

3-2016

Climate Change Decreases Nitrogen Pools and Mineralization Rates in Northern Hardwood Forests

Jorge Durán

Cary Institute of Ecosystem Studies

Jennifer L. Morse

Portland State University, jlmorse@pdx.edu

Peter M. Groffman

Cary Institute of Ecosystem Studies

John L. Campbell

USDA Forest Service's Northern Research Station

Lynn M. Christenson

Vassar College

See next page for additional authors

Let us know how access to this document benefits you.

Follow this and additional works at: https://pdxscholar.library.pdx.edu/esm_fac

Part of the [Environmental Sciences Commons](#)

Citation Details

Durán, J., Morse, J. L., Groffman, P. M., Campbell, J. L., Christenson, L. M., Driscoll, C. T., ... Vadeboncoeur, M. A. (2016). Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests. *Ecosphere*, 7(3). <http://doi.org/10.1002/ecs2.1251>

This Article is brought to you for free and open access. It has been accepted for inclusion in Environmental Science and Management Faculty Publications and Presentations by an authorized administrator of PDXScholar. For more information, please contact pdxscholar@pdx.edu.

Authors

Jorge Durán, Jennifer L. Morse, Peter M. Groffman, John L. Campbell, Lynn M. Christenson, Charles T. Driscoll, Timothy J. Fahey, Melany C. Fisk, Gene E. Likens, Jerry M. Melillo, Myron J. Mitchell, Pamela H. Templer, and Matthew A. Vadeboncoeur

Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests

JORGE DURÁN,^{1,13,14,†} JENNIFER L. MORSE,² PETER M. GROFFMAN,¹ JOHN L. CAMPBELL,³
LYNN M. CHRISTENSON,⁴ CHARLES T. DRISCOLL,⁵ TIMOTHY J. FAHEY,⁶ MELANY C. FISK,⁷
GENE E. LIKENS,^{1,8} JERRY M. MELILLO,⁹ MYRON J. MITCHELL,¹⁰ PAMELA H. TEMPLER,¹¹
AND MATTHEW A. VADEBONCOEUR¹²

¹*Cary Institute of Ecosystem Studies, 2801 Sharon Turnpike, Millbrook, New York 12545 USA*

²*Environmental Science and Management, Portland State University, PO Box 751 (ESM), Portland, Oregon 97207 USA*

³*Northern Research Station, USDA Forest Service, 271 Mast Road, Durham, New Hampshire 03824 USA*

⁴*Biology Department, Vassar College, 124 Raymond Ave., Poughkeepsie, New York 12604 USA*

⁵*Department of Civil and Environmental Engineering, Syracuse University, 900 S Crouse Ave, Syracuse, New York 13210 USA*

⁶*Department of Natural Resources, Cornell University, Ithaca, New York 14850 USA*

⁷*Department of Zoology, Miami University, 501 E High St, Oxford, Ohio 45056 USA*

⁸*Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269 USA*

⁹*Marine Ecological Laboratory, The Ecosystem Center, Woods Hole, Massachusetts 02543 USA*

¹⁰*Department of Environmental and Forest Biology, SUNY-College of Environmental Science and Forestry,
1 Forestry Dr, Syracuse, New York 13210 USA*

¹¹*Department of Biology, Boston University, 5 Cummington Street, Boston, Massachusetts 02215 USA*

¹²*Institute for the Study of Earth, Oceans and Space, Earth Systems Research Center,
University of New Hampshire, 8 College Road, Durham, New Hampshire 03824 USA*

¹³*Center for Functional Ecology, University of Coimbra, Calçada Martim de Freitas, Coimbra, 3000-456 Portugal*

Citation: Durán, J., J. L. Morse, P. M. Groffman, J. L. Campbell, L. M. Christenson, C. T. Driscoll, T. J. Fahey, M. C. Fisk, G. E. Likens, J. M. Melillo, M. J. Mitchell, P. H. Templer, and M. A. Vadeboncoeur. 2016. Climate change decreases nitrogen pools and mineralization rates in northern hardwood forests. *Ecosphere* 7(3):e01251. 10.1002/ecs2.1251

Abstract. Nitrogen (N) supply often limits the productivity of temperate forests and is regulated by a complex mix of biological and climatic drivers. In excess, N is linked to a variety of soil, water, and air pollution issues. Here, we use results from an elevation gradient study and historical data from the long-term Hubbard Brook Ecosystem Study (New Hampshire, USA) to examine relationships between changes in climate, especially during winter, and N supply to northern hardwood forest ecosystems. Low elevation plots with less snow, more soil freezing, and more freeze/thaw cycles supported lower rates of N mineralization than high elevation plots, despite having higher soil temperatures and no consistent differences in soil moisture during the growing season. These results are consistent with historical analyses showing decreases in rates of soil N mineralization and inorganic N concentrations since 1973 that are correlated with long-term increases in mean annual temperature, decreases in annual snow accumulation, and increases in the number of winter thawing degree days. This evidence suggests that changing climate may be driving decreases in the availability of a key nutrient in northern hardwood forests, which could decrease ecosystem production but have positive effects on environmental consequences of excess N.

Key words: carbon; global change; microbial activity mineralization; nitrification; nitrogen; soil frost.

Received 26 August 2015; revised 28 September 2015; accepted 30 September 2015. Corresponding Editor: D. P. C. Peters.

Copyright: © 2016 Durán et al. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

¹⁴ Present address: Department of Life Sciences, Centre for Functional Ecology, University of Coimbra, 3001-401, Coimbra, Portugal.

† E-mail: humia20@gmail.com

INTRODUCTION

Nitrogen (N) is an essential nutrient for all biota. In most temperate forest ecosystems, N is often a limiting nutrient that constrains primary production (Vitousek and Howarth 1991, LeBauer and Treseder 2008). In excess, N can contribute to environmental problems, such as acidification, emissions of N oxides, and eutrophication (Galloway et al. 2008, Porter et al. 2013). The global N cycle is strongly affected by anthropogenic activities through changes in atmospheric deposition, climate, and species composition (Aber et al. 1997). Global climate is projected to become significantly warmer, which will markedly alter N biogeochemical cycling across diverse ecosystems, including temperate forests (Pendall et al. 2008, Campbell et al. 2009). Temperate forest biomes contain approximately 10% of global soil carbon (C) stocks, and most terrestrial C sequestration at mid-latitudes in the Northern Hemisphere occurs in forest ecosystems (Rasmussen et al. 2006). Given the tight linkages between C and N cycles, there is a strong need to assess how changing climate will influence ecosystem processes that affect and are affected by N cycling to make accurate projections of regional and global ecological responses to environmental change (Vitousek et al. 1997).

