



## Red spruce ecosystem level changes following 14 years of chronic N fertilization

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### Abstract

In the early 1980s, nitrogen (N) deposition was first postulated as a cause of N saturation and spruce mortality across the northeastern US. In 1988, a series of high elevation spruce-fir forest N addition plots were established on Mt. Ascutney (southeastern) Vermont to test this hypothesis. The paired plots each received, in addition to ambient N deposition, 15.7 kg N ha<sup>-1</sup> year<sup>-1</sup> (low N addition), 31.4 kg N ha<sup>-1</sup> year<sup>-1</sup> (high N addition) or no N addition (control) from 1988 to 2002. Over the years, potential and annual in situ forest floor net N mineralization and net nitrification, foliar and forest floor elemental concentrations, and basal area growth by species were measured on each plot. Live basal area decreased by 18% on the low N addition plots, and by 40% on the high N addition plots between 1988 and 2002, while the control plots had a 9% increase in basal area over the same time period. Initially, none of the plots had measurable rates of in situ nitrification, but by 2002, 21% of the mineralized N was being annually nitrified on the high N addition plots, compared to no net nitrification on the control plots. We also observed a significant increase in forest floor N concentration on the high N plots from 1988 to 2000. Reductions in live basal area, and increased net nitrification suggest that we induced late stage N saturation on the high N addition plots. The low N addition plots exhibited symptoms of mid-stage N saturation, with a smaller reduction in live basal area and net N mineralization, and a smaller increase in net nitrification compared to the high N addition plot values. Other correlations between forest floor and vegetation composition and function, and N saturation will be discussed in the paper.

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### 1. Introduction

During the 1980s, high rates of spruce decline were observed across New England (McNulty et al., 1991) and the southern Appalachians (Peart et al., 1992). The interactions among pollutants made it difficult to

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identify the cause(s) of the decline. However, many papers have shown a direct link between experimentally applied nitrogen (N) and N saturation, and forest decline. N saturation (defined as N availability in excess of biological demand) has been cited as a contributor to forest mortality (Aber et al., 1989; Magill et al., 2000, 2004). High levels of forest floor N, relative to biological demand, can lead to foliar nutrient imbalance (Zoetl and Huettl, 1986; Cronan and Grigal, 1995), reduced red spruce cold tolerance (Sheppard, 1994; Schaberg et al., 2002), and increased freeze injury to foliage that in turn reduce carbohydrate reserves and increase the potential for secondary stressors that can cause tree mortality (DeHayes, 1992; Schaberg et al., 1997; Perkins et al., 2000). Other environmental stressors, such as drought (Johnson and Siccama, 1983; Driscoll et al., 2003) and aluminum toxicity in roots (Shortle and Smith, 1988), or nutrient leaching from the forest floor (Berg, 1986; Johnson et al., 1994; Adams, 1999) could act synergistically with N saturation to increase the potential for tree mortality on already pollutant-stressed forests.

The 1990 amendments to the United States (US) Clean Air Act (CAA) mandated a 50% reduction in sulfate deposition, and a 10% reduction in N deposition. From 1990 to 1999, sulfur dioxide decreased significantly across the eastern US as a result of technological improvements associated with the CAA, but there was a slight increase in nitrogen emissions during this same period (US Environmental Protection Agency, 2002).

In 1988, we established a series of N addition plots on Mt. Ascutney, Vermont to examine the influence of N inputs on *Picea rubens* (red spruce) and *Abies balsamea* (balsam fir) forest N cycling, tree mortality and forest growth (McNulty and Aber, 1993). Our objective was to observe how varying rates of N inputs altered N-cycling and forest health over the course of the experiment. We initially hypothesized that chronic, low level (i.e., less than  $40 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) amounts of added N could induce early stages of N saturation on the plots after several years of treatment. Previous publications noted changes in leaf and litter chemistry, forest growth and N mineralization rates on the Mt. Ascutney treatment plots (Aber et al., 1995; McNulty et al., 1996). Perkins et al. (2000) and Schaberg et al. (1997, 2002) noted that red spruce on the N addition plots on Mt. Ascutney had reduced frost hardiness and

cold tolerance, and increased leaf respiration rates. The N additions have also changed the forest floor microbial populations on Mt. Ascutney (Wallenstein, 2004). This paper provides an update of the continuing ecosystem changes on the control and two  $\text{NH}_4\text{Cl-N}$  addition treatments, and projects how plots may continue to change in response to additional N fertilization over the coming years.

