Regional patterns in foliar ^{15}N across a gradient of nitrogen deposition in the northeastern US

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Foliar δ^{15}N increased with increasing N deposition from Maine to NY, but decreased between 1987–1988 and 1999.

Abstract

Recent studies have demonstrated that natural abundance ^{15}N can be a useful tool for assessing nitrogen saturation, because as nitrification and nitrate loss increase, δ^{15}N of foliage and soil also increases. We measured foliar δ^{15}N at 11 high-elevation spruce-fir stands along an N deposition gradient in 1987–1988 and at seven paired northern hardwood and spruce-fir stands in 1999. In 1999, foliar δ^{15}N increased from −5.2 to −0.7‰ with increasing N deposition from Maine to NY. Foliar δ^{15}N decreased between 1987–1988 and 1999, while foliar %N increased and foliar C:N decreased at most sites. Foliar δ^{15}N was strongly correlated with N deposition, and was also positively correlated with net nitrification potential and negatively correlated with soil C:N ratio. Although the increase in foliar %N is consistent with a progression towards N saturation, other results of this study suggest that, in 1999, these stands were further from N saturation than in 1987–1988.
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1. Introduction

Anthropogenic inputs of nitrogen (N) to the atmosphere have increased N deposition significantly over the last century as a result of increased fossil fuel combustion and fertilizer use (Galloway et al., 2003). Elevated levels of N deposition have been associated with numerous detrimental ecological effects. These include soil and surface water acidification, plant nutrient imbalances which may ultimately result in declines in forest health and, over the long term, changes in species composition (Nihlgard, 1985; Aber et al., 1989; Schaberg et al., 2002). Trees at higher elevations are typically affected more than those at lower elevations, because of elevated N deposition and poor

site quality (Johnson et al., 1992; Lazarus et al., 2006). Different tree species may exhibit varying levels of susceptibility to elevated N inputs; often coniferous species, which have lower biological N demand and are better adapted to poorer quality/ low nutrient sites are impacted more by increases in N availability (Southern Forest Resource Assessment; SOFRA, 2002).

In the northeastern US, N deposition ranges from 4 to 13 kg N ha^{-1} year^{-1} in a gradient from east to west (Ollinger et al., 1993). This gradient may allow a space-for-time substitution—providing information about the likely impact of continued or increasing N inputs at sites with lower N deposition. McNulty et al. (1991) utilized this gradient almost twenty years ago to evaluate the impact of N deposition on red spruce (*Picea rubens* Sarg.) stands. They reported strong correlations between factors including foliar %N, forest floor %N, C:N, and nitrification with N deposition; they suggested that nutrient imbalances may be implicated in red spruce decline.

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Other regional assessments of N cycling across gradients in deposition, such as the NITREX study in Europe (Wright and van Breemen, 1995; Wright and Rasmussen, 1998), have also generated useful data for interpreting broad-scale patterns in ecosystem response to N deposition. For example, this study found forest floor N status and C:N to be an important determinant of ecosystem response to N inputs (Emmett et al., 1998; Gundersen et al., 1998).

In this study, we revisited some of the sites along the N deposition gradient in the northeastern US that had previously been evaluated by McNulty et al. (1991). Our gradient study may be useful in evaluating the time-course to N saturation for forested catchments receiving the moderate levels of N deposition that occur in the northeastern US (in contrast to higher levels of N deposition observed in Europe). Most evaluations of the N cycling and N status of an ecosystem rely on long-term monitoring of streamwater chemistry to develop input–output budgets of N for the ecosystem (Stoddard, 1994; Peterjohn et al., 1996; Aber et al., 1998). This approach provides much useful information about sites, including the seasonal and annual variability of N fluxes (e.g., Gilliam et al., 2001). However, the measurement intensity and long-term nature of such studies limits their use for large-scale regional assessments of N cycling or N saturation.

Stable N isotope ratios, in contrast, are a potentially valuable measure for regional assessments of N saturation. Stable isotope measurements of plant and soil have the advantages both of being feasible for investigating large areas and of providing, not simply instantaneous information, but rather, an integrated measure of the N cycling history of a site with a single sampling (Robinson, 2001). Isotopic fractionation occurs during enzymatic and other biological processes, discriminating against the heavier ^{15}N when chemical bonds are broken, such that the product generally has a lower ratio of $^{15}N/^{14}N$ than the remaining substrate (Mariotti et al., 1981; Robinson, 2001). During nitrification, the nitrate produced is depleted in ^{15}N relative to the ammonium substrate, so that in ecosystems with high nitrification and nitrate loss, the remaining N pools become enriched in ^{15}N (Nadelhoffer and Fry, 1994). These residual N pools include soil, vegetation and inorganic N pools (NH_4^+ and NO_3^-).

When used to record the effect of large disturbances in the N cycle, stable isotope measurements showed a clear relationship between elevated nitrification and nitrate loss induced by clear-cutting and increases in litter and organic soil horizon $\delta^{15}N$ (Pardo et al., 2002). Increases in foliar $\delta^{15}N$ were observed after N saturation was induced via N additions to a red spruce stand in Vermont (Pardo et al., 1998; McNulty et al., 2005). Näsholm et al. (1997) observed elevated foliar $\delta^{15}N$ in spruce stands in southern Sweden with elevated nitrate leaching. Another study (Pardo et al., 2001), also suggested that smaller disruptions of the N cycle (e.g., a period of elevated nitrification and nitrate loss, perhaps associated with N saturation) caused detectable increases in $\delta^{15}N$ values in organic soil horizons. These studies suggest that $\delta^{15}N$ could be used to compare sites with respect to N saturation. This approach was successfully applied to the NITREX study where measurements of plant and soil $\delta^{15}N$ increased along an N deposition gradient in Europe (Emmett et al., 1998).