Forests in northeastern North America are maturing, and atmospheric N inputs remain elevated, which would be expected to result in high rates of N leaching loss (Aber et al. 2003). Yet, in recent decades there have been remarkable, unexplained declines in nitrate concentrations and export from these forests (Goodale et al. 2003, Bernhardt et al. 2005, Bernal et al. 2012). Proposed explanations for these declines include recovery from past disturbances, decreases in atmospheric N deposition, increased losses of dissolved organic N, a longer growing season, increases in the N content of mineral soil, increased plant uptake or denitrification, and changes in hydrologic flow paths (Bernhardt et al. 2005, Bernal et al. 2012, Yanai et al. 2013, Morse et al. 2015). An unexplored factor that could also contribute to these declines is a decrease in internal N cycling via N mineralization (Vitousek and Howarth 1991). Many studies have shown N cycle processes to be highly sensitive to changes in precipitation and temperature and past studies provide some evidence that climate change may be driving decreases in

northern forest N cycling rates. Research at the Hubbard Brook Experimental Forest (HBEF; New Hampshire, USA) has consistently found greater rates of N mineralization and nitrification to be associated with cooler soil temperatures and higher soil water availability at high elevation compared to low elevation sites (Venterea et al. 2003, Groffman et al. 2009, Durán et al. 2014). These studies suggest that warmer temperatures leading to drier soils through greater evapotranspiration counteract the potential increases in activity anticipated with higher temperatures. Similarly, declines in N mineralization with declining snowpack suggest that changes in winter climate could also contribute to decreasing N-cycling rates over time (Durán et al. 2013, 2014).

Here, we coupled an elevation gradient analysis with long-term soil measurements to evaluate effects of climate change on N transformation rates. We also asked whether changes in climate can help explain the observed decreases in stream nitrate concentrations and ecosystem nitrate losses in northeastern North America over the past four decades (Campbell et al. 2007, Yanai et al. 2013). We exploited a natural elevation gradient that has little variation in soils or vegetation in a northern hardwood forest at the HBEF, but encompasses the variation in temperature that is projected to occur with climate change over the next 50–100 yr (Hayhoe et al. 2007). With this approach we evaluated the expected long-term effects of climate variation on soil N pools and transformation rates in northern hardwood forests. We also utilized historical data from the HBEF to investigate long-term changes in climate, N mineralization, and stream nitrate concentrations. We hypothesized that: (1) N mineralization and nitrification rates are slower in warmer, drier low elevation plots than in colder, wetter high elevation plots; (2) N mineralization rates have decreased over the past four decades along with climate change-driven variations in both dormant (i.e., winter) and growing (i.e., summer) season conditions; and (3) these decreases are linked to the historical, unexplained decrease in nitrate export in northeastern U.S. forests in recent decades.

METHODS

The study was conducted at the Hubbard Brook Experimental Forest long-term ecological

research (LTER) site (New Hampshire, USA). The climate is cool, humid, and continental. Average monthly air temperatures range from -9°C to 18°C . Average annual precipitation is 1400 mm. A snowpack usually persists from late December until mid-April. Soils are mostly Spodosols and Inceptisols developed from unsorted basal tills (75–100 cm deep, $\text{pH}\approx 4$; 32). The forest is dominated by northern hardwoods (*Acer saccharum*, *Fagus grandifolia*, and *Betula alleghaniensis*).

Elevation gradient

In October 2010, we established three high elevation (≈ 550 – 600 m, north facing) and three low elevation (≈ 375 – 500 m, south facing), 20 m diam., independent (separated by >300 m) plots. This gradient has relatively little variation in soils or vegetation with all plots located on well to moderately well-drained soils and dominated by sugar maple. The gradient encompasses variation in mean annual air temperature ($\approx 2^{\circ}\text{C}$) similar to what is projected to occur with climate change over the next 50–100 yr in northeastern North America (Hayhoe et al. 2007).

In each plot, we monitored soil temperature (ST) and volumetric water content (VWC) at 5 cm depth with Decagon 5TM[®] combination probes. We used a Federal snow sampling tube (Rickly Hydrological Company, Columbus, OH) to measure snow depth and snow water content, and installed frost tubes to measure soil frost depth biweekly (Hardy et al. 2001). We integrated the continuous measurements of snow depth, soil frost depth and snow water content to produce seasonally integrated, unitless indices of winter climate conditions (“snow”, “frost”, and “snow water”; see SI). As a measure of winter soil temperature variability, we calculated the SDL (standard deviation of log-transformed observations) of daily soil winter temperatures (hereafter referred as to “*frzthaw*”) (Durán et al. 2014).

In each plot, at each sampling date (Fall 2010, Winter 2010–2011, Spring 2011, Summer 2011, Fall 2011, Winter 2011–2012, Spring 2012, and Summer 2012), we measured *in situ* N mineralization and nitrification using the buried bag method (Robertson et al. 1999, Durán et al. 2012) and potential N mineralization and nitrification rates in laboratory incubations following Durán

et al. (2014). We selected 10 and 5 sampling points for measurements of potential and *in situ* N mineralization and nitrification rates, respectively. Soil samples were taken with a 5-cm diameter \times 20-cm depth intact core that allowed collection of the first ~ 15 cm of the soil profile. Horizon depths and mass do not vary with elevation in the hardwood zones at the HBEF (Johnson et al. 2000), therefore this sampling procedure produced a valid comparison of rates at different elevations.

For measurement of *in situ* rates, two intact soil cores were taken at each sampling point with one placed back into the soil for incubation (≈ 5 months for the winter incubations and ≈ 1 month for the rest of seasons) and one returned to the laboratory for extraction of inorganic N. Changes in inorganic N (ammonium plus nitrate) between the incubated and immediately extracted cores were used to calculate net mineralization, and changes in nitrate were used to calculate net nitrification. Annual estimates of N transformation rates were calculated by multiplying measured seasonal daily average rates by the length (in days) of each season (Groffman et al. 2009).

For measurements of potential rates, samples were stored at 4°C between sampling and analysis (less than 1 week), hand sorted to remove roots and rocks, mixed, and held at field moisture for all analyses. Analyses consisted of 10 d laboratory incubations of 10 g of soil at $\approx 25^{\circ}\text{C}$ and field moisture. As for *in situ* N transformation rates, changes in inorganic N (ammonium plus nitrate) between the lab-incubated and immediately extracted soil samples were used to calculate potential mineralization, and changes in nitrate were used to calculate potential nitrification. Soil organic matter content was determined by loss on ignition at 450°C for 4 h (Nelson and Sommers 1996). Soil gravimetric water content (GWC) was determined by drying at 60°C for 48 h (McInnes et al. 1994). Inorganic N in 2M KCl extracts was quantified colorimetrically using a flow injection analyzer (Lachat Quikchem 8100).