## 2. Materials and methods

### 2.1. Study plots

During June 1988, six  $15 \text{ m} \times 15 \text{ m}$  plots were established at an elevation of 762 m above sea level, on Mt. Ascutney, Vermont ( $43^\circ 26' \text{N}$ ,  $72^\circ 27' \text{W}$ ), where red spruce (*P. rubens* Sarg.) grew in large patches ( $>1 \text{ ha}$ ) at elevations above 725 m. Red spruce comprised  $>80\%$  of the total basal area in all plots; the remainder of the other tree species were divided between balsam fir (*A. balsamea*), red maple (*Acer rubrum*), mountain maple (*Acer spicatum*) and birch (*Betula* spp.) (McNulty and Aber, 1993). Study plots were randomly located within these red spruce patches. Before the N additions were applied, we used a *t*-test ( $p < 0.05$ ) to test for significant differences between plots. No statistically significant differences in stand structure, stand species composition, forest floor net N mineralization and net nitrification, and foliar chemistry existed between the plots (McNulty, 1991; McNulty and Aber, 1993).

All of the plots received approximately 1000 mm of precipitation per year in the form of 70% sleet or snow, and 30% as rain or mist (McNulty and Aber, 1993). The area is characterized by warm summers (i.e., average regional July air temperature  $22^\circ \text{C}$ ) and cold winters (i.e., average regional January air temperature  $-6^\circ \text{C}$ ) (USDC, 1968). Located on broad areas between rock outcrops, the plots were on Houghtonville soils classified as frigid Typic Haplothods. The soils were well drained and have 3–15% slopes (USDA Soil Conservation Service, 1989).

### 2.2. N additions

The paired control plots received no N fertilizer. Two pairs of plots were randomly selected to be

amended via a backpack sprayer with three equal doses of  $\text{NH}_4\text{Cl-N}$  during June–August from 1988 to 2002. Cl was chosen as input anion because we wanted to examine just the impact of  $\text{NH}_4$  on ecosystem structure and function in the absence of other nutrients (e.g., P) or acids (e.g., S). The treatments were applied topically, with just enough water to cause the fertilizer to go into solution as it was sprayed across the plots. The distributed fertilizer was then incorporated into the forest floor during rain events. The Kansas State University guidelines for irrigation of sensitive crops suggest that Cl concentrations below 142 mg Cl/L is low and less than 355 mg Cl/L is moderate. There are no set guidelines for red spruce, but based on road salt impacts on spruce and other conifers, red spruce would probably be considered to have moderate sensitivity. Therefore, our high N addition rates are at least three times below the levels known to begin having impact on vegetation.

At the time of establishment, the rates of N application were comparable to the high range of annual N deposition recorded in spruce-fir ecosystems located in industrialized regions in the US (e.g., 16 kg N  $\text{ha}^{-1} \text{year}^{-1}$  Friedland et al., 1991) and western Europe (e.g., 30–40 kg N  $\text{ha}^{-1} \text{year}^{-1}$  Grennfelt and Hultberg, 1986). Across New England, N deposition increases from east to west (Ollinger et al., 1993.), and the Mt. Ascutney plots were located slightly east of the area where signs of N saturation had been observed (McNulty et al., 1991). Prior to treatment, the plots did not exhibit any of the initial symptoms of N saturation as described by Aber et al. (1989).

Bulk precipitation collectors measured 5.4 kg N  $\text{ha}^{-1} \text{year}^{-1}$  in open areas adjacent to the research plots from 1989 to 1991 (McNulty and Aber, 1993). Dry and cloud N deposition can increase estimates of total N loading (Lovett and Kinsman, 1990; Ollinger et al., 1993), but neither of these N inputs were measured on the plots. Therefore, estimates of the total ambient plot N additions should be considered conservative estimates relative to the actual N loading. By supplementing the long-term inputs of anthropogenic N deposition with additional N fertilizer at a rate equal to high rates of N deposition found elsewhere, we hypothesized that N saturation could be induced on these plots.

