deposition of reactive N into terrestrial ecosystems is predicted to double (Galloway et al.

2004). These increases in temperature and N deposition could dramatically alter terrestrial C and N cycling, with consequences for water quality, air quality, and greenhouse gas emissions. Soil respiration comprises the majority of the C flux from terrestrial ecosystems to the atmosphere (Raich and Schlesinger 1992). Even a

10% loss in soil organic C worldwide is comparable to 30 years worth of anthropogenically emitted CO<sub>2</sub> (Kirschbaum 2000). The ability of soils to lose or retain N is equally important. Nitrogen can limit primary productivity, act as a pollutant in surface waters as NO<sub>3</sub><sup>-</sup>, contribute to the formation of tropospheric ozone as NO, and behave as a powerful greenhouse gas as N<sub>2</sub>O (Vitousek and Howarth 1991, Fenn et al. 1998).

Numerous studies over the past two decades have examined the long-term effects of either warming or N additions on soil biogeochemical processes (e.g., Aber et al. 1993, Peterjohn et al. 1994, McNulty et al. 1996, Magill et al. 1996, McHale et al. 1998, Rustad and Fernandez 1998). In the short term, soil warming has generally stimulated soil respiration and N mineralization (Peterjohn et al. 1994, McHale et al. 1998, Rustad and Fernandez 1998). Over time, warming has often ceased to stimulate soil CO<sub>2</sub> flux, even while maintaining high N mineralization rates (Luo et al. 2001, Melillo et al. 2004). Nitrogen fertilization has shown small, initial increases in soil respiration, but has generally suppressed soil CO<sub>2</sub> flux in chronically amended plots (Butnor et al. 2003, Bowden et al. 2004, Burton et al. 2004). Likewise, N additions have initially increased N mineralization, but elevated rates of N cycling have either returned to control levels (Aber et al. 1993, Magill et al. 1996) or declined after a few months or years of fertilization (McNulty et al. 1996). Further, many N amendment experiments have reported that between 85–100% of the added N is retained in the soil, even after 15–20 years of elevated N inputs (Christ et al. 1995, Magill et al. 2000). Thus where they occur separately, soil warming and N additions appear to have an opposite effect on soil C and N dynamics, with warming causing long term soil C losses and high N turnover, and N deposition resulting in soil C gain and low rates of N cycling.

While both increased temperatures and N fertilization can impact soil C and N cycles, much of the work to date that quantifies these effects have existed independently of one another. The segregation of these two ecological disturbances does not represent a real-world scenario where increased temperatures and N loads occur simultaneously, interacting in ways that may be difficult to predict. Warming can

negate the positive effect of nutrient additions on plant and microbial biomass (Chapin et al. 1995, Christensen et al. 1997, Rinnan et al. 2007). Increased temperatures and N inputs can also interact to synergistically increase soil N availability (Chapin et al. 1995, Turner and Henry 2010). Alternatively, the combined effect of warming and N additions can be purely additive and can be extrapolated from each treatment on its own (Zavaleta et al. 2003). Whether soil biogeochemical cycling in temperate forests will show an additive, annulling, or synergistic response to concurrent warming and N fertilization is unknown. Such multifactor studies have typically occurred in grassland and tundra ecosystems, and their findings may not be applicable to temperate, deciduous forests.

Most investigations of soil warming and N fertilization have also not explicitly addressed the role that season plays in mediating the response of soils to warming and N deposition. Yet numerous studies have demonstrated strong seasonal differences in soil C and N cycling that mirror patterns in temperature and plant productivity (Davidson et al. 1998, Jaeger et al. 1999, Bohlen et al. 2001, Groffman et al. 2001, Högberg et al. 2001, Knoepp and Swank 2002, Bowden et al. 2004, Mo et al. 2005, DeForest et al. 2006). That is, soil respiration and N mineralization tend to be greatest at the height of the growing season, when temperatures are warmest and root exudates of labile C are greatest. In contrast, soil C and N fluxes tend to be lowest when temperatures are cold and labile C is scarce. These seasonal differences in soil C and N cycling may be linked to compositional and functional differences in the belowground community. For example, soil respiration during summer may be primarily due to roots and rhizosphere microbes that utilize recent plant photosynthate (Boone et al. 1998, Epron et al. 2001, Högberg et al. 2001, Lipson et al. 2002, Schindlbacher et al. 2009). Respiration outside of this period, i.e., during the early spring, late fall, and winter, likely comes from saprotrophic microorganisms consuming recalcitrant substrates like cellulose and lignin (Lipson et al. 2002, Schmidt et al. 2004). Likewise, N mineralization and uptake during the spring and summer may be driven primarily by plant demand, while immobilization in fall and winter may be due to microbial N

requirements for decomposition (Jaeger et al. 1999, Lipson et al. 1999). Environmental changes such as warming and N fertilization may differentially impact these components of the soil ecosystem at different parts of the year. Failure to address season-specific changes in soil C and N cycling may neglect to identify certain organisms and processes affected by increased temperatures and N inputs.

Winter soil C and N cycling may be especially important to consider when evaluating the seasonal response of soils to warming and N fertilization. Until recently, winter has been perceived as a “dormant” period based on the belief that biological activity declines when temperatures grow cold (Campbell et al. 2005). However, research in areas with seasonal snowpack has demonstrated significant contributions of winter C and N fluxes to the total annual flux (Groffman et al. 2001, Knoepp and Swank 2002, Mo et al. 2005, Groffman et al. 2006, Kielland et al. 2006, Groffman et al. 2009). As a result, changes in winter soil C and N cycling due to higher temperatures and/or N inputs may have negative ecosystem consequences. Increased rates of winter N mineralization in the absence of plant demand could result in N leaching or gas loss (Fisk and Schmidt 1996, Turner and Henry 2010). Already winter soil respiration in temperate forests accounts for 15 to 30% of C fixed during the previous growing season (Goulden et al. 1996); larger winter CO<sub>2</sub> fluxes due to increased temperatures could augment that amount.

Most experimental and observational studies in temperate forests have not measured soil C and N fluxes during winter, often because of the logistical complications of sampling in snow. Instead they rely on over-winter incubations to measure winter N mineralization (e.g., Magill et al. 2000, Melillo et al. 2004, Turner and Henry 2010), and either linear interpolation or empirical models of the temperature-respiration relationship to estimate winter soil CO<sub>2</sub> fluxes (e.g., Savage and Davidson 2001, Melillo et al. 2004). These methods may not adequately capture winter responses to warming and N fertilization, and consequently may underestimate the impact of these disturbances on soil biogeochemical cycling.

The purpose of this study was to examine the

interactive effects of soil warming and N additions on soil C and N fluxes, with particular emphasis on the role that season plays in mediating this response. We hypothesized that: (1) both warming and N additions would stimulate soil respiration and N mineralization, but that the effect of warming would be much greater than that of N additions, (2) where warming and N additions occurred simultaneously, N fertilization would negate the positive effect of warming on soil respiration and N mineralization, (3) the impact of warming and N additions on soil respiration and N mineralization would vary seasonally, and (4) traditional methods of calculating winter fluxes would undervalue both total annual and winter soil respiration and N mineralization, thereby underestimating the effect of warming and N additions on C and N processes.

## MATERIALS AND METHODS

### *Site description and experimental design*

This study was conducted at the Soil Warming × Nitrogen Addition Study at the Prospect Hill Tract of the Harvard Forest Long Term Ecological Research Site in Petersham, Massachusetts, USA (42°50' N, 72°18' W). The forest at the site is comprised of even-aged, mixed hardwoods, including red oak (*Quercus rubra*), black oak (*Quercus velutina*), red maple (*Acer rubrum*), striped maple (*Acer pensylvanicum*), American beech (*Fagus grandifolia*), white birch (*Betula papyrifera*), and American chestnut (*Castanea dentata*). Soils are of the Gloucester series (fine loamy, mixed, mesic, Typic Dystrochrepts; Peterjohn et al. 1994). Mean annual air temperature at the Harvard Forest is 7°C, with summer temperatures as high as 32°C and winter temperatures as low as −25°C. Average total annual precipitation, including water equivalent of snow, is 1100 mm (Boose et al. 2002). Mean annual snowfall is 1700 mm and occurs primarily from December through February (NOAA 2009).