Historical data

To investigate whether potential or *in situ* N mineralization and nitrification rates have changed in recent decades and whether these changes could be linked to changes in climate, we analyzed published data from previous studies at the HBEF (Melillo 1977, Bohlen

et al. 2001, Groffman et al. 2001a, 2006, 2009, Houlton et al. 2003, Durán et al. 2014). The earliest soil N transformation data was produced by Melillo (1977) in 1973. To evaluate changes through time, we compared those data with *in situ* and potential N transformation data collected in 1994–1996 (Bohlen et al. 2001), 1997–1999 (Groffman et al. 2001a, 2006, Houlton et al. 2003), 2000–2004 (Groffman et al. 2006, 2009) and 2011–2012 (Durán et al. 2014) (described above). All these studies: (1) provided (or allowed us to calculate) estimates of annual potential or *in situ* N mineralization rates; (2) calculated ecosystem averages by sampling across the HBEF elevation gradient using similar elevation ranges, and (3) used the same methodology, except for Melillo (1977), whose methods differed slightly. Specifically, for *in situ* N mineralization, Melillo used a buried bag method similar to the remainder of our historical data, but soils were sieved before incubation. For potential N mineralization, Melillo (1977) also used lab incubations but calculated potential rates from leachate instead of from soil extractions. A final analysis was performed to examine relationships between soil N transformations (rates and pools) and nitrate export from the biogeochemical reference watershed at the HBEF (Likens 2013).

To identify possible physical drivers of changes in N cycling, and to investigate whether changes in N cycling could underlie recent declines in nitrate concentrations and export from forests in northeastern North America, we (following Hamburg et al. [2013]) calculated annual mean temperature (based on daily means which are reported as the mean of the daily minima and daily maxima temperatures) using data collected by the USDA Forest Service at the HBEF for the period from 1970 to 2012 (<http://www.hubbardbrook.org>). We also derived a secondary temperature-based climate metric, winter thawing degree-days (TDD) by calculating deviations of mean daily temperature from a base temperature of 0 °C for the period December 1st to March 31st. Finally, in this case we calculated an integrated snowpack measurement by multiplying the daily values of snow depth by the number of days the snowpack was present during the winter.

Statistical analysis

To test the effect of the elevation gradient on response variables, a distance-based permutational repeated measures ANOVA was fitted with elevation as the main categorical fixed factor, dividing our plots into three low elevation and three high elevation replicates (PERMANOVA [Anderson et al. 2008]). Significant differences between elevations were investigated using an *a posteriori* pairwise comparison with the PERMANOVA t-statistic, taking into account time dependencies between samplings. A maximum of 9999 permutations were used to obtain the Pseudo-*F* and *P*-values in each data set, and Monte Carlo correction was applied when necessary. Relationships between variables were explored with univariate linear regressions (OLS). Temporal trends in N transformation rates were explored with Mann–Kendall trend tests. Statistical analyses were carried out using R version 3.1.1 and Primer 6 and PERMANOVA+ (PRIMER-E Ltd, Plymouth, UK) statistical packages.

RESULTS AND DISCUSSION

The elevation gradient at the HBEF was effective in providing a wide range of climatic conditions. Low elevation plots had consistently warmer soil (Fig. 1), less snow accumulation, greater amounts of soil frost, and more soil freeze/thaw events in both winters of our study (Fig. 2). High elevation plots tended to have higher water content than low elevation plots, but the differences were not statistically significant (Fig. 1). These results confirm that the expected climate shift toward warmer temperatures in northern forest ecosystems might cause not only less winter snow accumulation, but also increases in soil freezing (Groffman et al. 2001a, Durán et al. 2013, 2014). Although we would intuitively expect warmer temperatures to decrease soil frost, our results support the alternative hypothesis that the loss of insulation associated with a reduced snowpack overwhelms the increase in air temperatures (Henry 2008). Our findings are consistent with other studies that anticipate increases in soil freezing and freeze/thaw events—which have been found to affect a wide variety of soil biogeochemical processes—in northern forests in the upcoming

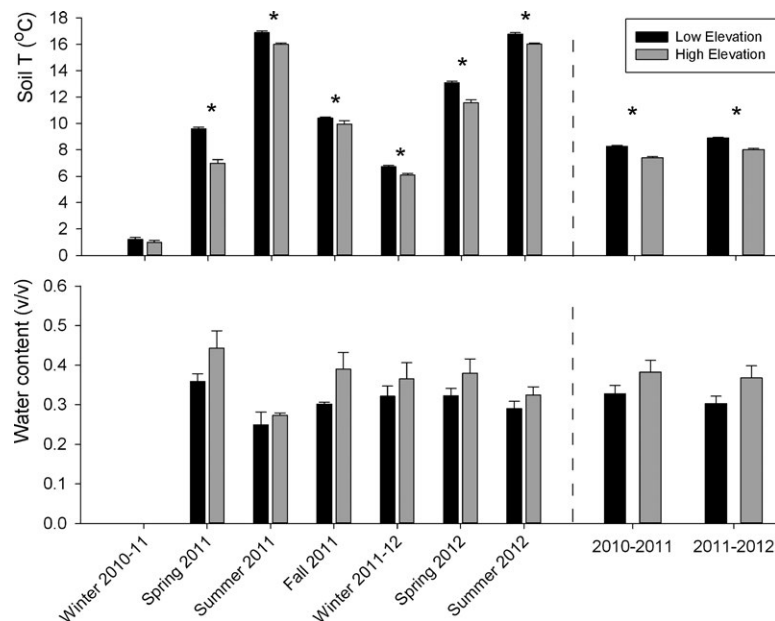


Fig. 1. Seasonal (left) and annual (right) estimates of soil temperature and water content measured in low (black bars) and high elevation (gray bars) plots. Values are mean with standard error. Asterisks refer to statistically significant ($P < 0.05$) differences between low and high elevation plots (distance-based permutational ANOVA).

decades (Schimel and Clein 1996, Durán et al. 2014).

We found a positive effect of elevation on both annual and individual date observations of *in situ* and potential nitrification (Pseudo- F [$P-F$] = 7.8, $P < 0.05$ and $P-F = 21.17$, $P < 0.01$, respectively) and N mineralization ($P-F = 5.17$, $P < 0.1$ and $P-F = 52.17$, $P < 0.005$, respectively) rates, and a significant effect of sampling date on *in situ* nitrification and N mineralization rates ($P-F = 2.66$, $P < 0.05$ and $P-F = 12.14$, $P < 0.001$, respectively; Fig. 3). Regression analyses revealed consistently negative relationships between annual N transformation rates and soil temperature (Table 1). In both years, annual *in situ* nitrification had a significant negative relationship with soil temperature ($P < 0.05$). Potential N mineralization rates were also negatively related to temperature ($P < 0.10$ in year 1 and $P < 0.05$ in year 2). Soil moisture did not show consistent relationships with N transformation rates.

There were strong relationships between winter climate and annual estimates of N supply. In the first year, annual *in situ* nitrification rates were negatively correlated with our proxy of winter freeze/thaw events ($P < 0.10$; Table 1), and

positively related with time-integrated estimates of winter snow and snow water content ($P < 0.05$ and $P < 0.10$, respectively). *In situ* N mineralization rates were positively related with snow ($P < 0.10$) and snow water ($P < 0.005$), and negatively with time-integrated estimates of soil frost ($P < 0.05$) and freeze/thaw cycles ($P < 0.05$). In the second year, *in situ* N mineralization rates were negatively related with soil frost and freeze/thaw events ($P < 0.05$), and positively related with snow ($P < 0.10$), and snow water ($P < 0.005$). *In situ* nitrification rates were only positively related with snow water ($P < 0.10$) in the second year. Potential N mineralization was negatively related with soil frost and freeze/thaw cycles ($P < 0.10$), and positively related with snow, and snow water ($P < 0.05$ and $P < 0.005$, respectively). Overall, lower elevation sites, with higher soil temperatures, smaller depth and duration of snowpack accumulation, and more soil frost and freeze/thaw events during the winter, had lower *in situ* and potential N transformation rates.