From 1988 to 2002, between 220 kg  $\text{NH}_4\text{Cl-N ha}^{-1}$  (15.7 kg  $\text{NH}_4\text{Cl-N ha}^{-1} \text{year}^{-1}$  “low N addition”

plots) and 440 kg  $\text{NH}_4\text{Cl-N ha}^{-1}$  (31.4 kg  $\text{NH}_4\text{Cl-N ha}^{-1} \text{year}^{-1}$  “high N addition” plots) were added to the treatment plots. In addition to the added N, at least 75 kg N  $\text{ha}^{-1}$  bulk deposition fell to all plots (assuming a bulk deposition rate of 5.4 kg N  $\text{ha}^{-1} \text{year}^{-1}$  for 14 years.). Therefore, this represents an addition of at least 75, 295 and 515 kg N  $\text{ha}^{-1}$  over the course of the experiment for the control, low and high N treatment plots, respectively. This N addition is equal to 7, 26 and 46% of the initial forest floor organic N content of 1150 kg N  $\text{ha}^{-1}$ , as measured before treatments began in 1988 (McNulty and Aber, 1993).

Following the analysis of 1998 foliar chemistry, we noted unexpected increases in the red spruce foliar N concentration on the control plots. In July 2000, we established five additional 10 m  $\times$  10 m control plots between 50 and 100 m from the original experimental plots to rule out any possibility that cross-plot N contamination (e.g., nitrate leaching for treatment plots, or accidental and un-noted fertilization of the control plots) was responsible for the control plot foliar N increase. Foliar and forest floor N concentrations, forest floor C:N, and forest floor potential and in situ N mineralization rates were measured on each of the five new and two original control plots, and four treatment plots using the previously established sampling protocols.

### 2.3. Basal area (BA) and stem density measurements

Prior to the start of N additions in June of 1988, we measured the basal area, species types and tree condition (i.e., living versus dead based on the presence or absence of leaves) on all trees  $>2$  cm in diameter measured 1.5 m above the forest floor. The number, size and species type of all trees within each plot were again measured in 1990, 1994, 1998 and 2002, and compared to initial measurements.

### 2.4. Foliar chemistry

Three randomly selected dominant red spruce trees were tagged on each plot at the beginning of the experiment. In July 1988, 1994, 1998 and 2002, we collected three randomly oriented, sunlit, or undershadowed branch samples that contained live needles from all age classes from each tagged tree using a pruning

pole. The clipped branches were placed in individual large paper bags and dried for 48 h at 70 °C. After drying, all branch needles had fallen into the bag. The contents of the bag were shaken to homogenize the age classes, and a randomly selected sub-sample was sieved through a 2 mm × 2 mm mesh screen to remove twigs and detritus, and then ground using a Wiley Mill. The samples were then placed in 60 mL acid-washed glass jars, dried for 24 h at 70 °C, and stored in darkness at 22 °C.

Percent foliar N and lignin were measured using near-infrared reflectance spectroscopy (Wessman et al., 1988; McLellan et al., 1991). After determination of N and lignin concentration, metal cation concentrations of the foliar samples were measured using 0.5 g of leaf tissue ashed at 450 °C for 4 h and added to 10 mL of plant buffer. One liter of buffer consisted of 300 mL HCl, 100 mL HNO<sub>3</sub>, 20 mL of 1000 ppm Mo Standard, and was brought to volume (Jones, 1988). The extracts were analyzed using inductively coupled argon plasma emission spectroscopy (Jarrell-Ash 965 Atomcomp).

### 2.5. Forest floor and air temperature

In July 2002, a Hobo H8 outdoor four channel external temperature logger was installed on each of the control, low and high N addition plots. Each logger contained one wide-range temperature TMCx-HA air sensor and three wide-range temperature TMCx-HA soil sensors. The air sensor was installed 2 cm above the forest floor and the soil sensors were installed at the base of the O horizon, at a depth from 6 to 10 cm from the top of the forest floor. Air and forest floor temperature measurements were recorded every hour, and the data were downloaded at least once a month except through winter months. Maximum air and forest floor temperatures were determined from these data.

### 2.6. Forest floor chemistry

In June 1988 (before the first N additions), and in July 1994, 1998 and 2000, 20 150 cm<sup>3</sup> forest floor samples, were collected from each plot and randomly composited into five samples per plot. The samples were sieved through a 5 mm × 5 mm mesh screen to remove all twigs, coarse roots and stones. Carbon (C) and N were measured in these samples using a Perkin-

Elmer model 240B CHN analyzer. Two (of the five) forest floor samples were randomly selected for pH measurements using a 1:2 (w/v) forest floor to CaCl<sub>2</sub> (0.01 M) solution. The forest floor samples were analyzed for Mg and Ca concentrations by placing 2.5 g of sieved sample into a 500 °C muffle furnace for 12 h. Two millilitres of distilled water, 4 mL of 6N HCl, and the ashed sample were added to a 50 mL volumetric flask that was filled to volume, mixed, and filtered prior to analysis. Each element was analyzed using the Inductive Coupled Plasma (ICP) analyzer at North Carolina State University, Raleigh, NC (Schumacher et al., 1995).