Using a completely randomized design, twenty-four 3×3 m plots were assigned one of four experimental treatments with six replicates per treatment: control (C), nitrogen addition (N), warming (W), and warming × N (WN). Average soil temperature in the heated plots (warming and warming × N) was continuously elevated

5°C above ambient using buried heating cables placed at 10 cm depth below the soil surface and spaced 20 cm apart. The 5°C temperature differential is the same as other soil warming studies at the Harvard Forest (Peterjohn et al. 1993) and falls within the range of worst-case-scenario model projections for increased global air temperature by the year 2100 (IPCC 2007). Cables were installed in the heated plots in October 2005, with the cables extending 10 cm outside each plot perimeter to minimize heat loss at the plot edge. Cables were not buried in unheated plots. Disturbance controls in an adjacent soil warming experiment showed no differences in fluxes of C and N as compared to undisturbed plots (Peterjohn et al. 1994), suggesting that the initial disturbance of burying the cables did not have lasting effects on soil processes. After recovering for ten months from cable installation, the system was activated in August 2006. The soil warming treatment resulted in an average 5°C temperature difference between heated and unheated plots (Fig. 1). Mean soil temperature for both sampling years was 9.9°C in unheated plots and 14.7°C in heated treatments. Minimum temperatures were 1.6°C to 4.7°C, and maximum temperatures were 21°C to 26°C for unheated and heated plots respectively.

Nitrogen additions were also initiated in August 2006. Nitrogen was applied in equal doses during the May to October growing season as an aqueous solution of  $\text{NH}_4\text{NO}_3$  at a rate equivalent to the low N plots at the Harvard Forest Chronic Nitrogen Addition Study ( $5 \text{ g N m}^{-2} \text{ y}^{-1}$ ). This fertilization rate was about eight times that of ambient N deposition, which has been calculated at  $0.66 \text{ g m}^{-2} \text{ y}^{-1}$  from eddy covariance estimates of wet and dry deposition at the Harvard Forest (Munger et al. 1998). As with the heating cables, the fertilizer is applied 10 cm outside of the plot boundary to reduce plot edge effects.

#### *Soil sampling and analysis*

Soils were sampled monthly between May 2007 and April 2009. On each sampling date, two 8 cm wide and 10 cm long cores were removed from each plot, separated into mineral and organic fractions, and bulked by soil horizon. Two more soil cores previously taken from an

adjacent area were used to back-fill the holes created by the sampling and marked with the date to prevent re-sampling in that location. During the growing season, sampling occurred at least two weeks following fertilizer application. In winter, a narrow hole was dug into the snowpack to access the soil. This hole was refilled with snow following soil sampling. The cores were transported back to the University of New Hampshire, passed through a 2 mm sieve to remove rocks and roots, and stored at 4°C for less than 48 hours prior to analysis. Gravimetric moisture was determined by drying the organic fraction at 60°C and the mineral soil at 105°C for 24 hours.

Net N mineralization was determined using the buried bag technique outlined by Westermann and Crothers (1980) and Eno (1960), where the values of extractable  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  were compared in initial and incubated soil cores. Incubations occurred in situ for approximately one month. While taking two sample cores for immediate analysis, a third core was removed, placed into an air-permeable plastic bag nested inside a 1-mm fiberglass mesh sac, and re-situated into the soil. During the following soil sampling four to five weeks later, the incubated core was removed, and the hole was back-filled and marked with a soil core from off plot. Within 48 hours of sampling, the mineral and organic fractions of both initial and incubated cores were extracted using 2M KCl, filtered, and stored at  $-20^\circ\text{C}$ . A BioTek Synergy HT microplate reader (Winooski, Vermont, USA) was used to analyze  $\text{NH}_4$  and  $\text{NO}_3$  in the soil extracts. Ammonium was quantified using the indophenol-blue method adapted for microtiter plates (Sims et al. 1995). Nitrate was assessed by the vanadium (III) reduction reaction (Braman and Hendrix 1989) modified for microplate assays (J. L. DeForest, *personal communication*). The detection limits for both  $\text{NH}_4$  and  $\text{NO}_3$  was 0.1 ppm.

#### *Soil respiration*

Net  $\text{CO}_2$  flux was measured bi-monthly from May 2007 through April 2009 using a static chamber technique (Raich et al. 1990, Peterjohn et al. 1993). In April 2006, permanent collars were situated into the soil, leaving about 10 cm above the soil surface. There was one collar per plot, or

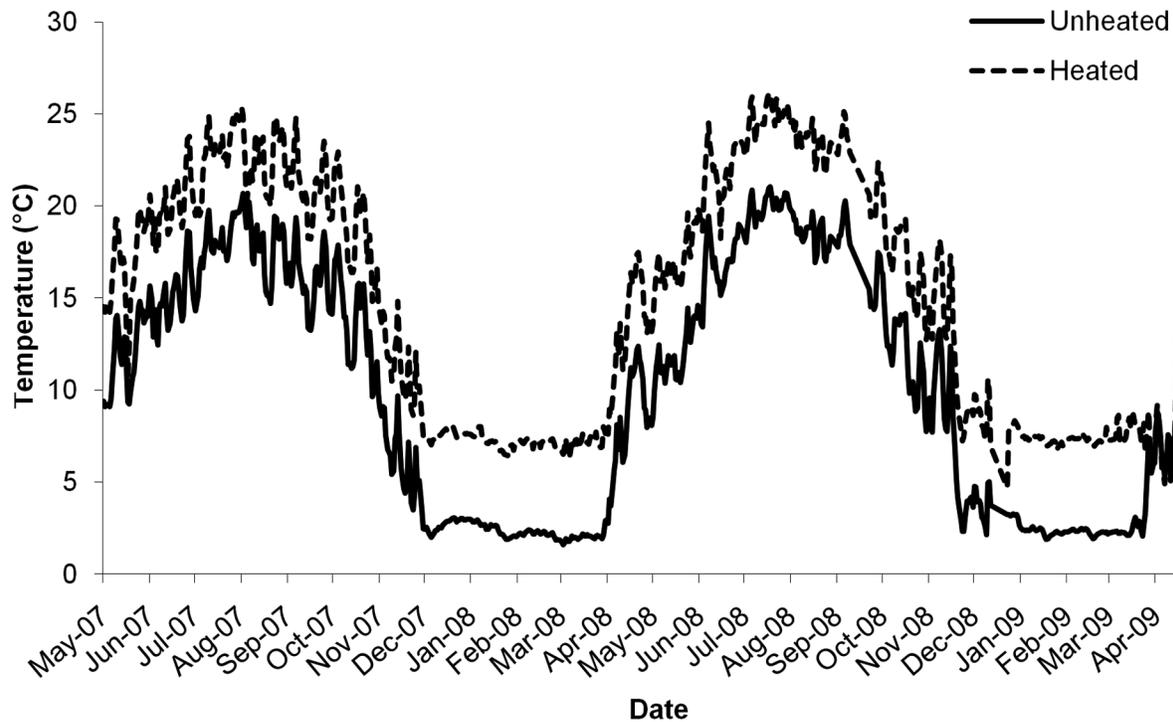


Fig. 1. Daily soil temperatures (°C) for unheated (control, N addition) and heated (warming, warming  $\times$  N) plots. The solid line depicts unheated plots, and the dashed line shows heated plots.

24 collars total. While spatial heterogeneity of soil respiration can be high (e.g., Rayment and Jarvis 2000), we believe that installing a single collar in each  $3 \times 3$  m plot adequately captured the spatial variability of the forest floor. Davidson et al. (2002) estimated that as few as 3 collars could be sampled per day in a several hundred square meter field site and be within  $\pm 40\%$  of the full site population mean. Here we have measured six collars per treatment per day in a field site whose plots total  $216 \text{ m}^2$ .

All sampling occurred when the average of the diel flux takes place: between 10:00 and 13:00 local time (Davidson et al. 1998). Before each sampling, the depth of the collar was measured to account for changes in chamber volume with litter inputs and decay. In winter, snow was removed as carefully as possible from collars, and was replaced upon completion of sampling to minimize disturbance. Once excavated, collars equilibrated with the atmosphere for one hour prior to sampling to release  $\text{CO}_2$  that had accumulated in the surrounding snow. Immediately prior to measurement, lids were placed

over the pre-installed collars to create respiration chambers. Ten ml headspace samples were taken from these chambers using air-tight, plastic syringes at zero, five, ten, and 15 minutes. Air temperature and pressure were measured concurrent with sampling. Following sampling, gas samples were immediately transported back to the University of New Hampshire and analyzed using a LI-COR LI-6252 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). The air temperature and pressure of the room in which the IRGA was located were also recorded. Fluxes were calculated from the linear increase in gas concentration over the 15 minute incubation, the volume of the chamber, and the surface area of the soil within the chamber. In addition, the rate calculations were corrected for differences between in situ air temperature and pressure and the atmospheric conditions of the lab where the gas analyzer was located. In the summer of 2008, we compared our static chamber measurements with those made on a dynamic, portable IRGA system and found fluxes to be well-correlated ( $r^2 = 0.86$ ) between the two techniques (A. Contosta,

*unpublished data*). As a result, we believe that our static chamber method provided reliable, in situ flux measurements.