Because stable isotopes provide direct insights into past N cycling by providing a record of the net effect of N transformations, a single sampling point can provide information that would require a long-term record of streamwater nitrate output and precipitation nitrate inputs. Furthermore, because plant $\delta^{15}N$ values are typically more closely linked to measures of soil N cycling than are foliar %N measurements, they may be a better early indicator of N saturation than foliar %N alone (Pardo et al., 2006). Combining measurements of foliar $\delta^{15}N$ and %N may provide the best information about N cycling dynamics at a given site, particularly for regional surveys.

In this study, we measured foliar $\delta^{15}N$ and N concentrations at hardwood and conifer sites across an N deposition gradient in the northeastern US to test the hypotheses that:

(i) Foliar $\delta^{15}N$ will increase with N deposition, nitrification and nitrification:mineralization
(ii) Foliar $\delta^{15}N$ and %N will decrease with forest floor C:N
(iii) Foliar $\delta^{15}N$ and %N will increase over time

2. Materials and methods

2.1. Site description/plot location

The study was conducted at 11 sites in the northeastern US across a gradient of increasing N deposition (Fig. 1). In 1987–1988, McNulty et al. (1991) established 161 10-m radius red spruce plots across this gradient at 11 sites at elevations ranging from 100 to 1460 m (Table 1). During the summer of 1999, we resampled 42 of the original red spruce-dominated plots at 6 sites (Fig. 1, Table 1). The original conifer plots at a seventh site, Mt. Moosilauke, were not located in 1999, so 12 new conifer plots were established at the site. Consequently, Mt. Moosilauke is included in the comparisons involving samples collected only in 1999, but not for any comparisons between 1987–1988 and 1999 surveys. In 1999, we also established 55 hardwood plots across the 7 sites within 100 m of the conifer plots. The dominant hardwood species included paper birch (*Betula papyrifera* Marsh.) at all sites; co-dominant species were red maple (*Acer rubens* Sarg.) at the Howland site, sugar maple (*Acer saccharum*) at Mount Washington, and yellow birch (*Betula alleghaniensis* Britt.) at the rest of the sites. Because site and plot selections were based primarily on N deposition input, species composition, elevation, slope, and aspect varied across sites and plots. Estimates of N deposition (wet + dry inorganic N) for the sites were standardized by use of a statistical model of atmospheric deposition, ClimCalc, described by Ollinger et al. (1993), which estimates wet and dry deposition as a function of latitude, longitude, and elevation. This model was modified slightly with updated dry deposition coefficients from Lovett and Rueth (1999).

2.2. Foliage analysis

Foliar samples were collected during the summer of 1999 from the seven sites indicated in Table 1 (Fig. 1). The method of plot selection is described in detail in Boggs et al. (2007). Green foliage was collected using a pole pruner (for shorter trees) or by shooting small branches from the canopy with a shotgun. Samples were collected from up to three randomly selected trees of up to three dominant or co-dominant species in each plot. Conifer foliage samples were split in half and for half of each sub-sample only current-year needles were kept; for the other half of the sample, all age classes of needles were kept. Leaves were stored and air dried in paper bags; they were then oven dried at 65 °C for 24 h. Dried foliar samples were pulverized in a shatterbox (SPEX Chemical and Sample Prep, model 8500, Metuchen, NJ), oven dried at 65 °C and loaded into tin capsules for isotope analysis. Isotopic analyses were performed using a Dumas combustion system in continuous flow mode (ANCA-SL Elemental Analyzer) followed by a PDZ Europa Scientific 20/20 mass spectrometer
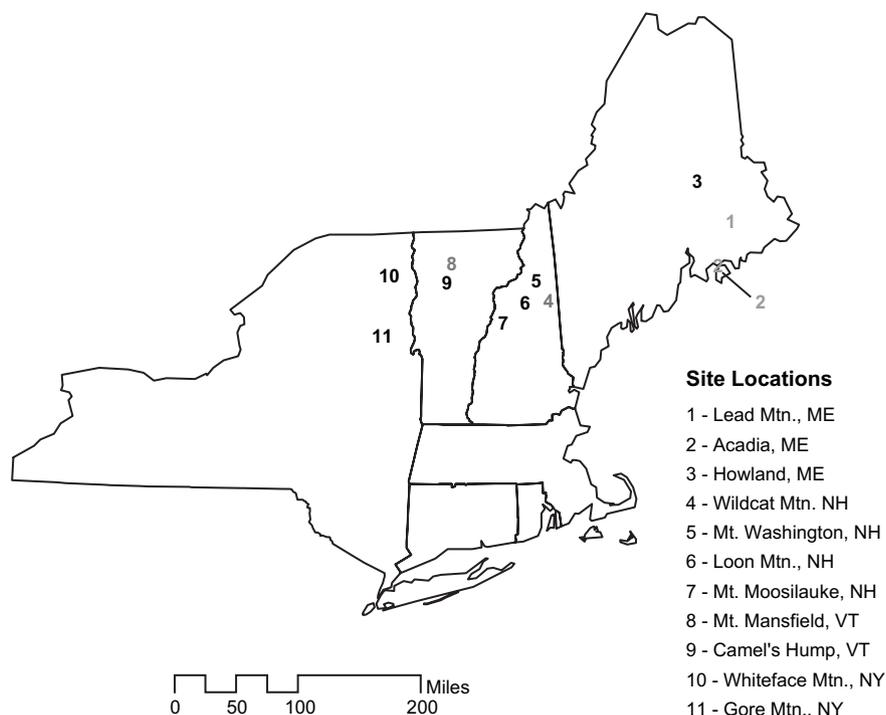
Site Locations