### 2.7. Forest floor net N mineralization and net nitrification

From July 1988 to June 1991, and from October 2001 to September 2002, 40 150 cm<sup>3</sup> forest floor samples were collected monthly (except samples left in the ground over winter, when samples incubated in situ from September to April), from each plot. Twenty samples from each plot were randomly combined into five samples per plot (to assure that there would be sufficient sample mass for analysis) and sieved through a 6.3 mm mesh screen to remove twigs, coarse roots, and stones. Approximately 10 g of each of the five composite samples from each plot were placed into 150 mL of 1 M KCl, shaken and set at room temperature for 48 h. After 48 h, approximately 20 mL of solution was extracted using a vacuum pump and frozen at 0 °C until the samples were analyzed for NH<sub>4</sub>-N and NO<sub>3</sub>-N concentrations (Hart et al., 1994).

The 20 remaining samples from each plot were placed into 1 mil ( $2.54 \times 10^{-4}$  mm) thick polyethylene bags, replaced into the forest floor and incubated in situ for 28 days. After 28 days, the 20 incubated samples were excavated, composited, sieved, and extracted as previously described. Initial and incubated extracts were run on a TRAACS 800 Auto-analyzer for NH<sub>4</sub>-N (Technicon Industrial Systems, 1978) and NO<sub>3</sub>-N (Technicon Industrial Systems, 1977) concentrations. Net annual N mineralization was calculated as the sum of monthly-incubated sample N (NH<sub>4</sub>-N + NO<sub>3</sub>-N) minus initial monthly sample N (NH<sub>4</sub>-N + NO<sub>3</sub>-N). Net annual nitrification was calculated as the sum of monthly-incubated sample NO<sub>3</sub>-N minus initial monthly sample NO<sub>3</sub>-N.

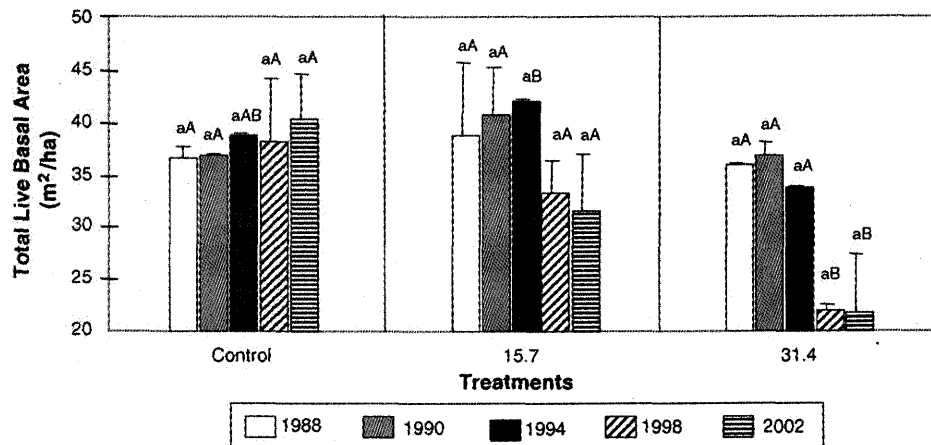


Fig. 1. Total live basal area on six spruce-fir plots (two paired N addition treatments and one paired control) on Mt. Ascutney, Vermont from 1988 to 2002). Standard error bars are shown. Means with the same letter are not significantly different, year vs. year within treatment (lowercase) and treatment vs. treatment within year (uppercase).

In May 1988 and July of 1994, 2000 and 2002, 40 forest floor samples were collected from each plot and sieved. Twenty of the sieved samples were used to determine initial  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations, as previously described. The other 20 samples were incubated at  $22^\circ\text{C}$  for 28 days. After 28 days, incubated  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations were measured as previously described, to determine the net N mineralization and net nitrification potential for each plot.