Although higher temperatures have often been observed to increase rates of microbial activity, in these forests this effect might have been overcome by warmer winter temperatures leading to

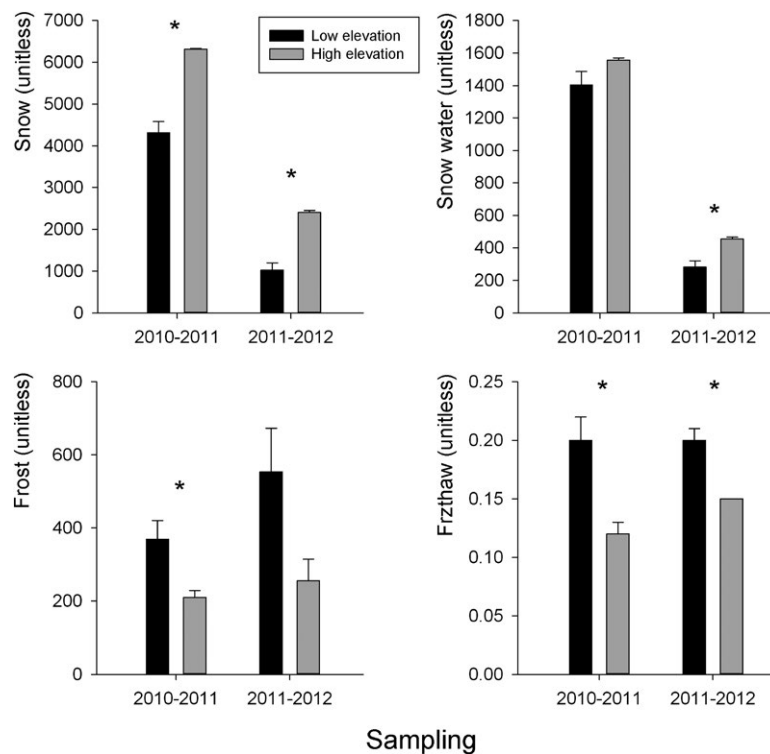


Fig. 2. Annual estimates of “snow” (Snow), “snow water” (SW), “soil frost” (Frost) and the variability in soil winter temperature (*frzthaw*) measured in low (black bars) and high elevation (gray bars) plots in the 2010–2011 and 2011–2012 winters. Values are mean with standard error. Asterisks refer to statistically significant ($P < 0.05$) differences between low and high elevation plots (distance-based permutational ANOVA).

a reduced snowpack, more soil frost, and more soil freeze/thaw events (Paul 2007). In a previous study in the same forest, we showed that a reduced snowpack, together with the associated increases in soil frost dynamics (more soil freeze/thaw events during the winter) in lower elevation plots may stress and/or kill microbial populations, decreasing organic matter decomposition and N transformation rates (Durán et al. 2014). In contrast, in higher elevation plots with greater snowpack and insulation that prevents soils from freezing, belowground biological processes can continue uninterrupted during the winter (Brooks et al. 2011). These results are consistent with those of Morse et al. (2015), who found that soil denitrification, at the same sites, was also strongly affected by elevation, with lower rates occurring at low elevation plots.

The stronger relationships between winter rather than summer climate variables and annual rates of mineralization and nitrification sug-

gest that winter conditions, and changes in them, are a key factor in driving N supply in northern hardwood forests. These results are somewhat surprising because it is generally thought that the bulk of annual biotic soil activity occurs during summer (Likens 2013). Previous studies at the HBEF and elsewhere have also found that soil temperature and moisture are strong drivers of soil activity, especially during the growing season (Groffman et al. 2009). Here, however, we observed negative relationships between soil temperature and annual activity and no consistent relationships with soil moisture. These results build on previous work (Durán et al. 2014) showing that changes in winter conditions have marked effects on microbial activity well into the growing season.

We acknowledge that factors other than winter climate likely contributed to the elevation effects on N mineralization and nitrification that we observed. For example, differences in soil or plant