1 - Lead Mtn., ME
2 - Acadia, ME
3 - Howland, ME
4 - Wildcat Mtn. NH
5 - Mt. Washington, NH
6 - Loon Mtn., NH
7 - Mt. Moosilauke, NH
8 - Mt. Mansfield, VT
9 - Camel's Hump, VT
10 - Whiteface Mtn., NY
11 - Gore Mtn., NY

Fig. 1. Site locations. Sites sampled in 1987—1988 and 1999 are shown in boldface. Sites sampled only in 1987—1988 are shown in gray type.

(Center for Stable Isotope Biogeochemistry at the University of California, Berkeley). The standard deviation of the 10% of samples analyzed in triplicate was 0.13‰; the precision of the analysis for National Institute of Standards and Technology apple leaf standard, NIST 1515 (mean $\delta^{15}N = 0.71$‰) used as an internal standard was ±0.14‰ (SD).

Isotope data are reported as $\delta^{15}N$ values, which represent the per mil (‰) difference between the isotopic composition of the sample and that of atmospheric dinitrogen:

$$\delta^{15}N = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000 \qquad (1)$$

Table 1
Site descriptions

Location	Long.	Lat.	N deposition kg ha^{-1} year^{-1}	Elevation range (m)	Conifer plots 1987—1988	Conifer plots 1999	Hardwood plots 1999	Species sampled
Howland, ME	−68°40′	45°20′	5.0	80—100	5	10	9	*Abies balsamea, Acer rubrum, Betula alleghaniensis, Betula papyrifera, Picea rubens, Pinus strobus, Thuja occidentalis, Tsuga canadensis*
Lead Mtn., ME	−68°10′	44°50′	5.2	90—240	7			*Picea rubens*
Acadia, ME	−68°22′	44°20′	5.6	10—60	8			*Picea rubens*
Mt. Washington, NH	−71°16′	44°17′	8.4	630—1460	40	14	11	*Abies balsamea, Acer rubrum, Acer saccharum, Betula alleghaniensis, Betula papyrifera, Betula populifolia, Fagus grandifolia, Picea rubens, Sorbus americana*
Loon Mtn., NH	−71°37′	44°02′	8.9	690—910	20	7	7	*Abies balsamea, Betula alleghaniensis, Betula papyrifera, Picea rubens, Sorbus americana, Tsuga canadensis*
Camel's Hump, VT	−72°53′	44°20′	9.3	590—1040	9	8	7	*Abies balsamea, Acer rubrum, Betula alleghaniensis, Betula lenta, Betula papyrifera, Picea rubens, Sorbus americana*
Wildcat Mtn. NH	−71°13′	44°16′	9.4	930—1210	20			*Picea rubens*
Mt. Moosilauke, VT	−71°49′	44°01′	10.1	810—1400	11	12	5	*Abies balsamea, Betula papyrifera, Picea rubens*
Mt. Mansfield, VT	−72°48′	44°31′	10.2	900—1210	4			*Abies balsamea, Picea rubens*
Whiteface Mtn., NY	−73°54′	44°24′	10.7	840—1190	20	9	9	*Abies balsamea, Betula alleghaniensis, Betula papyrifera, Picea rubens, Sorbus americana*
Gore Mtn., NY	−74°02′	43°41′	11.0	880—1010	17	7	7	*Abies balsamea, Betula alleghaniensis, Betula papyrifera, Picea rubens*

where R_{sample} represents the isotope ratio (^{15}N/^{14}N), and $R_{standard}$ is ^{15}N/^{14}N for atmospheric N$_2$, or 0.0036765.

2.3. Forest floor analysis

We collected 20 Oe + Oa horizon sub-samples to a depth of up to 15 cm from randomly selected locations in each of the 10-m radius plots, brushing away the Oi horizon prior to sampling and removing any mineral soil from the bottom of the sample. At each plot, the 20 sub-samples were composited into 5 samples, stored in 25-μm thick polyethylene bags and kept at 5 °C until time of analysis. The five composite samples were sieved using a 6-mm screen. Net potential nitrification and N mineralization were calculated based on a ratio of wet weight to dry weight that was determined by weighing 10.0 g of the sieved sample, then oven drying the sample at 65 °C for 48 h prior to re-weighing. Another 10.0-g replicate was placed in 150 ml of 1 M KCl, shaken for 30 min, and centrifuged to collect the extract. The extract of these initial samples was frozen at 0 °C until analysis for ammonium and nitrate concentrations. The remaining forest floor sample material from each bag was incubated for 28 days at 22 °C, and then extracted as previously described. We used a TRAACS 800 auto-analyzer to analyze the initial and incubated extract for NH$_4^+$ (Technicon Industrial Systems, 1978) and NO$_3^-$ (Technicon Industrial Systems, 1977). Monthly potential net nitrification was calculated as the incubated sample nitrate minus the initial sample nitrate. Monthly net potential N mineralization was calculated as the incubated sample (NH$_4^+$ + NO$_3^-$) minus the initial sample (NH$_4^+$ + NO$_3^-$).