There were two reasons why we opted for snow removal over other winter respiration sampling strategies such as placing a lid on top of the snow (e.g., Savage and Davidson 2001, Groffman et al. 2006) or inserting CO<sub>2</sub> sensors into the snowpack (e.g., Monson et al. 2006b, Schindlbacher et al. 2007). First, placing a lid on top on the snowpack to create a respiration chamber can substantially underestimate soil CO<sub>2</sub> flux due to lateral diffusion (Schindlbacher et al. 2007). Second, both the snow-top chamber and the CO<sub>2</sub> sensor approach require the presence of snow at all sampling sites. This was not the case in the present study, where heated plots were often snow free even when unheated plots were covered with as much as 20 cm of snow. Because the snowpack can trap gases emitted from soil (Monson et al. 2006b), our measurements of winter flux did not necessarily represent CO<sub>2</sub> diffusing through the snow into the atmosphere at the time of sampling. However, since the CO<sub>2</sub> that accumulates in the snowpack is eventually released to the atmosphere, we believe that our winter soil respiration measurements are valid for comparing respiration rates across seasons.

### Statistical analysis

All statistical analyses were conducted in R 2.9.2 (R Development Core Team 2009). We used a mixed model approach with the *nlme* package (Pinheiro et al. 2009) to examine differences among experimental treatments and across seasons for soil CO<sub>2</sub> flux, net N mineralization, and soil moisture. Each dependent variable (soil CO<sub>2</sub> flux, net N mineralization, and soil moisture) was log-transformed to meet the normality and homoscedasticity assumptions of the mixed-effects model. Fixed effects included the warming and N addition treatments and their interaction with one another and with season, which was defined as both a categorical and continuous variable as described below. Soil horizon was also included as a fixed effect for net N mineralization and soil moisture, which were measured in both the mineral and organic fractions.

Seasons were defined as winter, spring, sum-

mer or autumn based on the phenology of the site using twenty-year records of bud-break, leaf-out, senescence, and leaf-fall for the Harvard Forest (O'Keefe 2000). Spring was delineated as the time between the initial swelling of buds and when more than 80% of leaves had reached their full size. For the three dominant canopy species on the site—red maple, red oak, and black oak—this approximated the period between April 1 and May 30. With the same dataset, the start of autumn was determined as the time when greater than 20% of leaves had changed color, which was around September 1. Although most trees had lost all of their leaves by October 31, autumn actually extended to late November, after which soil temperatures precipitously declined (Fig. 1). Thus spring occurred from April 1 to May 31, summer from June 1 to August 30, autumn from September 1 to November 30, and winter from December 1 to March 31. Winter also coincided with daily air temperatures at or below 2.2°C (Boose et al. 2002), which typically defines the “dormant” period outside the growing season (Schwartz et al. 2006).

In addition to defining season as a categorical variable, we also explicitly modeled the seasonal cycle as the periodic function:

$$\sin(2 \times \pi \times \text{adjusted Julian day}) + \cos(2 \times \pi \times \text{adjusted Julian day}). \quad (1)$$

Adjusted Julian days were the sampling dates scaled between 0 and 3 to represent the three calendar years over which soil respiration and N mineralization measurements occurred. These were obtained by assigning Julian days to the sampling date for 2007, Julian days + 365 for 2008, Julian days + 730 for 2009, and dividing all values by 365. The inclusion of this periodic function both explicitly modeled the temporal autocorrelation structure of the data (Crawley 2007) and also allowed for the examination of whether the experimental treatments shifted the seasonal cycle.

Models were chosen with backward selection, starting with a beyond optimal model containing all hypothesized fixed effects and their interactions as per Zuur et al. (2009). After creating the beyond optimum model of fixed effects, random effects were chosen by comparing models constructed with no random component, a random intercept (plot), and a random intercept (plot)

plus a random slope (warming and/or N addition). Models containing no random effects were fit as generalized linear models (*gls* in R), and models with random effects were fit as linear mixed-effects models (*lme* in R) (Pinheiro and Bates 2000). For the variables soil moisture and net N mineralization, soil horizon was nested within plot as part of the random intercept. The optimal random effects structure was determined with the likelihood ratio test of restricted maximum likelihood (REML) estimates for each model (Zuur et al. 2009). The resulting *P*-values were corrected to account for the fact that this test occurred on the boundary and therefore violated the assumption that the likelihood values compared followed a  $\chi^2$  distribution. After choosing the random effects, the autocorrelation structure was selected using Akaike's Information Criteria (AIC). Fixed model components were subsequently selected in a backwards selection process by examining *P*-values for all fixed effects and interactions estimated with a maximum likelihood (ML) fit as recommended by Zuur et al. (2009). All statistical tests were performed at  $\alpha = 0.05$  level. After completing model selection, the final model was refit with the REML method for reporting significant differences among group means. Model verification consisted of visually inspecting residuals for normality and homoscedasticity.

Seasonal and annual soil CO<sub>2</sub> respiration was estimated by linearly interpolating fluxes between two sampling dates, and then adding these values to obtain the total flux for the year, or for winter, spring, summer, and autumn. That is:

$$R = \Sigma F_{m,k} \Delta t_k \quad (2)$$

where *R* represents either the annual, winter, spring, summer, or fall estimate of soil respiration,  $\Delta t_k = (t_k - t_{k-1})$  is the difference in days between adjacent field sampling dates, and  $F_{m,k}$  is the average CO<sub>2</sub> flux, in units of mg C m<sup>-2</sup> d<sup>-1</sup>, for the ( $t_k, t_{k-1}$ ) sampling interval (Wang et al. 2010). In order to obtain daily CO<sub>2</sub> flux rates for linear interpolation, we multiplied our hourly flux measurements by 24 hours. Because winter measurements of soil respiration are not usually taken into account when calculating annual and winter fluxes, we also used Eq. 2 to estimate annual and winter CO<sub>2</sub> respiration using data

collected during the growing season (i.e., April through November). Finally, we used linear regressions to compare field-season only estimates of winter and annual flux with those obtained from the entire data set.

Although our daily, seasonal, and annual estimates were based on hourly measurements made once per week, we believe that our approach adequately captured temporal variability of soil respiration on diel and weekly timescales. Savage and Davidson (2003) reported that daily soil respiration rates extrapolated from single, hourly measurements made between 10:00 and 13:00 were in strong agreement with daily respiration rates obtained from hourly autochamber measurements added together for the same timeframe. In addition, Savage and Davidson (2003) showed that over a 58-day span, linear interpolation of hourly fluxes, made once per week and with a manual system, could produce identical estimates of total soil respiration when compared to the sum of hourly fluxes made continuously with an autochamber.

Seasonal totals of N mineralization were determined by adding the net N mineralized over the incubation period for winter, spring, summer and fall for each sampling year. An ice storm in December of 2008 prevented incubating cores from December 2008 to January 2009. The amount of N mineralized during this period was estimated by interpolating N fluxes between December and January using the method outlined above for soil respiration.

Relative treatment effects were described as percentages, and were calculated as follows:

$$\% \text{effect} = ((\text{treatment} - \text{control}) / \text{control}) \times 100.$$

## RESULTS

Soil respiration, N mineralization, and soil moisture data are presented within the context of our hypotheses, and are thus organized into categories of season, annual fluxes, N additions, warming, and warming  $\times$  N. Final model output statistics for showing the effect of season, warming and/or N additions on these variables are located in Tables 1–3. Model selection statistics are presented in the Appendix.

Table 1. Final model output for gravimetric soil moisture (g H<sub>2</sub>O/g soil) for the organic and mineral soil horizons.

Fixed Effect	Estimate	SE	DF	<i>t</i> -value	<i>P</i> -value
Organic Horizon					
Winter	0.340	0.057	494	5.947	<0.0001
Spring	-0.097	0.056	494	-1.746	0.082
Summer	-0.126	0.051	494	-2.480	0.014
Fall	-0.606	0.054	494	-11.282	<0.0001
Warming	-0.091	0.069	22	-1.308	0.204
Mineral Horizon					
Winter	-0.569	0.062	490	-9.228	<0.0001
Spring	-0.050	0.040	490	-1.247	0.213
Summer	-0.176	0.036	490	-4.920	<0.0001
Fall	-0.286	0.038	490	-7.571	<0.0001
Warming	-0.198	0.082	22	-2.418	0.024

Notes: The structure of the final models for both the organic horizon and the mineral fraction was:  $\ln(\text{Moisture}) \sim \text{Warming} + \text{Season}$ . Model output includes parameter estimates, standard errors of the estimates (SE), degrees of freedom (DF), *t*-values and *P*-values for fixed effects.

Table 2. Final model output for net N mineralization ( $\mu\text{g N g soil}^{-1} \text{ d}^{-1}$ ).