#### 2.7.1. Statistical analysis

All correlations were derived from lumped data per plot. All data analyses were completed using SYSTAT Version 9 and all possible significant differences between treatment means were determined using Tukey test ( $p < 0.05$ ). Correlation analyses were determined using Pearson correlation ( $p < 0.05$ ). The Tukey test was used to determine if the mean of the measured ecosystem parameters varied between plots during the same year or within the same plot between years.

### 3. Results and discussion

#### 3.1. Basal area change

We found no significant differences in the amount of total live basal area between plots at the beginning

of the experiment. Between 1988 and 1990, total live basal increased more on the low and high N addition plots compared to the control, but the differences were not statically significant given the small sample size ( $n = 2$ ), and high intra-treatment plot variability (Fig. 1). However, by 1994, significant differences were observed in treatment level live basal area. The low N addition plots had significantly more live basal when compared to the amount of basal area on the high N addition plots. Measurements from 1998 to 2002 indicated that the high N addition plots had significantly less live basal area than the control and low N addition plots, and the low N addition plots were not significantly different than the control plots. These results suggest that the low N addition plots continued to experience enhanced growth due to the N additions 6 years after treatments began, while the high N addition plots rapidly moved from a slight enhanced growth phase to a decline phase by 2002. Additionally, measurements from 2002 indicated that live basal area had increased by 20% on the control plots, and decreased by 20 and 43% on the low and high N addition plots, respectively. The basal areas on the low N and control plot were not statistically significant due to increased intra-treatment plot basal area variability. Research by Magill et al. (2004) found no biomass accumulation and a mortality rate of 56% in a high N addition, mature, red pine (*Pinus resinosa*) fertilization experiment after 15 years of treatment. Increased forest basal area, such as occurred in our

Table 1

Mean foliar chemistry on Mt. Ascutney, Vermont spruce-fir plots collected from 1988 to 2002

TRT	Year	N (g/kg)	Ca (g/kg)	Mg (g/kg)	Mg:N	n
Control	1988	8.8 (0.57)aA	–	–	–	2
Control	1990	10.0 (0.14)aA	3.5 (0.87)abA	1.1 (0.02)aA	0.119 (0.001)aA	2
Control	1994	8.5 (0.53)bA	3.5 (0.27)abA	0.6 (0.06)bA	0.069 (0.003)aA	2
Control	1998	10.0 (0.06)aA	4.5 (0.09)aA	1.0 (0.10)aA	0.051 (0.06)aA	2
Control	2002	8.8 (0.28)aA	2.0 (0.09)bA	0.5 (0.09)bA	0.061 (0.01)aA	2
15.7	1988	9.0 (0.10)aA	–	–	–	2
15.7	1990	13.5 (0.84)bB	2.6 (0.55)aA	1.1 (0.07)aA	0.084 (0.001)aA	2
15.7	1994	13.0 (0.50)bB	3.5 (0.74)aA	0.8 (0.04)abA	0.062 (0.01)abA	2
15.7	1998	15.6 (0.42)cB	3.6 (2.10)aA	1.0 (0.21)abA	0.059 (0.011)abA	2
15.7	2002	14.6 (0.10)bcB	1.8 (0.39)aA	0.5 (0.01)bA	0.035 (0.001)bB	2
31.4	1988	9.1 (0.64)aA	–	–	–	2
31.4	1990	13.4 (0.50)bB	2.6 (0.65)aA	1.3 (0.15)aA	0.097 (0.015)aA	2
31.4	1994	14.7 (1.20)bB	3.4 (0.31)aA	0.7 (0.10)bA	0.047 (0.003)bB	2
31.4	1998	18.4 (0.95)cC	2.7 (0.74)aA	0.7 (0.14)bA	0.038 (0.006)bB	2
31.4	2002	15.6 (0.44)bcB	1.5 (0.05)aA	0.4 (0.07)bA	0.027 (0.004)bB	2

(–) Missing data. Means with the same letters are not significantly different (Tukey,  $p < 0.05$ ), year vs. year within treatment (lowercase) and treatment vs. treatment within year (uppercase). The standard error for the mean of each value is in parenthesis.

control plots was characteristic of early stage N saturation (i.e., fertilization phase), and decreased forest basal area, such as occurred in the high N addition plots is characteristic of late stage N saturation (Aber et al., 1989).