Percent forest floor C and N were determined by combusting each forest floor sample in pure oxygen environment using a Perkins-Elmer CHN 2400 analyzer to collect and measure the resulting combustion gases.

2.4. Statistical analysis

We examined differences in δ^{15}N and %N between current-year and all-age class needles using a paired two-tailed *t*-test ($\alpha = 0.05$). We calculated plot-wise means by species and used these values for all of our general statistical analyses to minimize pseudo-replication. This aggregation lumped the 1163 foliar samples into 562 species-level means for 249 plots, with 1−5 species per plot. For foliar δ^{15}N and %N, the effects of site, year, and species were tested using Proc GLM and compared using the Student−Newman−Keuls statistic ($\alpha = 0.05$) for control of type I error from multiple comparisons. The assumption of normality was met. Homogeneity of variance was tested by plotting predictors against residuals. A square root transformation was performed on the %N data to satisfy the assumption of homogeneity of variance. We examined the potential relationships between δ^{15}N, %N and C:N in foliage and N deposition, forest floor C:N, nitrification, mineralization and nitrification: mineralization using Spearman's rank correlation analysis ($\alpha = 0.05$). We used non-parametric methods, because they are appropriate when data exhibit non-linear relationships. All statistical analyses were performed using SAS® 2004 (Version 9.3).

3. Results

3.1. Differences in foliar δ^{15}N and N concentration by needle age class

For samples collected in 1999, we compared the chemistry of current-year needles to that of needles from all age classes combined to evaluate whether the more biologically active current-year needles were a more sensitive indicator of site N cycling than all age classes of needles combined. Current-year needle δ^{15}N was indistinguishable from δ^{15}N of needles from all age classes combined except for red spruce at Howland where it was significantly lower (0.6‰; $p = 0.002$) and Whiteface where it was significantly higher (0.4‰; $p = 0.0003$). At all sites, foliar N concentration was higher for current-year needles than for needles from all age classes combined. The difference

at Howland, for red spruce was 0.11%, while the difference at Mt Washington was 0.4%.

3.2. Patterns in foliar δ^{15}N and N concentration with N deposition and forest floor N cycling measures

Foliar δ^{15}N was correlated with N deposition ($r = 0.56$, $p < 0.0001$ for both current and all-aged needles; Fig. 2a; Table 2) for conifer plots in 1999. Similarly, for all-aged needles, the correlation between foliar N concentration and N deposition was significant ($r = 0.55$, $p < 0.0001$; Fig. 2b; Table 2); for current-year needles, the correlation ($r = 0.36$, $p < 0.0001$) was also significant. Foliar N concentration for the conifer plots in 1999 was significantly correlated with forest floor C:N ($r = -0.62$, $p < 0.0001$ for all-aged needles; $r = -0.54$, $p < 0.0001$ for current-year needles; Fig. 3, Table 2).

In 1987−1988, conifer foliar N concentration was significantly correlated with forest floor C:N ($r = -0.58$, $p < 0.0001$; Table 2) and other measures of forest floor N cycling, except N mineralization for which there was no significant correlation (Table 2). Conifer foliar N concentration was also
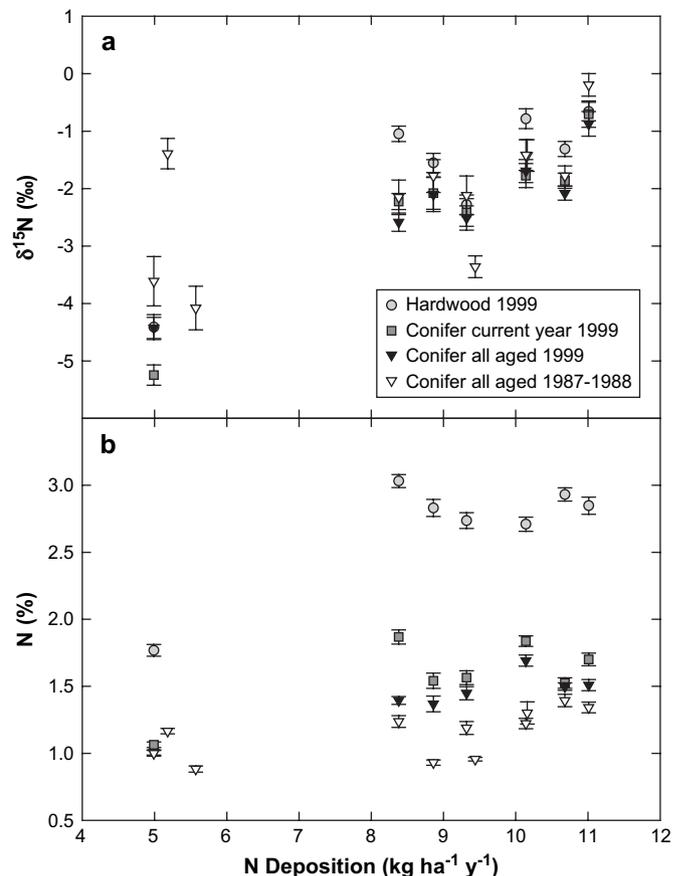
Fig. 2. Foliar δ^{15}N and % N vs. N deposition for hardwoods and conifers. Foliar δ^{15}N (a) and N concentration (b) are shown across the N deposition gradient in the northeastern US for hardwoods and conifers. In 1987−1988 only all age classes of needles were collected; in 1999, paired samples of current year and all aged needles were collected from each tree. Means by site are shown with standard error bars.