Fixed Effect	Estimate	SE	DF	<i>t</i> -value	<i>P</i> -value
Winter	-0.964	0.125	933	-7.725	<0.0001
Spring	-1.363	0.162	933	-8.412	<0.0001
Summer	-1.087	0.203	933	-5.353	<0.0001
Fall	-0.427	0.164	933	-2.598	0.010
sin (2 $\pi$ AJD)	-0.251	0.093	933	-2.709	0.007
cos (2 $\pi$ AJD)	-1.196	0.110	933	-10.897	<0.0001
Warming	0.336	0.070	22	4.802	<0.0001
N Additions	0.148	0.070	22	2.114	0.035
O Horizon	0.978	0.070	22	13.983	<0.0001

Notes: The structure of the final model was:  $\ln(\text{N Mineralization}) \sim \text{Warming} + \text{N Additions} + \text{Season} + \text{Horizon} + (\sin(2\pi\text{AJD}) + \cos(2\pi\text{AJD}))$ . The abbreviation 2 $\pi$ AJD denotes  $2 \times \pi \times$  adjusted Julian day, the explicit model of seasonal cycle. Model output is as in Table 1.

Table 3. Final model output for soil respiration ( $\text{mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ).

Fixed Effects	Estimate	SE	DF	<i>t</i> -value	<i>P</i> -value
Winter	3.177	0.092	900	34.396	<0.0001
Spring	0.155	0.112	900	1.377	0.169
Summer	1.040	0.145	900	7.151	<0.0001
Fall	0.777	0.123	900	6.325	<0.0001
sin (2 $\pi$ AJD)	-0.177	0.058	900	-3.041	0.002
cos (2 $\pi$ AJD)	-0.378	0.072	900	-5.258	<0.0001
Warming $\times$ sin (2 $\pi$ AJD)	-0.108	0.082	900	-1.315	0.189
Warming $\times$ cos (2 $\pi$ AJD)	-0.278	0.102	900	-2.723	0.007
Warming $\times$ Winter	0.486	0.142	900	3.415	0.001
Warming $\times$ Spring	-0.094	0.160	900	-0.592	0.554
Warming $\times$ Summer	-0.417	0.206	900	-2.022	0.04
Warming $\times$ Fall	-0.148	0.174	900	-0.851	0.395
N Additions $\times$ Winter	0.012	0.097	900	0.120	0.905
N Additions $\times$ Spring	0.139	0.120	900	1.152	0.250
N Additions $\times$ Summer	0.199	0.105	900	1.891	0.059
N Additions $\times$ Fall	0.254	0.110	900	2.316	0.021
Warming $\times$ N $\times$ Winter	0.028	0.159	22	0.178	0.860
Warming $\times$ N $\times$ Spring	-0.040	0.172	900	-0.234	0.815
Warming $\times$ N $\times$ Summer	-0.219	0.149	900	-1.475	0.141
Warming $\times$ N $\times$ Fall	-0.296	0.155	900	-1.908	0.057

Notes: The structure of the final model was:  $\ln(\text{Soil Respiration}) \sim \text{Warming} \times \text{N Additions} \times \text{Season} + \text{Warming} \times (\sin(2\pi\text{AJD}) + \cos(2\pi\text{AJD}))$ . Model output is as in Table 1. The abbreviation of 2 $\pi$ AJD is as in Table 2.

### *Seasonal patterns of soil moisture, nitrogen mineralization, and soil respiration*

Soils showed pronounced seasonal differences in moisture, N mineralization, and soil respiration. The forest floor was wettest in winter and early spring and significantly drier in summer ( $P = 0.01$ ) and autumn ( $P < 0.0001$ ) (Table 1, Fig. 2). A drought in the summer of 2007 caused soil moisture values in the O horizon to drop in August in all the experimental manipulations. The same seasonal pattern occurred in the mineral fraction, with significantly drier soils in summer ( $P < 0.0001$ ) and autumn ( $P < 0.0001$ ) as compared to winter. In addition, soil moisture varied between mineral and organic soil fractions ( $P < 0.0001$ ), and the effect of warming on soil moisture was horizon dependent (warming  $\times$  horizon:  $P < 0.05$ ). As a result, differences in gravimetric soil moisture among treatments and sampling dates were measured separately for mineral and organic fractions (see the Appendix).

Unlike moisture, N mineralization and soil respiration were lowest in winter and spring and highest in summer and fall. Starting from a winter minimum, N mineralization increased through April and May (spring:  $P < 0.0001$ ) to reach an annual maximum from mid July to mid August (summer:  $P < 0.0001$ ) (Table 2, Fig. 3). Although N fluxes subsequently declined during September and October, they remained higher in autumn than they were in winter (autumn:  $P = 0.01$ ). In addition to these seasonal differences in N mineralization, the sin ( $P = 0.007$ ) and cos ( $P < 0.0001$ ) models of the seasonal cycle indicated a strong, periodic trend in N cycling. January 2007 appeared to deviate from this trend when a mid-winter thaw increased net N mineralization to rates typical of late spring and early autumn. Following this mid-winter boost, N mineralization returned to amounts near zero for the subsequent two months. For the 2007–2008 sampling year, the lowest fluxes were between 2 and 10% of the largest mineralization rates, while in 2008–2009, the lowest mineralization rates were less than 1% of the highest annual fluxes. Since  $\text{NO}_3^-$  concentrations were rarely above the detection limit of 0.1 ppm in both initial and incubated cores, net N mineralization represented ammonification and not nitrification. While the organic fraction had significantly higher rates of mineralization than the mineral

soil (horizon:  $P < 0.0001$ ), there were no treatment by horizon interactions. Consequently, N flux rates in mineral and organic fractions were analyzed together, with horizon as both a fixed effect and nested within plot in the temporal autocorrelation structure of the model (Appendix).

Soil respiration also displayed a distinct seasonal cycle ( $P < 0.0001$  for the sin and cos models) (Table 3, Fig. 4). Fluxes in summer and autumn were significantly higher than fluxes in winter and spring (summer:  $P < 0.001$ , autumn:  $P < 0.0001$ ). The lowest fluxes of the year were about 10% of the largest respiration rates.

### *Estimates of seasonal and annual fluxes*

Winter N mineralization comprised 2 to 17% of the total annual mineralization, with much higher winter fluxes in 2007–2008 than in 2008–2009. Spring made up 5–18%, summer 29–66%, and fall 20–50% of net annual N mineralization (Table 4). Winter  $\text{CO}_2$  fluxes comprised 10–14% of the total annual soil respiration, spring 12–16%, summer 46–50%, and fall 20–32% (Table 5). Total annual and seasonal fluxes of C and N varied between the two sampling years. Annual net N mineralization was higher in the control plots in 2007–2008 as compared to 2008–2009. By contrast, annual soil respiration was larger in 2008–2009 than it was in 2007–2008.

Soil respiration measurements taken during the field season consistently underestimated winter fluxes, particularly in the unheated plots (Fig. 5). Linear interpolation of fluxes between the end of November and the beginning of April were an average 35% lower in the control plots ( $r^2 = 0.56$ ,  $P = 0.005$ ) and 25% lower in the N addition treatment ( $r^2 = 0.07$ ,  $P = 0.41$ ) compared to interpolation of fluxes between bi-monthly winter sampling dates. Winter flux estimates were only 13% lower in the warming plots ( $r^2 = 0.73$ ,  $P = 0.001$ ), and 5% lower in the warming  $\times$  N treatment ( $r^2 = 0.72$ ,  $P = 0.001$ ) when using field-season data as compared to the year-round data set. Because interpolation of field-season data across the winter months disproportionately underestimated winter respiration in unheated plots, the apparent treatment effect of warming during winter would be greater when using this approach. However, linear interpolation of field season data over the entire year generated almost