### 3.2. Spruce foliar chemistry

Red spruce foliar N concentration had not changed significantly on the control plots from 1988 to 2002 (Table 1). However, by 1990, both the low and high N addition plots had significantly higher red spruce foliar N concentrations compared to the control plots values (Table 1). Bauer et al. (2004) also found that in long-term N amended study of a red pine forest, foliar N increased significantly on the high N treatment plots. In 1998, the high N addition plots had statistically higher red spruce foliar N concentrations than both the control and low N addition plots, and the low N addition plots had higher red spruce foliar N concentrations than did the control trees (Table 1). These foliar N concentrations were higher than any of the values reported for red spruce across New England (Huntington et al., 1990; McNulty et al., 1991; Nadelhoffer et al., 1999; ACCP, 2005) and may represent the upper limit of the red spruce foliar N concentrations under N addition field conditions.

After 1998, red spruce foliar N concentrations began to decrease on all plots (including the controls).

Red spruce foliar Mg concentrations decreased significantly on all the plots from 1990 to 2002 (Table 1). This decrease in foliar Mg suggests that the red spruce foliar Mg is responding to both N fertilizer and anthropogenic N deposition. The foliar Ca concentrations did not change significantly on the treatment plots. Schaberg et al. (2002), however, noted a significant reduction in membrane-associated calcium, increased foliar respiration, reduced cold tolerance and increased freezing injury for both the low and high N addition plots compared to control levels. Foliar Mg:N did not change significantly on controls plots from 1988 to 2002. However, foliar Mg:N was significantly lower in 2002 when compared to 1988 in both the low and high N addition plots. The increases in concentrations of red spruce foliar N (except on the control plots) suggest that red spruce foliar N concentrations are responding to N fertilizer.

### 3.3. Forest floor chemistry

The low and high N addition plots did not have significantly different forest floor N concentrations in 2000, compared to the control plots (Table 2). From 1988 to 2000, low and high N addition plot forest floor

Table 2  
Mean forest floor chemistry on Mt. Ascutney, Vermont spruce-fir plots collected from 1988 to 2000

TRT	Year	N (g/kg)	C (g/kg)	C:N	n
Control	1988	12.2 (1.6)aA	357 (45)aA	29.4 (0.1)aA	2
Control	1994	13.6 (0.5)aA	435 (38)aA	32.1 (0.8)bA	2
Control	1998	15.0 (0.4)aA	438 (23)aA	29.3 (0.7)aA	2
Control	2000	15.6 (0.9)aA	452 (19)aA	29.1 (0.5)aA	2
15.7	1988	14.7 (0.1)aA	429 (31)aA	29.2 (0.4)aA	2
15.7	1994	14.7 (0.5)aA	440 (24)aA	29.9 (0.08)aB	2
15.7	1998	15.0 (0.5)aA	429 (5.3)aA	28.7 (1.3)aA	2
15.7	2000	16.5 (1.2)aA	464 (8.2)aA	28.3 (0.9)aA	2
31.4	1988	13.0 (0.4)aA	393 (21)aA	30.0 (0.9)aA	2
31.4	1994	14.0 (0.6)abA	416 (38)aA	29.7 (0.4)aB	2
31.4	1998	14.9 (0.6)abA	420 (21)aA	28.3 (0.2)aA	2
31.4	2000	16.5 (1.1)bA	442 (3.9)aA	26.9 (1.5)aA	2

Means with the same letters are not significantly different (Tukey,  $p < 0.05$ ), year vs. year within treatment (lowercase) and treatment vs. treatment within year (uppercase). The standard error for the mean of each value is in parenthesis.

N concentration increased by 11 and 21%, respectively (Table 2). The increase in forest floor N concentration between 1988 and 2000 occurred following at least a 22% (low N addition plots) and 38% (high N addition plots) increase in plot added N, relative to pretreatment forest floor N mass. However, between 1988 and 2000, control plot forest floor N concentration increased 22% even though bulk N deposition accounted only for a 6% ( $65 \text{ kg N ha}^{-1}$ ) increase in total forest floor N concentration, assuming that forest floor mass has remained constant since first measured in 1988 (McNulty et al., 1996). The lack of a measured increase in in situ net N mineralization on the control plots suggest that forest floor decomposition rates have not increased sufficiently to account for a large decrease in forest floor biomass. Therefore, it seems likely that inputs of unmeasured cloud and dry N deposition are contributing to the increase in forest floor N% on both the control and N addition plots.