Table 2
Statistical summary of correlation analysis for foliar δ^{15}N and % N with N deposition and forest floor N cycling measures

| | Conifer | | | | | | | | | | | | Hardwood 1999 | | | | | |
| | All-aged 1987–1988 | | | | | | All-aged 1999[a] | | | | | | Current-year 1999[a] | | | | | | | | | | | |
	δ^{15}N			%N			δ^{15}N			%N			δ^{15}N			%N			δ^{15}N			%N		
N deposition kg ha⁻¹ year⁻¹	**0.43**	<.0001	174	**0.46**	<.0001	174	**0.56**	<.0001	137	**0.55**	<.0001	137	**0.56**	<.0001	137	**0.36**	<.0001	120	**0.40**	<.0001	120	**0.29**	0.003	105
Forest Floor C:N	**−0.51**	<.0001	60	**−0.58**	<.0001	60	**−0.52**	<.0001	135	**−0.62**	<.0001	135	**−0.46**	<.0001	135	**−0.54**	<.0001	118	**−0.49**	<.0001	118	**−0.38**	<.0001	103
Forest floor nitrification mg kg⁻¹ day⁻¹	**0.45**	<.0001	174	**0.51**	<.0001	174	**0.33**	0.0002	137	**0.32**	0.0002	137	**0.28**	0.0002	137	**0.31**	0.0007	120	**0.53**	<.0001	120	**0.49**	<.0001	105
Forest floor mineralization mg kg⁻¹ day⁻¹	0.11	0.2	174	0.08	0.3	174	**0.21**	0.01	137	**0.26**	0.003	137	**0.23**	0.01	137	**0.33**	0.0002	120	0.17	0.08	120	0.17	0.08	105
Forest floor nitrification: min mg mg kg⁻¹ day⁻¹	**0.50**	<.0001	174	**0.55**	<.0001	174	**0.26**	0.0002	137	**0.27**	0.0002	137	**0.20**	0.03	137	**0.33**	0.0002	120	**0.46**	<.0001	120	**0.34**	0.0003	105

The table includes Spearman rank correlation coefficients, p value, and number of observations; significant correlations are in bold type (α=0.05).

[a] Only six sites were included in these analyses, because conifer plots at Mt. Moosilauke were not re-sampled.

significantly correlated with N deposition (Table 2). In 1999, foliar C:N ranged from 52 to 28 with increasing N deposition.

Hardwood foliar δ^{15}N and N concentration were significantly correlated with forest floor nitrification ($r = 0.53$ and $r = 0.49$, $p < 0.0001$; Figs. 2 and 3, Table 2). Other significant correlations included foliar δ^{15}N and N concentration with N deposition ($r = 0.40$ and $r = 0.29$, $p < 0.0001$; Fig. 2, Table 2). Hardwood foliar C:N ranged from 28 to 16 along the N deposition gradient.

3.3. Patterns in foliar δ^{15}N and N concentration over time

There were significant differences in conifer foliar δ^{15}N and N concentration over time, by species and by site (Table 3). Conifer foliar δ^{15}N decreased over time and foliar N concentration increased ($p < 0.0001$; Table 3). Differences in foliar δ^{15}N and N concentration among sites are shown in Table 3. Howland had significantly lower foliar δ^{15}N and N concentration than the other sites, Gore had higher foliar δ^{15}N than the other sites, and Loon had a foliar N concentration intermediate to Howland and the four other sites which were equivalent (Table 3).

3.4. Species patterns in foliar δ^{15}N and N concentration

In general, we observed that foliar δ^{15}N and N concentration tended to be higher for hardwoods than conifers. Balsam fir foliar δ^{15}N and N concentration were higher than red spruce ($p < 0.0001$; Table 3). Correlations between foliar δ^{15}N and N concentration and N deposition were stronger for red spruce than balsam fir (Table 4). For hardwoods, the correlation between foliar δ^{15}N and N deposition (Table 4) was significant only for paper birch ($r = 0.30$, $p = 0.03$) and red maple ($r = 0.75$, $p = 0.005$).