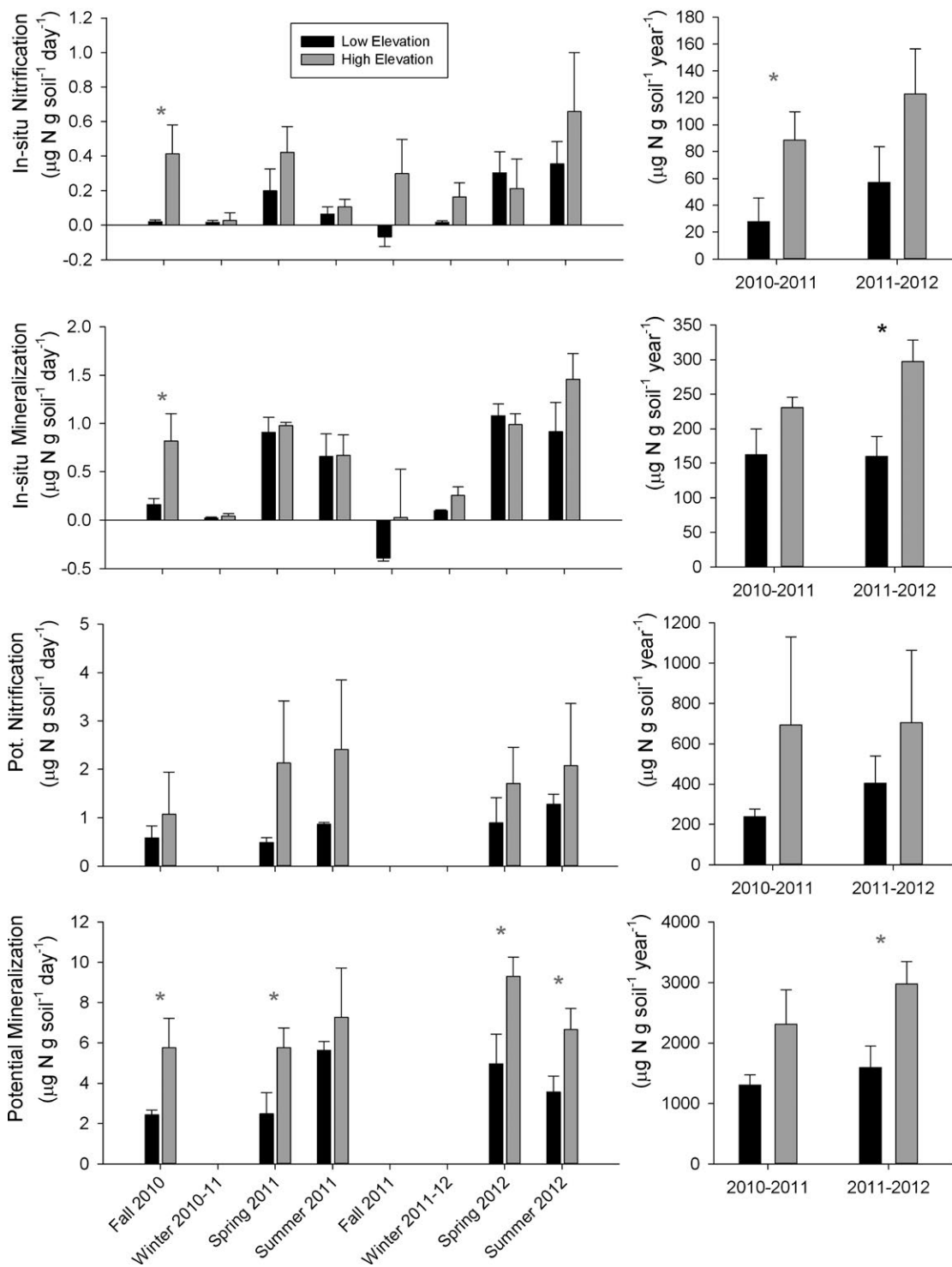


Fig. 3. Seasonal (left) and annual (right) estimates of *in situ* and potential nitrification and N mineralization (Nitrification, Mineralization, PN, and PNM, respectively) rates measured in low (black bars) and high elevation (gray bars) plots. Values are mean with standard error ($n = 3$). Asterisks indicate statistically significant ($P < 0.05$) differences between low and high elevation plots (distance-based permutational ANOVA).

Table 1. Univariate linear regressions (ordinary least-squares [OLS]) between soil temperature (Temp), soil water content (WC), “snow”, “snow water” (SW), “soil frost”, and the soil freeze/thaw cycles index (“Frzthaw”; see Materials and methods section) as independent variables, and in situ and potential nitrification and N mineralization rates (In situ Nit, In situ Nmin, Pot Nit, and Pot Nmin, respectively) in 2010–2011, and in 2011–2012.

Independent variable	In situ Nit		In situ Nmin		Pot Nit		Pot Nmin	
	β	R ²	β	R ²	β	R ²	B	R ²
2010–2011								
Temp	-82.38	0.82*	-83.75	0.52	-665.68	0.37	-1266.22	0.54 ^a
WC	394.87	0.20	998.09	0.76*	-2751.98	0.07	-370.44	0.00
Snow	0.03	0.68*	0.04	0.62 ^a	0.23	0.23	0.49	0.44
SW	0.28	0.59 ^a	0.44	0.88**	1.67	0.14	3.20	0.22
Frost	-0.29	0.45	-0.45	0.69*	-1.59	0.09	-4.36	0.29
Frzthaw	-657.60	0.57 ^a	-956.20	0.73*	-4865.78	0.15	-9778.06	0.35
2011–2012								
Temp	-85.35	0.52	-159.08	0.81*	-450.08	0.25	-1661.82	0.78*
WC	390.31	0.12	869.16	0.27	150.89	0.00	10 213.32	0.32
Snow	0.05	0.51	0.10	0.84 ^a	0.27	0.22	1.07	0.79*
SW	0.44	0.60 ^a	0.79	0.86**	2.29	0.28	8.42	0.87**
Frost	-0.18	0.45	-0.34	0.69*	-0.85	0.17	-3.40	0.62 ^a
Frzthaw	-1186.78	0.39	-2385.45	0.84*	-4828.12	0.34	-23 211.46	0.59 ^a

Note: ^aP < 0.1, *P < 0.05, **P < 0.005, ***P < 0.001.

C and N stocks or quality with elevation may have influenced N dynamics. Although we did not measure total stocks of C and N in this study, past studies have not found significant variation in these stocks with elevation within the hardwood vegetation zones at the HBEF (Johnson et al. 2000, Bohlen et al. 2001). Given that vegetation is quite similar along the gradient, differences in organic C and N quality are also unlikely. Differences in soil moisture during summer could also have played a role in the elevation differences that we observed. While there was no significant difference in summer soil moisture with elevation in this study, there was a trend for higher soil moisture at high elevation, and significant differences have been observed in previous studies at the HBEF (Venterea et al. 2003, Groffman et al. 2009). There is a clear need for more detailed analysis of the multiple factors that vary with elevation during both the growing and dormant seasons. More specific measurements of microbial communities, enzymatic activities, and gross rates of N mineralization, immobilization and nitrification would be particularly useful.

Our elevation gradient results are consistent with the hypothesis that a warmer world may lead to decreases in the supply of inorganic N

in northern forests (Groffman et al. 2009, Durán et al. 2014) but see (Melillo et al. 2011), confirming past studies that have suggested that a climatic shift toward warmer annual temperatures in these forests may lead to a net decrease in inorganic N supply, which could exacerbate N limitation (LeBauer and Treseder 2008, Durán et al. 2014). This hypothesis is also supported by our analyses of long-term observations of soil microbial processes. Annual *in situ* and potential N mineralization and nitrification rates have decreased since 1973, although the trends were only significant for the potential rates ($P < 0.05$; Fig. 4). There has been a parallel and significant decrease in soil inorganic N concentrations over the same period (Fig. 4). Comparison of data from different studies over time is problematic due to subtle differences in methods and sites. However, all of the studies compared here provided (or allowed us to calculate) estimates of annual rates and were based on sampling across the HBEF elevation gradient using similar elevation ranges. Most importantly, *in situ* and potential rates as well as soil inorganic N pools all showed a similar decrease with time.

The idea that winter climate change is reducing N mineralization is, in some ways, not consistent