Forest floor C concentrations increased over time on both the control and treatment plots, and the values were not statistically different between treatments at any time during the experiment (Table 2). The intra-treatment forest floor C concentration was also not significantly higher in 2000 compared to 1988 for both the control and treatment plots (Table 2).

The changes in forest floor C:N ratio were not as pronounced as either the forest floor N or C concentrations because both N and C increased over time. Magill et al. (2004) found that in a red pine N amended forest, organic horizon C:N ratio was

significantly higher in a treated plot ( $15 \text{ g N m}^{-2} \text{ year}^{-1}$ ) when compared to the control. On average the C:N value was lower on the high N addition plots in 2000, but the large standard error prevented the values from being statistically different from the 1988 values (Table 2). Ironically, the 2000 measurement of forest floor C:N ratio was the lowest on the new control plots, and significantly lower than on the original control plots.

It does not appear that the large reductions in live biomass on the low and high N addition plots were incorporated into the forest floor given the modest increase of forest floor C concentration and reduction in forest floor C:N. Instead, it is possible that the large influx of N-poor detritus from dying tree roots and stems could be immobilizing the added N on the treatment plots, and thus minimizing the impacts on forest floor N cycling to date. Increased nitrification, with the potential for increased nitrate leaching on the high N addition plots could be another reason that the forest floor N concentration and C:N ratio have not changed significantly compared to control plot values. Therefore, it appears that that long-term N deposition is having a significant influence on increasing forest floor N% on all the plots, but that the additional N fertilizer was largely immobilized or tied up into coarse woody debris or leached from the ecosystem (McNulty and Aber, 1993).

Across all plots, forest floor pH was very low (2.7–3.0) at the beginning of N fertilization in 1988, and had not changed significantly between or within plots

when measured during the 1990, 1994, 2000 or 2002 collections (i.e., pH ranged from 2.6 to 3.0).

### 3.4. Temperature

The July 2002 maximum forest floor temperature (at approximately 10 cm depth) was significant correlated ( $R^2 = 0.99$ ,  $p = 0.04$ ,  $n = 3$ ) with N addition. The temperature was warmest in August when above forest floor air temperature reached 39 °C on the high N addition plots, and coldest in November when the temperature fell to –10 °C on the low N addition plots.

### 3.5. Net N mineralization and net nitrification

After the first 2 years of treatment, annual in situ forest floor net N mineralization was four times higher on the N treatment plots, compared to the control plot values (Fig. 2). Gundersen et al. (1998) also found that net N mineralization responded positively with increased N addition. However, in our study inter-plot net N mineralization variability increased with increasing net N mineralization rates so the increase between control and treatment net N mineralization rates were not statistically significant. However, we feel that it is still instructive to examine the general trends despite the statistical limitations. From 1988 to 1989 there was an increase in in situ net N

mineralization with increasing N additions (McNulty and Aber, 1993). However, this relationship was short lived, and by 1991, in situ net N mineralization had leveled off on the high N addition plots, but was still increasing on the low N addition treatments. When in situ net N mineralization was remeasured in 2002, the net N mineralization was lowest in the high N addition and highest in the low N addition plots (Fig. 2). However, the high and low N addition plots did not have significantly different in situ net N mineralization rates from that of the controls (Fig. 2). Aber et al. (1989) suggested that increased recalcitrant organic matter or that suppression of humus degrading enzymes in the presence of high N availability could account for the long-term reduction in net N mineralization with increasing N availability.

Net N mineralization potential almost doubled from 1988 to 2002 on the control plots (Fig. 2). We also sampled in situ net N mineralization on the five newly established control plots to assure that the control plots were not compromised by N leaching from adjacent N addition plots. The net N mineralization potential was not significantly different between the new and old control plots. The potential net N mineralization rates on Mt. Ascutney are now similar to 1987 measurements from Mt. Washington, New Hampshire (McNulty et al., 1991). On Mt. Ascutney, net N mineralization potential peaked in the 2000 measurement for both the low and high N