4. Discussion

These results suggest that comparisons of needle δ^{15}N are robust across different age classes and, therefore, that foliar δ^{15}N may be used to compare needles collected using different age-class composites. As expected, foliar N concentration was generally higher in the more biologically active current-year needles (Reich et al., 1992). The differences between current-year and all-age class needle δ^{15}N that we observed in red spruce at either end of the deposition gradient may provide some insight into N cycling at theses sites. Factors that regulate foliar δ^{15}N include δ^{15}N of the source of plant N and the rate of N transformations that cause isotopic fractionation. The pattern observed at Howland (lighter δ^{15}N in current-year needles coupled with a small increase in foliar N concentration over time; Table 3) may be consistent with greater N retranslocation relative to uptake compared to other sites. (If there is any fractionation on retranslocation, the retranslocated N should have a lower δ^{15}N than N that had not been retranslocated). This pattern should occur at a site with a tighter N economy (necessitated by lower N availability).
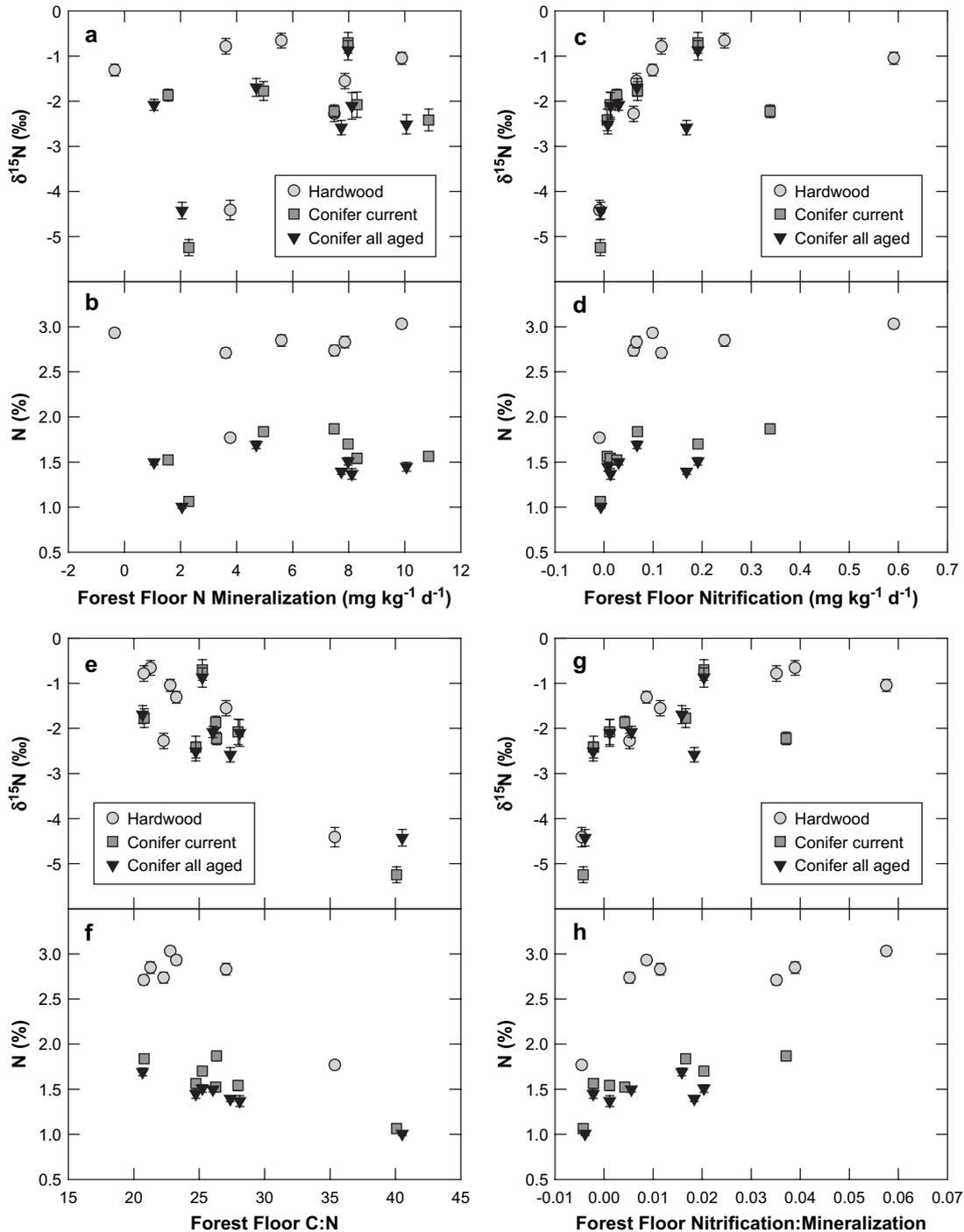
Fig. 3. Foliar $\delta^{15}N$ and %N vs. forest floor mineralization, nitrification, C:N, and nitrification:mineralization for hardwoods and conifers. Foliar $\delta^{15}N$ and %N are shown versus forest floor N mineralization potential (a, b), net nitrification potential (c, d), forest floor C:N (e, f), and nitrification:mineralization (g, h) for hardwoods and conifers. Paired samples of current year and all aged needles were collected from each tree. Means by site for 1999 are shown with standard error bars.

We expected foliar $\delta^{15}N$ to be correlated with N deposition largely because of the cascade of effects of increasing N deposition that ultimately leads to increased nitrification (Aber et al., 1989). Therefore, we expected that forest floor C:N and nitrification would be most closely linked to foliar $\delta^{15}N$, because it is these local drivers of N cycling that ultimately lead to increases in $\delta^{15}N$ of inorganic N taken up by plants. For conifers, this proved true in 1987–1988, but for the more recent sampling, correlations between foliar $\delta^{15}N$ and

N deposition were stronger than between foliar $\delta^{15}N$ and nitrification. During the early period, there was a strong gradient in net nitrification potential; however, during the more recent sampling, we did not observe such a strong pattern (Boggs et al., 2007). It is generally assumed that increases in foliar $\delta^{15}N$ with N deposition such as we observed during both sampling periods would be caused by increases in nitrification along the N deposition gradient. Another possible mechanism would be the $\delta^{15}N$ of the deposition itself. In some cases

Table 3
ANOVA and post hoc comparison of foliar $\delta^{15}N$ and %N by site, sample year, and species