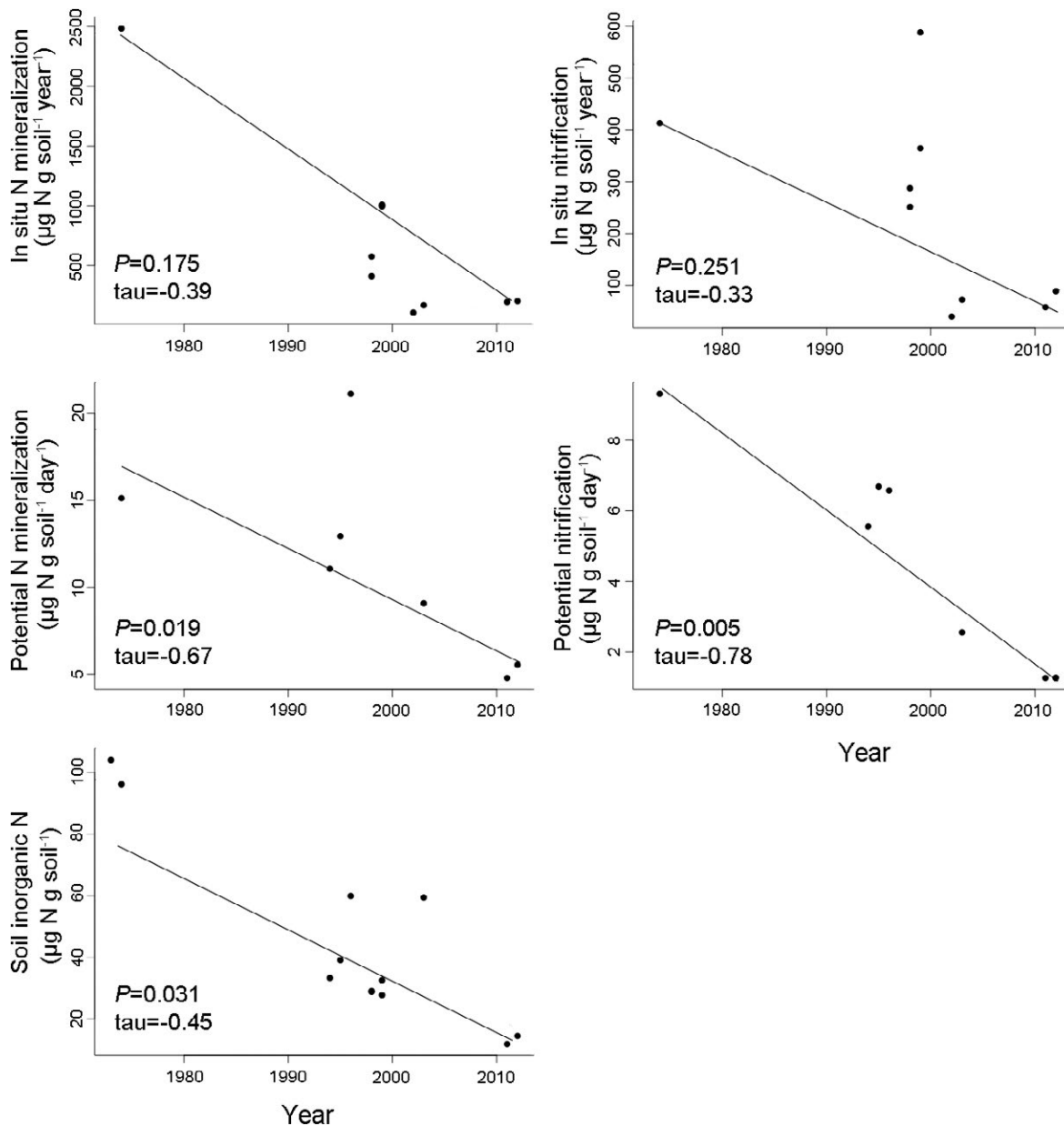


Fig. 4. Mann–Kendall analysis and Sen slopes for changes in *in situ* and potential nitrification and N mineralization rates and in soil inorganic N content in the Hubbard Brook Experimental Forest, New Hampshire, USA.

with results from the HBEF and elsewhere showing increases in hydrologic losses of N with decreases in snow depth and increases in soil freezing (Fitzhugh et al. 2001, Matzner and Borken 2008, Campbell et al. 2014). It is important to note that these increases in loss were driven primarily by root mortality and decreases in

plant uptake and not to any stimulation of N mineralization and nitrification (Groffman et al. 2001b, Tierney et al. 2001, Cleavitt et al. 2008, Comerford et al. 2013). Indeed, the increase in hydrologic losses with soil freezing likely reduce pools of soil labile N and contribute to the long-term declines in N availability.

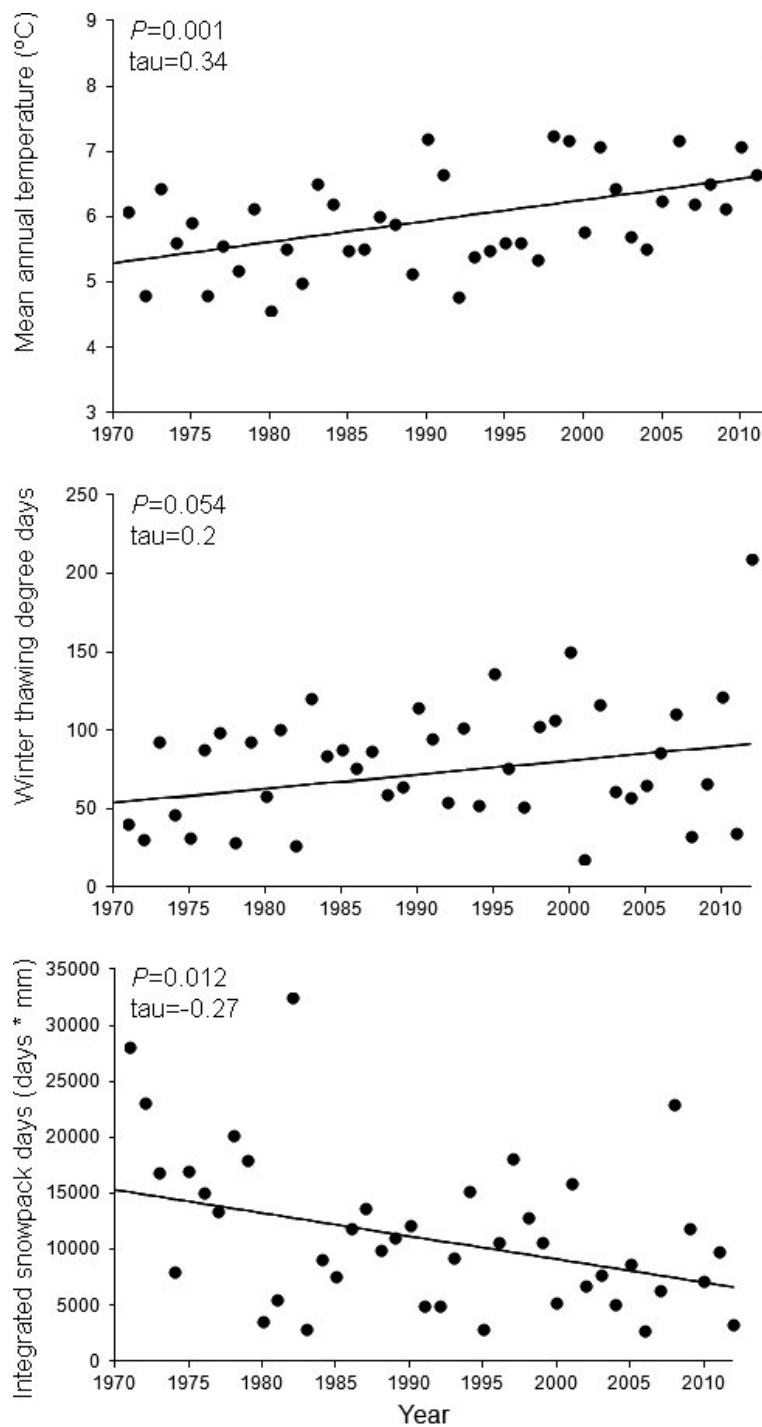


Fig. 5. Mann-Kendall analyses and Sen slopes for the long-term records of mean annual air temperature (490 m), winter thawing degree days (490 m), and integrated snowpack days (560 m) at the Hubbard Brook Experimental Forest, New Hampshire, USA.