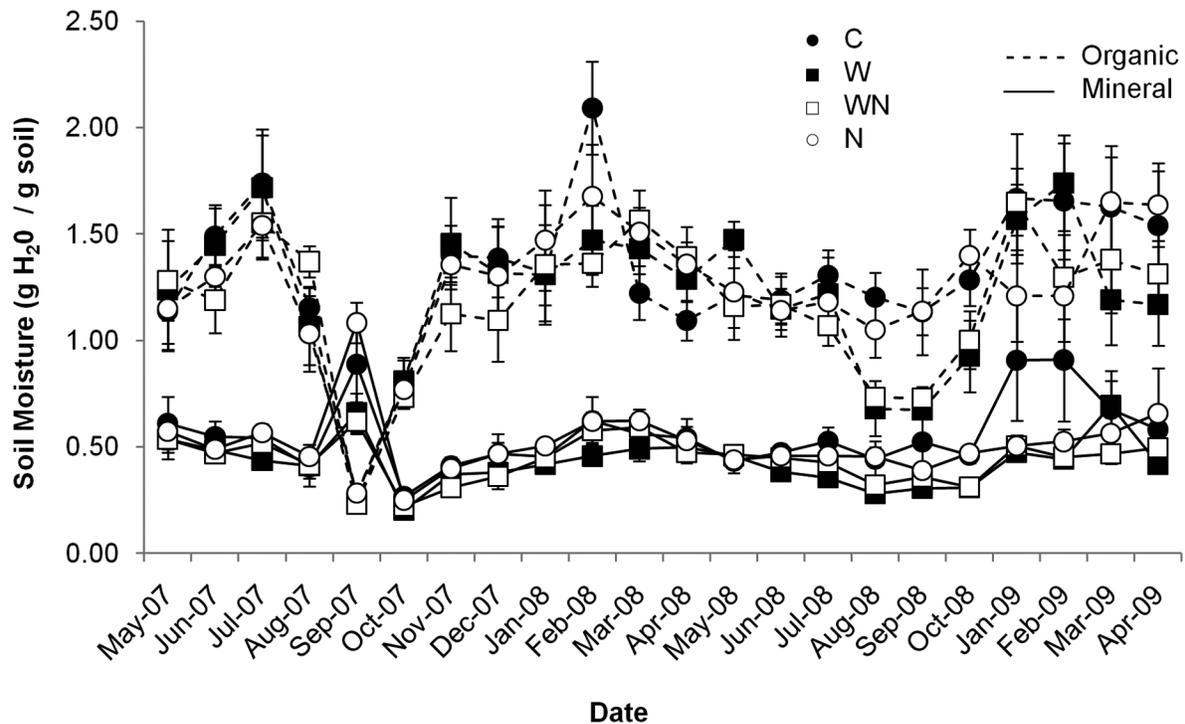


Fig. 2. Soil moisture ( $\text{g H}_2\text{O} / \text{g soil}$ ) for mineral and organic fractions in control (C), warming (W), warming  $\times$  N (WN), and N addition (N) plots. The solid line shows moisture in the mineral soil horizon, while the dashed line displays soil moisture in the organic horizon. Solid circles indicate the control treatment, solid squares warming, open squares warming  $\times$  N, and open circles N addition. Each data point represents the mean of six replicates, and error bars indicate  $\pm 1$  SE.

identical estimates of annual flux as data taken year-round ( $r^2 = 0.99$ ,  $P < 0.0001$  for all four treatments, Fig. 5). The small contribution of winter respiration to the total annual flux meant that even large shortfalls in winter estimates did not translate to large deficits in annual estimates.

#### Nitrogen additions

Nitrogen amendments increased both N mineralization and soil respiration relative to the control treatment. Over the entire study period, N additions increased N mineralization by an average of 8% irrespective of season ( $P = 0.03$ ) (Table 2, Fig. 3). By contrast, the increase in  $\text{CO}_2$  flux in N amended plots occurred only in summer and autumn (N addition:  $P > 0.05$ , N addition  $\times$  summer:  $P = 0.06$ , N addition  $\times$  autumn:  $P = 0.02$ ) (Table 3, Fig. 4). Nitrogen additions neither altered the seasonal cycle of N mineralization nor that of soil respiration ( $P > 0.05$  for interactions between N additions and sin

and cos models of both variables, Tables 2 and 3). There was no effect of N additions on soil moisture ( $P > 0.05$ , Table 1).

#### Soil warming

Soil warming decreased soil moisture but increased both N mineralization and soil respiration. Over the entire study period, moisture was an average 9% lower in the O horizon ( $P > 0.05$ ) and 19% lower in the mineral soil ( $P = 0.02$ ) of heated plots (Table 1, Fig. 2). Despite this lower moisture, net N mineralization rates were an average 83% higher in warmed plots ( $P < 0.0001$ ) both during the height of the growing season and during winter ( $P > 0.05$  for warming  $\times$  season) (Table 2, Fig. 3). Warming also increased soil  $\text{CO}_2$  flux an average of 44% over the control plots throughout the study (Fig. 4). However, the effect of warming varied seasonally, and was least pronounced during winter (warming  $\times$  winter:  $P = 0.001$ ) and summer

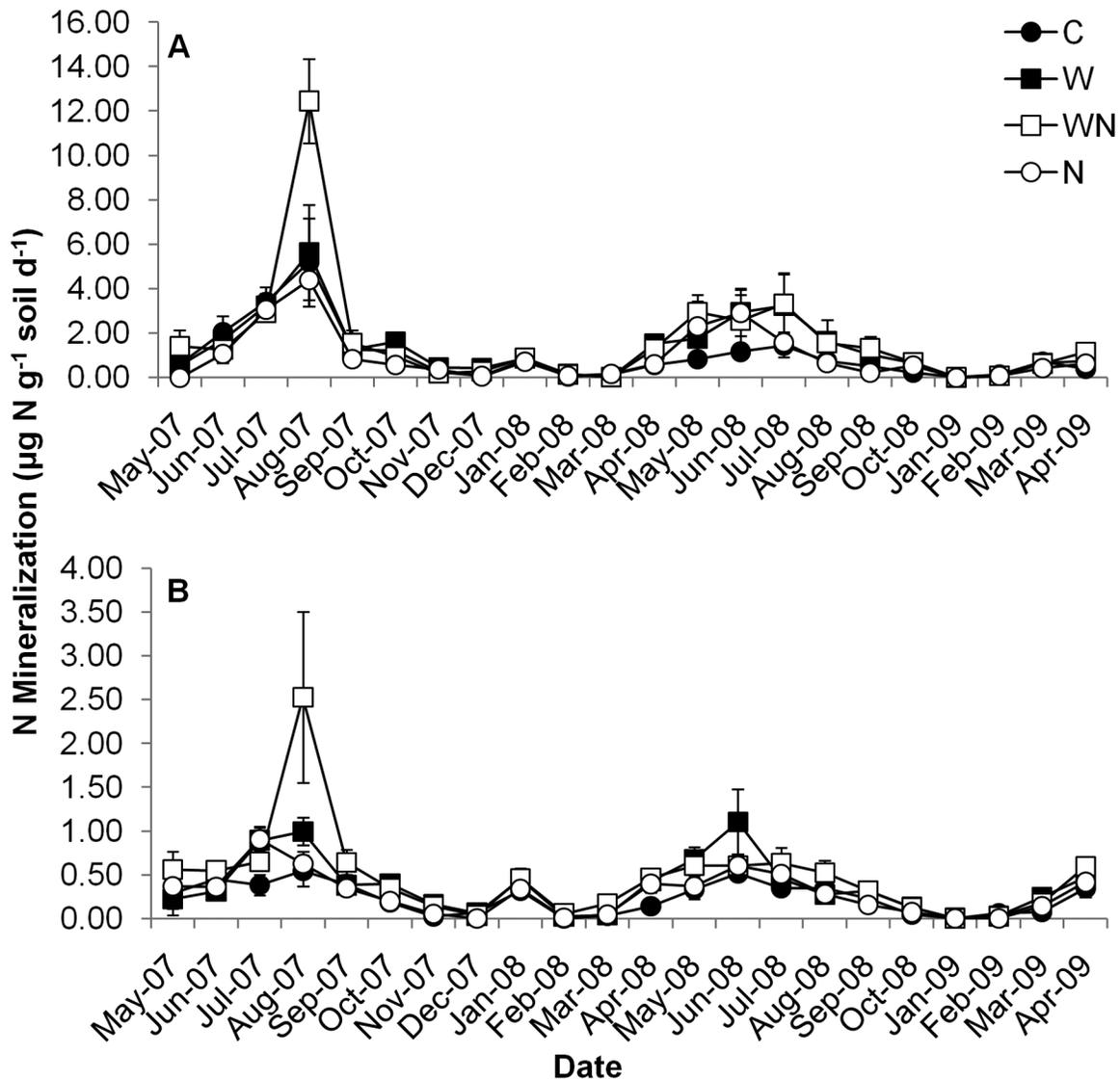


Fig. 3. Net N mineralization ( $\mu\text{g N g soil}^{-1} \text{d}^{-1}$ ) for (A) organic and (B) mineral soil horizons in control (C), warming (W), warming  $\times$  N (WN), and N addition (N) plots. Symbols and data points are as in Fig. 2.

(warming  $\times$  summer:  $P = 0.04$ , Table 3).

In addition, warming altered the seasonal cycle of soil respiration ( $P = 0.007$  for warming  $\times$  cos model), decreasing its periodicity (Table 3, Fig. 4). Average respiration in the warming only plots on April 22, 2008 was  $38 \text{ mg C m}^{-2} \text{ h}^{-1}$ —a value that the control treatment did not reach until two weeks later, when average respiration was  $33 \text{ mg C m}^{-2} \text{ h}^{-1}$ . Likewise, average respiration in the warming treatment on September 23, 2008 was  $111 \text{ mg C m}^{-2} \text{ h}^{-1}$ —a rate that the control plots

had not exhibited since August 25, when fluxes were  $112 \text{ mg C m}^{-2} \text{ h}^{-1}$ . Warming did not alter the seasonal cycle of N mineralization ( $P > 0.05$ , Table 2).