ANOVA	$\delta^{15}N$		%N	
Site	**22.00**	<0.0001	**17.17**	<0.0001
Year	**14.97**	0.0001	**15.85**	<0.0001
Species	**15.77**	<0.0001	**109.00**	<0.0001
Post hoc comparisons				
Site means				
Camels' Hump	−2.27 b		1.20 a	
Gore Mt.	−0.47 a		1.20 a	
Howland	−4.90 c		1.00 c	
Loon Mt.	−1.89 b		1.11 b	
Mt. Washington	−2.40 b		1.22 a	
Whiteface Mt.	−1.86 b		1.21 a	
Year means				
1988	−1.49 a		1.13 a	
1999	−2.60 b		1.18 b	
Species means				
Balsam fir	−1.67 a		1.29 a	
Red Spruce	−2.63 b		1.07 b	

ANOVA results include the *F* statistic and *p* value; significant correlations are in bold type ($\alpha = 0.05$). None of the interactions was significant. Within each post hoc comparison group, means with the same letter are not significantly different.

(Bauer et al., 2000; Pardo et al., 2006), the ^{15}N signature of N deposition may have a significant impact on the foliar $\delta^{15}N$. These cases include sites either where the deposition has a very distinct isotopic signature and very high magnitude (such as sites severely impacted by NH_3 volatilization from animal husbandry) or where direct canopy uptake represents the dominant part of foliar nutrition. In the northeastern US, studies have shown that most N deposition is cycled biologically within the ecosystem (Burns and Kendall, 2002; Pardo et al., 2004). Microbial cycling of N will alter the ^{15}N signature of N from deposition. This, combined with the fact that N mineralization tends to be greater than N deposition, suggests that the isotopic signature of deposition, which has been reported to increase along this deposition gradient for nitrate (Elliott, 2005), is unlikely to influence foliar $\delta^{15}N$ unless direct canopy uptake is the dominant source of N for foliage. Therefore, it is most likely that the patterns in foliar $\delta^{15}N$ across the deposition gradient were still driven by increased nitrification along the deposition gradient—although the range of nitrification rates observed was considerably smaller in 1999 than in 1987−1988.

Species differences in foliar $\delta^{15}N$ values have been reported in the northeastern US (Nadelhoffer et al., 1999; Templer, 2001; Pardo et al., 2006; Pardo et al., 2007). These differences in foliar $\delta^{15}N$, such as those we observed in this study, may be caused by a variety of factors including local nitrification rate, NH_4^+ versus NO_3^- uptake preference, rooting depth, phenology, and mycorrhizal association. The fact that we see stronger correlations for red spruce and red maple foliar $\delta^{15}N$ and N concentration with N deposition suggests either that these species are, in fact, responding directly or indirectly to N deposition or that other controlling factors vary along the same gradient. It is not possible for us to determine, based on the

Table 4
Statistical summary of correlation analysis for foliar $\delta^{15}N$ and % N with N deposition analyzed by species

N deposition $kg\ ha^{-1}\ year^{-1}$ vs.	1987–88 all-aged $\delta^{15}N$ (r, p, n)	%N (r, p, n)	1999 all-aged $\delta^{15}N$ (r, p, n)	%N (r, p, n)	1999 current year $\delta^{15}N$ (r, p, n)	%N (r, p, n)
Balsam fir	0.20, 0.2, 55	**0.39**, 0.003, 55	**0.37**, 0.003, 62	**0.27**, 0.04, 62	**0.36**, 0.006, 58	−0.054, 0.7, 58
Red spruce	**0.41**, <0.0001, 119	**0.40**, <0.0001, 119	**0.62**, <0.0001, 63	**0.63**, <0.0001, 63	**0.71**, <0.0001, 63	**0.63**, <0.0001, 58

N deposition $kg\ ha^{-1}\ year^{-1}$ vs.	1999 $\delta^{15}N$ (r, p, n)	%N (r, p, n)
Mountain ash	−0.19, 0.5, 12	**−0.69**, 0.01, 12
Paper birch	**0.30**, 0.03, 56	0.083, 0.5, 56
Red maple	**0.75**, 0.005, 12	**0.752**, 0.005, 12
Yellow birch	0.31, 0.2, 22	0.14, 0.5, 22

The table includes Spearman rank correlation coefficients, *p* value, and number of observations; significant correlations are in bold type ($\alpha=0.05$).

results from this study, which factor controls these foliar ^{15}N patterns.

Further, several unexpected temporal patterns complicate our interpretation. We expected foliar δ^{15}N and N concentration to increase over time due to progression toward N saturation caused by continuing N deposition at these sites. The decreases in foliar δ^{15}N coupled with increases in N concentration are puzzling. The decrease in foliar δ^{15}N over time, however, is consistent with the lower nitrification rates measured in the more recent sampling. The pattern of decreasing foliar δ^{15}N, forest floor %N, and nitrification (Boggs et al., 2007) suggest that the sites along this N deposition gradient are not further along the path of N saturation than they were in 1988. In fact, the lower nitrification rates may suggest that these sites are less impacted (their N cycling is less disturbed) than they were in 1987–1988. Goodale et al. (2003) observed a decrease in streamwater nitrate concentration in streams in New Hampshire from 1975 to 1995. During this period, N deposition did not change. The exact cause for the streamwater nitrate decrease was not identified; however, Goodale et al. suggest that climatic factors may have contributed to the regional decrease in streamwater nitrate concentrations over time (2003) and that these decreases may be linked to increases in DOC (2005). Such factors may have also indirectly influenced foliar δ^{15}N.