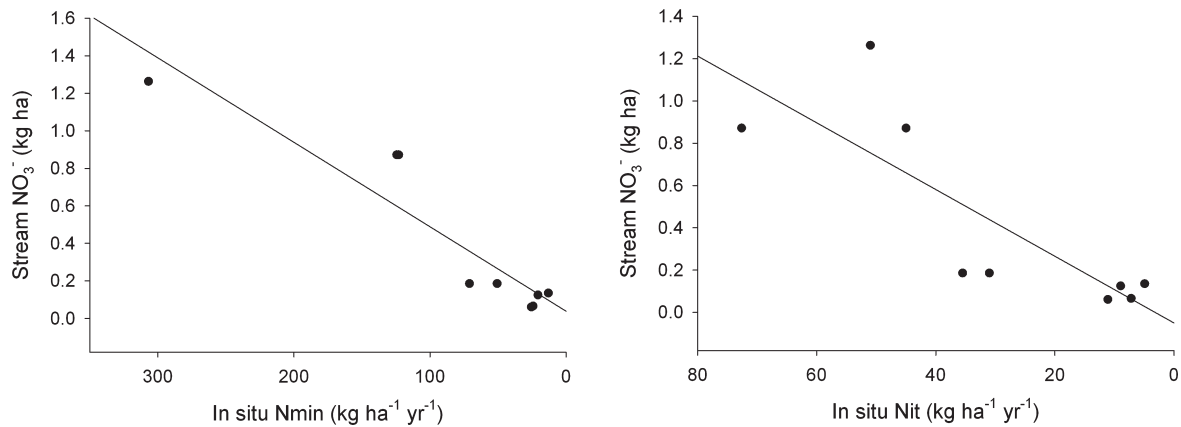


Fig. 6. Relationships between annual *in situ* N mineralization and nitrification rates, and the annual watershed nitrate export at the Hubbard Brook Experimental Forest from 1973 to 2012.

The results from both our experimental and historical analyses strongly suggest that the ability of the forest ecosystem to mineralize N has decreased in recent decades, which has led to a decrease in the concentration of inorganic forms of N in the soil and surface waters. The mechanisms behind these long-term trends remain to be determined. However, synchronous with the above-described decreases in N mineralization rates and soil inorganic N content, the HBEF has experienced a gradual and significant increase in mean annual air temperature, decrease in annual snow accumulation, and increase in the number of winter thawing degree days (Fig. 5). This synchrony, together with the consistency between the elevation gradient and the long-term measurements, provides linkages between the capacity of HBEF soils to mineralize N and changes in climate, snow accumulation, and soil frost dynamics over the past 40 yr (see above and Durán et al. 2014).

In recent decades, there have been remarkable and yet unexplained declines in nitrate concentrations and hydrological export from forests in northeastern North America (Bernhardt et al. 2005, Bernal et al. 2012, Likens 2013, Yanai et al. 2013). We hypothesized that a climate change-driven decrease in internal N production via decreases in N mineralization may be contributing to these declines (Vitousek and Howarth 1991, LeBauer and Treseder 2008). Consistent with this hypothesis, we found significant relationships between N mineralization and nitrification rates and watershed nitrate export over the past 40 yr

(Fig. 6). The limitations of our database necessitate caution when suggesting causal relationships, but these results highlight a strong need to determine whether the patterns observed at the HBEF are in fact due to changes in climate, and whether these dynamics were occurring in other forested watersheds that have also shown declining nitrate export (Goodale et al. 2003).

Our results suggest that climate change may be markedly decreasing the availability of a key nutrient in northern hardwood forests. Declines in N supply associated with climate could have important effects on the productivity of these forests and limit their ability to respond to projected increases in temperature, moisture and atmospheric CO₂, which would have the potential to increase forest ecosystem productivity over coming decades. On the other hand, these declines could mitigate the potential for these forests to become N saturated (a condition that can negatively affect forest productivity and surrounding aquatic ecosystems) or to emit N₂O, a powerful greenhouse gas whose production strongly depends on soil N transformation processes (Morse et al. 2015). The intriguing patterns reported here highlight a clear need for further studies on the mechanism(s) by which changes in winter climate affect soil conditions and N cycle processes during the growing season. We anticipate that these effects are likely to be complex and dynamic, given that the nature and extent of climate change and ecosystem response to this change are regulated by multiple factors.

ACKNOWLEDGMENTS

This research was supported by grants from the U.S. National Science Foundation programs in Ecosystem Studies, Long-Term Ecological Research and Long-Term Ecological Research in Environmental Biology and from the Andrew W. Mellon Foundation. J.D. was supported by a Fulbright fellowship of the Spanish Ministry of Education and by a FCT Research Fellowship of the Portuguese Ministry of Education and Science (SFRH/BDP/87966/2012).