#### Soil warming $\times$ nitrogen additions

There were no interactive effects of combined soil warming  $\times$  N additions on N mineralization or soil respiration ( $P > 0.05$ , Tables 2 and 3). Simultaneous warming  $\times$  N had a marginally significant effect on soil respiration, but only in

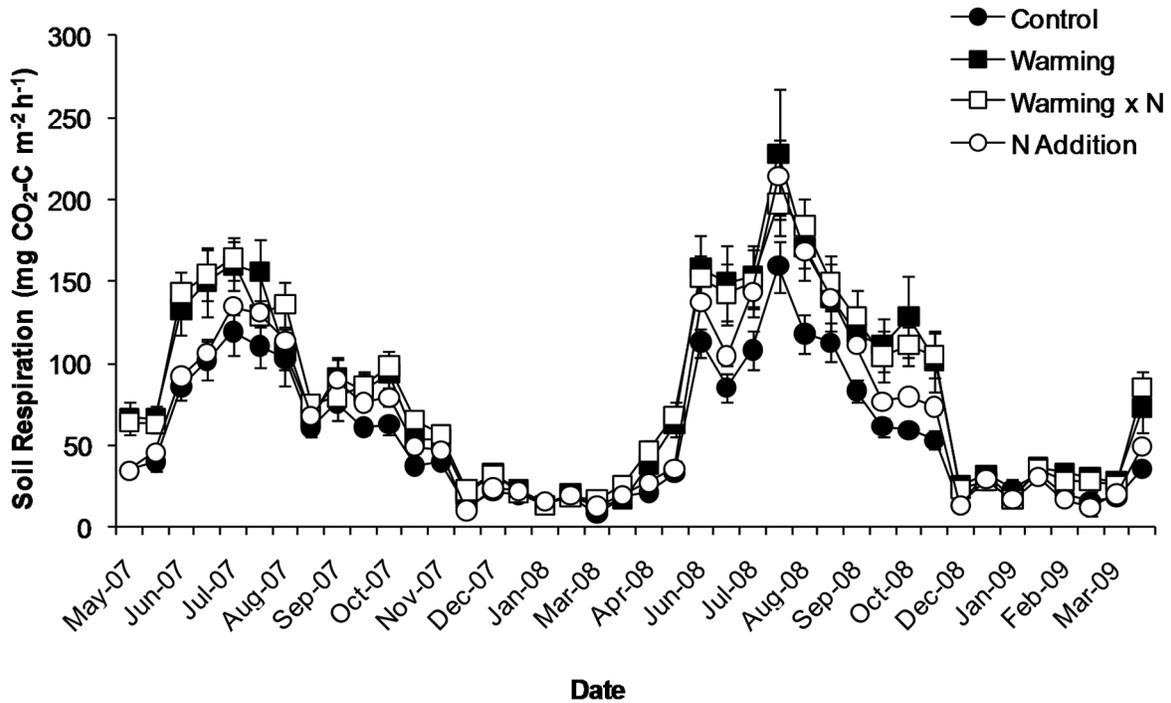


Fig. 4. Soil respiration ( $\text{mg CO}_2\text{-C m}^{-2}\text{ h}^{-1}$ ) in control (C), warming (W), warming  $\times$  N (WN), and N addition (N) plots. Symbols and data points are as in Fig. 2.

Table 4. Net N mineralization ( $\mu\text{g N g soil}^{-1}\text{ y}^{-1}$  or  $\text{season}^{-1}$ ) for control, warming, warming  $\times$  N, and N addition plots.

Year	Treatment	Annual	Winter	Spring	Summer	Autumn
2007–2008	Control	482 $\pm$ 55	55 $\pm$ 14	26 $\pm$ 9	197 $\pm$ 50	204 $\pm$ 49
	Warming	565 $\pm$ 42	96 $\pm$ 15	59 $\pm$ 16	230 $\pm$ 34	179 $\pm$ 57
	Warming $\times$ N	633 $\pm$ 91	70 $\pm$ 20	68 $\pm$ 10	185 $\pm$ 39	309 $\pm$ 72
	N Addition	430 $\pm$ 51	59 $\pm$ 10	41 $\pm$ 11	167 $\pm$ 26	162 $\pm$ 43
2008–2009	Control	242 $\pm$ 50	7 $\pm$ 3	44 $\pm$ 12	136 $\pm$ 27	57 $\pm$ 15
	Warming	466 $\pm$ 92	25 $\pm$ 9	54 $\pm$ 12	242 $\pm$ 63	146 $\pm$ 35
	Warming $\times$ N	531 $\pm$ 130	21 $\pm$ 4	65 $\pm$ 11	313 $\pm$ 110	133 $\pm$ 26
	N Addition	354 $\pm$ 55	18 $\pm$ 5	39 $\pm$ 7	234 $\pm$ 46	69 $\pm$ 13

Note: Data represent mean of six replicates  $\pm$  1 SE.

Table 5. Soil respiration ( $\text{g C m}^{-2}\text{ y}^{-1}$  or  $\text{season}^{-1}$ ) for control, warming, warming  $\times$  N, and N addition plots.

Year	Treatment	Annual	Winter	Spring	Summer	Autumn
2007–2008	Control	409 $\pm$ 38	55 $\pm$ 2	58 $\pm$ 4	188 $\pm$ 9	127 $\pm$ 6
	Warming	549 $\pm$ 51	75 $\pm$ 5	87 $\pm$ 7	277 $\pm$ 23	179 $\pm$ 13
	Warming $\times$ N	565 $\pm$ 31	74 $\pm$ 5	82 $\pm$ 7	271 $\pm$ 21	181 $\pm$ 7
	N Addition	466 $\pm$ 17	62 $\pm$ 3	69 $\pm$ 4	227 $\pm$ 15	142 $\pm$ 8
2008–2009	Control	475 $\pm$ 28	52 $\pm$ 3	62 $\pm$ 3	218 $\pm$ 15	97 $\pm$ 5
	Warming	707 $\pm$ 86	72 $\pm$ 5	85 $\pm$ 7	322 $\pm$ 28	148 $\pm$ 11
	Warming $\times$ N	711 $\pm$ 71	70 $\pm$ 7	84 $\pm$ 6	333 $\pm$ 30	142 $\pm$ 16
	N Addition	588 $\pm$ 22	63 $\pm$ 3	76 $\pm$ 5	273 $\pm$ 23	125 $\pm$ 9

Note: Data represent mean of six replicates  $\pm$  1 SE.

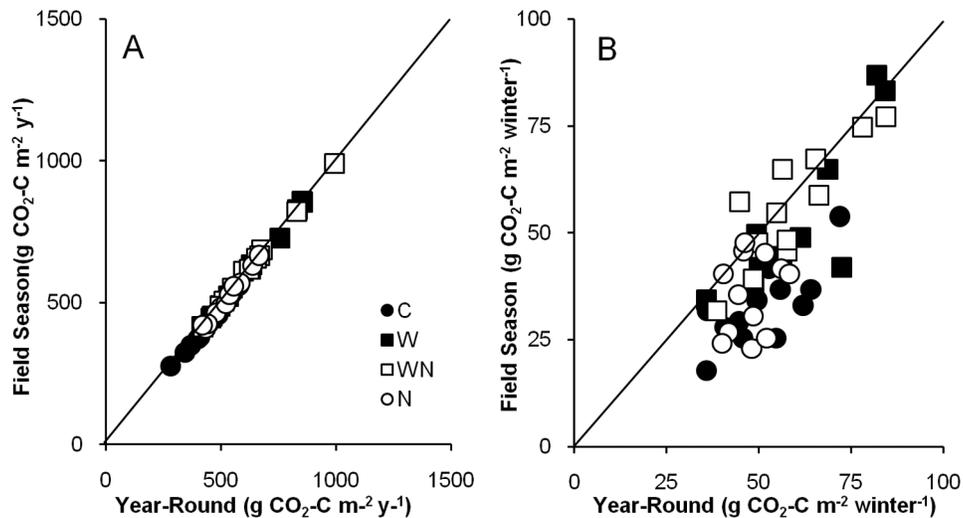


Fig. 5. Comparison of soil respiration ( $\text{mg CO}_2\text{-C m}^{-2} \text{h}^{-1}$ ) estimates using measurements taken year-round versus during the field season only for (A) annual fluxes and (B) winter fluxes in control (C), warming (W), warming  $\times$  N (WN), and N addition (N) plots. Symbols and data points are as in Fig. 2.

autumn (warming  $\times$  N  $\times$  autumn:  $P = 0.06$ , Table 3). Since average soil respiration in the warming  $\times$  N plots was as much as 13% lower than fluxes in the warming only treatment during this season, this interaction was likely the result of an annulling effect of N additions on soil warming.