An alternative explanation for the increase in foliar N concentration coupled with the decrease in foliar δ^{15}N may be that response of some parameters to elevated N deposition is cyclic and that these parameters may respond in cycles of different length. Previous studies have reported a cyclic response of streamwater N loss to disturbance. Periods of high N loss are often followed by periods of reduced N loss, because the post-disturbance pulse of N loss removed the most labile N from the system. Following the initial N loss, the system is less saturated with N and may go into a period of N retention. N losses may then return to pre-disturbance levels after the period of low losses. This pattern is seen both after major disturbances such as clear cutting (HBEF, Pardo et al., 1996) and after smaller disturbances, such as soil freezing, which led to short-term elevated streamwater nitrate losses followed by a period of very low losses across the northeastern US (Mitchell et al., 1996). The magnitude of the N pool in the soil and forest floor is such that very small fluctuations in the total soil N pool could account for large fluxes at the ecosystem scale relative to deposition inputs (Huntington et al., 1988). For example, the change in streamwater nitrate export reported by Goodale et al. (2003) could be accounted for by an accumulation of <0.1% of the total soil N pool per year. While this does not suggest that immobilization of N in soil is the cause of the reduced streamwater nitrate, it is certainly possible. It is, however, not possible to measure such a change directly by measuring soil or forest floor N concentration. The soil potentially represents a very large sink for immobilizing N.

The elevated foliar N concentrations, taken in combination with lower nitrification rates may suggest that plants were out-competing microbes for available N during 1999. If this

were the case, it could be the result of unfavorable conditions for N cycling microbes, or of an unexplained robustness of trees—i.e., an increase in N sequestration in above-ground biomass. Some studies in the region (Siccama, unpublished data; http://www.hubbardbrook.org.), however, suggest that net N uptake has been negligible during this period for second growth forests. Another study suggests that conditions may, indeed, be increasingly unfavorable for nitrifying bacteria as N availability increases (Wallenstein et al., 2006). Wallenstein et al. (2006) report that availability of labile C limits nitrification at N-addition studies in the northeast. One of the sites included in their study was Mt Ascutney in southern Vermont where N saturation was induced at a poor spruce-fir site with annual N additions (beginning in 1988) of only 15 kg N ha^{-1} year^{-1} (McNulty and Aber, 1993; McNulty et al., 1996, 2005). At Mt Ascutney, recently, although live basal area has decreased more than 40% on the ammonium addition plots (15 and 31 kg N ha^{-1} year^{-1}), nitrification decreased while foliar N increased (McNulty et al., 2005). The data from Mt Ascutney follow the same pattern as we observed in this study, suggesting that, certain response parameters (e.g., nitrification) may be temporarily suppressed even as the site continues toward N saturation. While Mt Ascutney is very sensitive to atmospheric deposition, due, in part to its shallow soils, it is an instructive example of how precarious the balance in the N cycle can be, and how easily disrupted are the tight N cycling and low N losses associated with a site that is not at N saturation. Nonetheless, it is difficult to assess whether the pattern we observed at spruce-fir sites across the region suggests a low point in cycling of soil N (nitrification) as they continue on toward N saturation or whether the sites have simply moved away from N saturation. We did not see evidence of widespread health declines or mortality, which indicates that these sites are not near the levels of decline reported at Mt. Ascutney. Given the sometimes rapid decline of forest ecosystems in response to air pollution or experimentally elevated inputs of atmospheric pollutants (McNulty and Aber, 1993; Schaberg et al., 2002), it is not clear what path these sites may follow. Because we did not measure hardwood stands in the earlier sampling, it is not possible to evaluate whether their low nitrification rates indicate an earlier phase in the N saturation continuum.

A final explanation of the increase in foliar N concentration over time may reflect patterns in red spruce physiology and response to stress across this region. Lazarus et al. (2006) reported that conifers in the western part of our study region were more stressed than trees in the eastern part of the region. For red spruce, that stress is associated with reduced freezing tolerance and increased winter injury. One mechanism that conifers use when they are stressed is to drop older needles (Schulze, 1989; Beyschlag et al., 1994). This means that the fraction that current year needles represent increases, hence the %N of the composite of all age class needles would also increase.

5. Conclusions

It is difficult to interpret the results of this study because some of the data suggest that the sites are progressing toward

N saturation (increases in foliar $\delta^{15}N$ and N concentration across the deposition gradient and the increase in foliar %N over time for conifers). Other patterns (the decline in nitrification, forest floor %N, and foliar $\delta^{15}N$ over time) do not suggest direct movement of these sites along the continuum of N saturation. Similarly, at many sites across the region, stream water nitrate concentration has decreased to very low levels, which is consistent with movement away from the condition of N saturation (Goodale et al., 2003). There are several possible interpretations of these results. They may suggest that, for low to moderate levels of deposition, the rate of progression towards N saturation is highly variable and may be more clearly observable only over longer time periods or larger N deposition gradients. Or they may suggest that a systematic decrease in nitrification (as observed at Mt. Ascutney: McNulty et al., 2005) and streamwater N loss occurs on the path toward N saturation. Finally, these results may suggest that these sites are, at this time, moving away from the condition of N saturation.

Future research is necessary to evaluate these scenarios. Such research could include determining whether N is being immobilized in the ecosystem and if so, where. Future work could include evaluating the factors that control N availability in these ecosystems (labile C limitation, climate, immobilization) and how susceptible they are to disruption of the N cycle.

Acknowledgments

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