LITERATURE CITED

- Aber, J. D., S. V. Ollinger, and C. T. Driscoll. 1997. Modeling nitrogen saturation in forest ecosystems in response to land use and atmospheric deposition. *Ecological Modelling* 101:61–78.
- Aber, J. D., C. L. Goodale, S. V. Ollinger, M. L. Smith, A. H. Magill, M. E. Martin, R. A. Hallett, and J. L. Stoddard. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? *BioScience* 53:375–389.
- Anderson, M., R. Gorley, and K. Clarke. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. Primer-E Ltd., Plymouth, UK.
- Bernal, S., L. O. Hedin, G. E. Likens, S. Gerber, and D. C. Buso. 2012. Complex response of the forest nitrogen cycle to climate change. *Proceedings of the National Academy of Sciences* 109:3406–3411.
- Bernhardt, E. S., et al. 2005. Can't see the forest for the stream? In-stream processing and terrestrial nitrogen exports. *BioScience* 55:219–230.
- Bohlen, P. J., P. M. Groffman, C. T. Driscoll, T. J. Fahey, and T. G. Siccama. 2001. Plant-soil-microbial interactions in a northern hardwood forest. *Ecology* 82:965–978.
- Brooks, P. D., P. Grogan, P. H. Templer, P. Groffman, M. G. Öquist, and J. Schimel. 2011. Carbon and nitrogen cycling in snow-covered environments. *Geography Compass* 5:682–699.
- Campbell, J. L., et al. 2007. Long-term trends from ecosystem research at the Hubbard Brook Experimental Forest. General Technical Report NRS-17, U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, Pennsylvania, USA.
- Campbell, J. L., et al. 2009. Consequences of climate change for biogeochemical cycling in forests of northeastern North America. *Canadian Journal of Forest Research* 39:264–284.
- Campbell, J. L., A. M. Socci, and P. H. Templer. 2014. Increased nitrogen leaching following soil freezing is due to decreased root uptake in a northern hardwood forest. *Global Change Biology* 20:2663–2673.
- Cleavitt, N. L., T. J. Fahey, P. M. Groffman, J. P. Hardy, K. S. Henry, and C. T. Driscoll. 2008. Effects of soil freezing on fine roots in a northern hardwood forest. *Canadian Journal of Forest Research* 38:82–91.
- Comerford, D., P. Schaberg, P. Templer, A. Socci, J. Campbell, and K. Wallin. 2013. Influence of experimental snow removal on root and canopy physiology of sugar maple trees in a northern hardwood forest. *Oecologia* 171:261–269.
- Durán, J., J. L. Morse, and P. M. Groffman. 2012. Comparison of in situ methods to measure N mineralization rates in forest soils. *Soil Biology and Biochemistry* 46:145–147.
- Durán, J., A. Rodríguez, J. L. Morse, and P. M. Groffman. 2013. Winter climate change effects on soil C and N cycles in urban grasslands. *Global Change Biology* 19:2826–2837.
- Durán, J., J. L. Morse, P. M. Groffman, J. L. Campbell, L. M. Christenson, C. T. Driscoll, T. J. Fahey, M. C. Fisk, M. J. Mitchell, and P. H. Templer. 2014. Winter climate change affects growing-season soil microbial biomass and activity in northern hardwood forests. *Global Change Biology* 20:3568–3577.
- Fitzhugh, R. D., C. T. Driscoll, P. M. Groffman, G. L. Tierney, T. J. Fahey, and J. P. Hardy. 2001. Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry* 56:215–238.
- Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. C. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892.
- Goodale, C. L., J. D. Aber, and P. M. Vitousek. 2003. An unexpected nitrate decline in New Hampshire streams. *Ecosystems* 6:75–86.
- Groffman, P. M., C. T. Driscoll, T. J. Fahey, J. P. Hardy, R. D. Fitzhugh, and G. L. Tierney. 2001a. Colder soils in a warmer world: a snow manipulation study in a northern hardwood forest ecosystem. *Biogeochemistry* 56:135–150.
- Groffman, P. M., C. T. Driscoll, T. J. Fahey, J. P. Hardy, R. D. Fitzhugh, and G. L. Tierney. 2001b. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry* 56:191–213.
- Groffman, P. M., M. C. Fisk, C. T. Driscoll, G. E. Likens, T. J. Fahey, C. Eagar, and L. H. Pardo. 2006. Calcium additions and microbial nitrogen cycle processes in a northern hardwood forest. *Ecosystems* 9:1289–1305.
- Groffman, P. M., J. P. Hardy, M. C. Fisk, J. T. Fahey, and C. T. Driscoll. 2009. Climate variation and soil car-

- bon and nitrogen cycling processes in a northern hardwood forest. *Ecosystems* 12:927–943.
- Hamburg, S. P., M. A. Vadeboncoeur, A. D. Richardson, and A. S. Bailey. 2013. Climate change at the ecosystem scale: a 50-year record in New Hampshire. *Climatic Change* 116:457–477.
- Hardy, J. P., P. M. Groffman, R. D. Fitzhugh, K. S. Henry, A. T. Welman, J. D. Demers, T. J. Fahey, C. T. Driscoll, G. L. Tierney, and S. Nolan. 2001. Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. *Biogeochemistry* 56:151–174.
- Hayhoe, K., et al. 2007. Past and future changes in climate and hydrological indicators in the US Northeast. *Climate Dynamics* 28:381–407.
- Henry, H. A. L. 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change* 87:421–434.
- Houlton, B. Z., C. T. Driscoll, T. J. Fahey, G. E. Likens, P. M. Groffman, E. S. Bernhardt, and D. C. Buso. 2003. Nitrogen dynamics in ice storm-damaged forest ecosystems: implications for nitrogen limitation theory. *Ecosystems* 6:431–443.
- Johnson, C. E., C. T. Driscoll, T. G. Siccama, and G. E. Likens. 2000. Element fluxes and landscape position in a northern hardwood forest watershed ecosystem. *Ecosystems* 3:159–184.
- LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379.
- Likens, G. E. 2013. *Biogeochemistry of a forested ecosystem*, Third edition. Springer-Verlag, New York, New York, USA.
- Matzner, E., and W. Borken. 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science* 59:274–284.
- McInnes, K. J., R. W. Weaver, and M. J. Savage. 1994. Soil water potential. Pages 53–58 in R. W. Weaver, editor. *Methods of soil analysis, part 2 – microbiological and biochemical properties*. Soil Science Society of America, Madison, Wisconsin, USA.
- Melillo, J. M. 1977. Mineralization of nitrogen in northern hardwood forest ecosystems, PhD dissertation. Yale University, New Haven, Connecticut, USA.
- Melillo, J. M., et al. 2011. Soil warming, carbon–nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences* 108:9508–9512.
- Morse, J. L., J. Durán, and P. M. Groffman. 2015. Denitrification and greenhouse gas fluxes in a northern hardwood forest: the importance of snowmelt and implications for ecosystem N budgets. *Ecosystems* 18:520–532.
- Nelson, D. W., and L. E. Sommers. 1996. Total carbon, organic carbon and organic matter. Pages 961–1010 in D. L. Sparks, editor. *Methods of soil analysis. Part 3. Chemical methods*. Soil Science Society of America, Madison, Wisconsin, USA.
- Paul, E. A., editor. 2007. *Soil microbiology, ecology, and biochemistry*, Third edition. Academic Press, Amsterdam.
- Pendall, E., L. Rustad, and J. Schimel. 2008. Towards a predictive understanding of belowground process responses to climate change: have we moved any closer? *Functional Ecology* 22:937–940.
- Porter, E. M., W. D. Bowman, C. M. Clark, J. E. Compton, L. H. Pardo, and J. L. Soong. 2013. Interactive effects of anthropogenic nitrogen enrichment and climate change on terrestrial and aquatic biodiversity. *Biogeochemistry* 114:93–120.
- Rasmussen, C., R. J. Southard, and W. R. Horwath. 2006. Mineral control of organic carbon mineralization in a range of temperate conifer forest soils. *Global Change Biology* 12:834–847.
- Robertson, G. P., D. Wedin, P. M. Groffman, J. M. Blair, E. A. Holland, K. A. Nadelhoffer, and D. Harris. 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification and carbon turnover. Pages 258–271 in G. P. Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins, editors. *Standard soil methods for long term ecological research*. Oxford University Press, New York, New York, USA.
- Schimel, J. P., and J. S. Clein. 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology and Biochemistry* 28:1061–1066.
- Tierney, G. L., T. J. Fahey, P. M. Groffman, J. P. Hardy, R. D. Fitzhugh, and C. T. Driscoll. 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry* 56:175–190.
- Venterea, R. T., G. M. Lovett, P. M. Groffman, and P. A. Schwarz. 2003. Landscape patterns of net nitrification in a northern hardwood-conifer forest. *Soil Science Society of America Journal* 67:527–539.
- Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115.
- Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7:737–750.
- Yanai, R. D., M. A. Vadeboncoeur, S. P. Hamburg, M. A. Arthur, C. B. Fuss, P. M. Groffman, T. G. Siccama, and C. T. Driscoll. 2013. From missing source to missing sink: long-term changes in the nitrogen budget of a northern hardwood forest. *Environmental Science & Technology* 47:11440–11448.