## DISCUSSION

### *Seasonal patterns of soil moisture, soil respiration and net N mineralization*

Soils showed strong seasonal differences in moisture, soil CO<sub>2</sub> flux, and net N mineralization. Seasonal cycles of soil respiration and N mineralization largely followed changes in soil temperatures, with CO<sub>2</sub> and N fluxes highest in summer and lowest during the coldest parts of the year. This pattern has been reported in other seasonal studies of soil C and N dynamics at the Harvard Forest and at similar temperate forest ecosystems (Davidson et al. 1998, Jaeger et al. 1999, Bohlen et al. 2001, Groffman et al. 2001, Savage and Davidson 2001, Knoepp and Swank 2002, Bowden et al. 2004, Mo et al. 2005, DeForest et al. 2006, Groffman et al. 2009). Soil moisture did not show this pattern, but exhibited the seasonal characteristics typical of forests in the region, with less moisture in summer and early autumn, and more moisture in winter, spring,

and late fall (Davidson et al. 1998, Savage and Davidson 2001, DeForest et al. 2006, Turner and Henry 2010).

Like other systems with seasonal snow cover, our data suggest that soils are active in winter, and that winter transformations of soil C and N can contribute substantially to the total annual flux. While winter net N mineralization was low compared to summer rates, it was similar to that of spring, and comprised 2 to 17% of the total annual mineralization. The contribution of winter N mineralization to the annual amount was similar to other studies in temperate regions (Knoepp and Swank 2002, Groffman et al. 2009, Turner and Henry 2010). Winter N mineralization also showed high interannual variation, with winter contributing 11 to 17% of annual net N mineralization in 2007–2008 and 2 to 5% of annual mineralization in 2008–2009. The very low N fluxes in the winter of 2008–2009 may have resulted from a later onset of snowfall, which may have suppressed N mineralization due to soil freezing. Groffman et al. (2009) reported a fourfold difference in winter mineralization between two sampling years and attributed the much lower N flux in the second year to earlier, deeper soil frost and thinner snow pack. Alternatively, a mid-winter thaw may have caused the much higher N mineralization in

2007–2008. In January 2008, air temperatures the week prior to sampling were 8.5°C compared to the average –7°C for the rest of the month. The higher temperatures may have stimulated mineralization, freed N from microbial cells lysed during freeze-thaw, released N trapped in the snow, or all of the above. In any case, this mid-winter increase in N mineralization may be overlooked in studies that allow buried bags to incubate from late fall through April or May (e.g., Kielland et al. 2006, Miller et al. 2009, Turner and Henry 2010). Such a sampling scheme may provide an estimate of total winter N mineralization, but it may not capture the response of soils to fluctuating winter temperatures that are expected to be more common as climate changes.

As with N mineralization, soil respiration during the coldest months of the year contributed substantially to the total annual flux. Winter respiration comprised between 10–14% of the total CO<sub>2</sub> respired throughout the year. Other studies in temperate forests show comparable winter respiration rates and winter contributions to annual respiration (Mo et al. 2005, De Forest et al. 2006). In addition, CO<sub>2</sub> fluxes quantified beneath the snowpack approximated eddy covariance measurements at the Harvard Forest for daily ecosystem respiration during the leafless part of the year: 42 to 84 mg C m<sup>-2</sup> h<sup>-1</sup> (Munger et al. 2004). Consistent with our hypothesis, our data also indicated that obtaining accurate estimates of winter soil respiration requires sampling throughout the winter months, not interpolating the winter flux between November and April. When we used field-season only data to determine winter respiration, we underestimated total winter flux by 5 to 35%. This was especially the case for unheated plots. However, annual flux calculations based on field-season data were almost identical to those generated with both year-round and field-season measurements. Consequently, a sampling campaign conducted during the April through November field season typical for the northeastern US should be adequate when the objective is to determine annual soil respiration. When the purpose of a study is to more closely examine winter soil respiration dynamics, researchers should consider measuring fluxes during the winter season. This may be especially important when evaluating the effects of increased temper-

atures on winter soil respiration; the apparent treatment effect of warming on soil respiration was 40 to 72% higher when using field-season only data to estimate total winter flux than when using measurements taken year-round.

#### *Seasonal response of soils to nitrogen additions*

As we predicted, N fertilization generally accelerated CO<sub>2</sub> respiration and N mineralization, though the stimulatory effect was less than that of soil warming. Over the entire study period, N additions increased net N mineralization an average 8% over the control treatment. The small overall increase in N mineralization was similar to the findings for the first few years of other N fertilization experiments in the northeastern US (e.g., Aber et al. 1993, Christ et al. 1995, McNulty et al. 1996, Magill et al. 1996). In addition, N fertilization boosted N mineralization rates in all seasons, with N fluxes as much as 70% higher than the control treatment during winter.

Winter increases in N mineralization in the absence of plant demand could result in large N losses from the system as leachate (Fisk and Schmidt 1996) or as N<sub>2</sub>O flux (Groffman et al. 2006). However, lysimeter samples taken at 60 cm depth during the growing season showed that dissolved inorganic N levels in the N addition plots were no different from the controls (A. Contosta, *unpublished data*), suggesting that these soils exhibit the same high N retention observed in the Harvard Forest Chronic Nitrogen Addition Experiment (Aber et al. 1998). Higher rates of winter N mineralization in the N addition treatment could also stimulate N<sub>2</sub>O flux, but since the small amount of nitrification that occurred in this study took place during the growing season, denitrification probably did not release the mineralized N to the atmosphere during winter. In fact, the higher winter N mineralization rates in fertilized plots only occurred during the 2007–2008 mid-winter thaw, and may have resulted from the release of N sequestered in microbial biomass. Schmidt et al. (2004) showed that both microbial biomass N and total microbial biomass can be higher in winter in fertilized plots as compared to unfertilized ones. This could allow for higher rates of N mineralization as microbial cells lyse during a melt event.

In addition to accelerating N mineralization, N additions increased soil CO<sub>2</sub> flux. Several studies have also reported short-term increases in soil respiration in response to N additions (Thirukumar and Parkinson 2000, Bowden et al. 2004, Waldrop et al. 2004), which may result from higher microbial utilization of labile carbon (Aber et al. 1998), increased microbial access to plant litter compounds (Craine et al. 2007), and/or increased root productivity (Bowden et al. 2004). In the long term, a significant decrease in CO<sub>2</sub> flux from chronically amended soils is typically observed (e.g., Nohrstedt et al. 1989, Butnor et al. 2003, Bowden et al. 2004, Burton et al. 2004), which could be due to labile C depletion (Neff et al. 2002, Frey et al. 2004), reduced microbial biomass (Compton et al. 2004, DeForest et al. 2004, Frey et al. 2004, Allison et al. 2007), and/or reduced root biomass (Bowden et al. 2004). The stimulation in soil respiration that we observed likely represents the initial phase of the response, which may be followed by longer-term declines in CO<sub>2</sub> flux in the N addition treatment.

Consistent with our hypothesis, the increased rates of soil respiration with N fertilization were seasonally dependent, occurring only in summer and autumn. The lack of response in winter and spring may have been due to the experimental manipulation since fertilization only occurred between May and October. However, N fertilization accelerated N mineralization throughout the year, suggesting that the season-specific response in soil respiration was not an experimental artifact. Rather, the treatment by season interaction may be related to phenological differences in soil C and N availability and microbial community dynamics. For example, N additions could stimulate C acquisition in order to balance the C:N ratio of microbial biomass (Schimel 1988, Hart et al. 1994). Yet labile C from root exudates and litter leachate may not become available for this purpose until summer and fall, which could account for why N fertilization only stimulated soil respiration during this time.

#### *Seasonal response of soils to warming*

As we predicted, warming reduced soil moisture, increased both soil respiration and N mineralization, and the effect of warming was larger than that of N additions. Lower soil

moisture is typical for many heating experiments (e.g., Peterjohn et al. 1993, Rustad and Fernandez 1998, Allison and Treseder 2008, Hagedorn et al. 2010) and may inhibit root and microbial activity if it falls below a certain threshold (e.g., 0.12 g m<sup>-3</sup> volumetric water content, Davidson et al. 1998). The lower effect of warming on soil respiration in summer suggests that drier conditions in heated plots may have limited C cycling during this season.

Over both sampling years and across all seasons, heating accelerated N mineralization rates by an average of 83%. This increase is of the same magnitude as other investigations showing a doubling of N mineralization within the first few years of a warming experiment (e.g., Peterjohn et al. 1994, Hartley et al. 1999). The stimulatory effect of warming on N mineralization showed no treatment by season interactions, occurring throughout the year and even during winter. Increased rates of winter net N mineralization in the warming treatment could result in the same N losses as higher rates of winter N mineralization in the fertilized plots: denitrification, N<sub>2</sub>O flux, and/or export of inorganic N below the rooting zone. As with the N addition treatment, the lack of difference in DIN in lysimeter water between control and warming plots and the almost nonexistent nitrification in any of the treatments during winter render each of these potential fates of the mineralized N unlikely. However, increased rates of winter N mineralization were much more common in the warming treatment than they were in the N addition plots, occurring both during the winter thaw of 2008 and during January and March of 2009.

In addition to accelerating N mineralization, soil warming increased soil CO<sub>2</sub> flux by an average 44%. This was slightly less than the 60% increase that Peterjohn et al. (1994) reported for the first year of experimental warming, but is equivalent to values reported for the first few years of other soil warming studies (e.g., Rustad and Fernandez 1998, Schindlbacher et al. 2009, Hagedorn et al. 2010). The positive response of soil respiration to warming varied seasonally, and was higher in spring and fall than it was during winter and summer (Table 3). During summer, lower soil moisture in heated plots may have limited both root and microbial respiration.

During winter, the absence of root respiration may account for the lower response of CO<sub>2</sub> flux to warming. Nevertheless, enhanced heterotrophic respiration in winter could augment the 15–30% of C already lost from the system that was fixed during the previous growing season (Goulden et al. 1996), and could result from greater microbial access to C substrates due to the presence of liquid water.

Soil warming also decreased the periodicity of the soil respiration seasonal cycle, in particular showing higher soil respiration rates earlier in spring. The doubling of soil respiration in the warming plots in late March and April as compared to the controls may have been due to the earlier disappearance of snowpack and soil temperatures well above freezing, both of which could have deepened the active layer responsible for soil respiration sooner (Rayment and Jarvis 2000, Mo et al. 2005). The pulse of water from snowmelt may also have prompted soil microbes to substantially increase respiration rates (Monson et al. 2006a). Whatever the mechanism, increased springtime respiration rates are more likely to occur as climate warming extends spring several weeks into what was historically the winter season (Hayhoe et al. 2007). The accompanying shifts in spring respiration could both alter soil respiration dynamics throughout the year and contribute to interannual variability in CO<sub>2</sub> flux (Goulden et al. 1996, Savage and Davidson 2001).

#### *Interactions among season, soil warming, and N additions*

Contrary to our hypothesis, this study showed no interactive effects of simultaneous warming and N fertilization on soil respiration and N mineralization. We observed a marginally significant interaction between warming × N on soil respiration. This occurred in autumn, overlapped with one of the two seasons in which N additions impacted soil respiration (summer and fall), and indicated an annulling effect of N additions on the warming treatment. On several dates in the autumns of 2007 and 2008, average soil respiration in the warming × N plots was as much as 13% lower than fluxes in the warming only plots. Environmental conditions may have been conducive on these occasions for producing the lower fluxes in the warming × N plots. Several

months of fertilization, higher rates of N mineralization, decreased plant N demand, and N inputs in senescing litter may have increased soil N levels high enough to partially suppress the stimulatory effect of warming on soil respiration.

Instead of showing a synergistic response, the warming × N plots generally displayed the same rates of soil respiration and N mineralization as the warming only treatment. The lack of interaction between warming and N additions may have resulted from differences in the magnitude of the treatment effects, a lag time between the initiation of the treatments and the ecosystem response, and/or divergent pathways through which the treatments affected the system. A 5°C increase in soil temperature may perturb soil processes much more strongly than an eightfold increase in atmospheric N deposition. Indeed, the relative changes in soil respiration and N mineralization in response to warming and N additions support this idea. Over the course of the study, the warming plots increased N mineralization by 83% and soil respiration by 44%, whereas the N addition plots only boosted N mineralization by 8% and soil CO<sub>2</sub> flux by 14%. However, the smaller impact in the N fertilized plots may not just be the result of a smaller disturbance to the system, but could be due to a lag between the initiation of fertilizer application and the ecosystem response. After all, soils at the Harvard Forest Chronic Nitrogen Addition experiment showed small immediate reactions to N fertilization, such as slight increases in N mineralization and CO<sub>2</sub> respiration (Aber et al. 1993, Bowden et al. 2004). Other responses, such as suppression of litter decay and export of DIN below the rooting zone, took three to five years to manifest (Magill and Aber 1998, Magill et al. 2000).

Alternatively, the disparity between the effects of warming and N additions on soil C and N cycling may be related to different ways in which they trigger the system. Increased temperatures change the kinetics of microbial and root activity, at least initially speeding up the rates of many biochemical reactions and thus accelerating soil respiration and N mineralization. Nitrogen additions can also stimulate these processes in the short term, but for stoichiometric reasons related to the C:N ratio of microbial biomass, plant litter, and soil organic matter that may not produce as

dramatic an effect as warming. The long term effects of warming and N fertilization on soil biogeochemical processes can also differ. The reduced soil respiration rates often observed in chronically warmed plots can be attributed to thermal acclimatization of soil microbes to higher temperatures (Bradford et al. 2008) and/or depletion of labile C (Melillo et al. 2002, Bradford et al. 2008). Lower CO<sub>2</sub> fluxes in chronically fertilized plots can also result from diminished labile C supplies (Frey et al. 2004). However, suppressed decomposition of litter and SOM in N amended soils (Magill and Aber 1998, Frey et al. 2004, Knorr et al. 2005) may play just as important a role. If the short and long term pathways through which soil warming and N fertilization affect soil processes diverge too widely, then they may not be able to overlap to interactively affect soil biogeochemical processes.

Because this study represents two and a half years of soil warming and N additions, we are not in a position to scale up our findings to make long-term forecasts of regional C and N dynamics. Results from longer-running soil warming and N fertilization studies show that the response of soils to global change drivers can vary over time, such that initial response differs from the response after ten or twenty years (e.g., Melillo et al. 2002, Magill et al. 2004). With this in mind, concurrent warming and N deposition may interact over longer timescales despite the lack of significant, short-term interactions. Likewise, the interface between season, warming, and N additions may evolve with sustained elevated temperatures and N inputs. A combination of long-term, manipulative experiments and modeling studies are needed to address future seasonal changes in soil C and N budgets with simultaneous warming and N deposition.

## CONCLUSION

The objective of this study was to examine interactions among season, soil warming, and N additions on soil C and N cycling. Of particular interest were wintertime responses that often are excluded from investigations of soils and global change. We determined that traditional approaches of estimating winter N mineralization may fail to capture dynamic shifts in soil N cycling that occur during freeze-thaw. Likewise,

common methods for calculating soil CO<sub>2</sub> fluxes between November and April significantly underestimated winter respiration, particularly in unheated soils. As a result, we recommend that measurements of winter N mineralization and soil respiration become research priorities for examining small scale temporal dynamics of winter nutrient cycling. This is especially important since global change drivers such as warming and N additions increased CO<sub>2</sub> and N fluxes during the winter months. Elevated rates of winter N mineralization in the absence of plant demand may result in the loss of this N from the system. In addition, higher rates of winter soil respiration signal larger losses of CO<sub>2</sub> fixed during the previous growing season, as well as enhanced microbial access to previously unavailable substrates. Season-specific responses also suggest that certain components of the below-ground community are more responsive to warming and N additions than others. They also indicate that the environmental conditions necessary for a biogeochemical response may only occur at certain times of the year. The marginally significant interaction between warming and N took place only in autumn, when the combination of six months of N additions, elevated rates of N mineralization, decreased plant N demand, and an influx of N-rich plant litter may have raised soil N levels high enough to dampen the positive response of soil respiration to warming. The lack of interaction between warming × N may also have resulted from differences in the magnitude of their disturbance, lags between the initiation of the treatments and ecosystem response, and/or disparate pathways through they disrupted the system. In combination with modeling studies, additional research at this and other multiple-manipulation experiments will be necessary to determine long-term, seasonal changes in soil C and N dynamics with warming and N deposition.

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## APPENDIX

Table A1. Random effects and autocorrelation model specifications for gravimetric soil moisture, net N mineralization, and soil respiration.

Variable	Random effects	L	Δ DF	P	Autocorrelation	Δ AIC	Δ DF
Moisture							
O Horizon	Plot	25.50	1	<0.0001	ARMA	26.76	2
M Horizon	Plot	103.68	1	<0.0001	None	0	0
N mineralization	None	1.36	0	0.51	ARMA	2.54	2
Soil Respiration	Plot, Warming	110.43	3	<0.0001	ARMA	25.76	2

Notes: For the random effects, plot was modeled as a random intercept and warming as a random slope. The likelihood ratio (L) and P-values refer to the random effects structure in a linear mixed effects (*lme*) model tested against a generalized least squares (*gls*) model, with P-values shown in their uncorrected form for testing on the boundary. The Δ degrees of freedom (Δ DF) column for random effects indicates the increase in degrees of freedom by adding random effects to a *gls* model, while the Δ DF column for autocorrelations refers to the increase in degrees of freedom from adding an autocorrelation structure. The Δ Akaike's Information Criterion (Δ AIC) column indicates the decrease in AIC by adding an autocorrelation structure. The ARMA autocorrelation structure indicates an autoregressive moving average. The names O Horizon and M Horizon represent organic and mineral soil horizons.