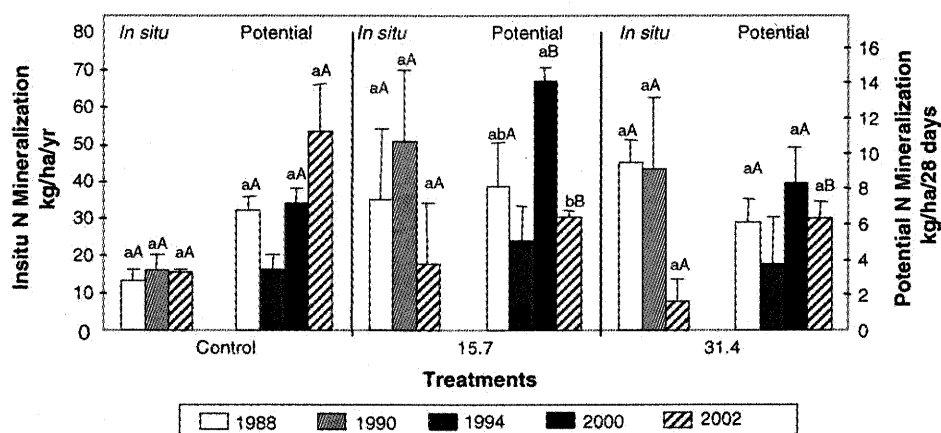


Fig. 2. In situ and potential N mineralization on six spruce-fir plots (two paired N addition treatments and one paired control) on Mt. Ascutney, Vermont from 1988 to 2002. Standard error bars are shown. Means with the same letter are not significantly different, year vs. year within treatment (lowercase) and treatment vs. treatment within year (uppercase). Potential N mineralization from 1994 was not included in the statistical analysis because we only had the mean value (one data point) for each treatment.



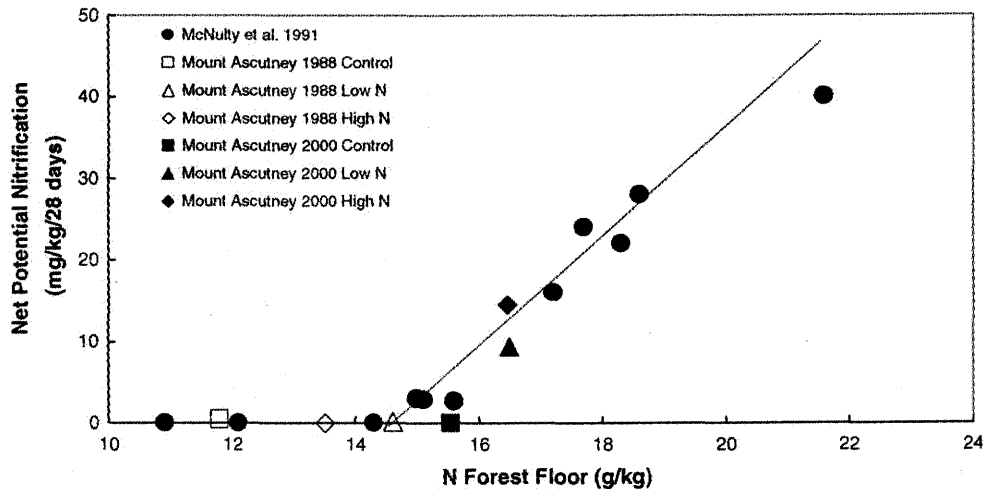


Fig. 3. Comparison of the relationships between potential net nitrification and forest floor N from McNulty et al. (1991) gradient and Mt. Ascutney 1988 and 2000.

addition plots (Fig. 2), with the low N addition plots' net N mineralization potential being significantly higher than the control and high N addition plots. The net N mineralization potential on the control plots was significantly higher than net N mineralization potential on the low N addition and high N addition plots during the 2002 measurement.

Twelve years on ambient N deposition on the control plot have increased both the N% of the forest floor and foliage. However, the control plot in situ forest floor net N mineralization has been stable compared to the N mineralization potential, which has generally increased over time. This suggests that although more N is available on the control plots, other environmental factors, such as soil temperature may be limiting in situ N mineralization.

There was not a consistent pattern between potential and in situ N mineralization on the treatment plots. The loss of canopy cover increased soil temperature on treatment plots compared to controls. Also, N mineralization on both the in situ and potential measurements may have been reduced by a lack of carbon substrate. Wallenstein (2004) observed a 50% reduction in the organic soil microbial biomass content and substrate, and over a 50% reduction in induced respiration rates in the high N addition plots relative to the control plot rates. These findings are consistent with the reduced levels of net N mineralization potential that were observed on the treatment

plots. Similarity in potential and in situ forest floor temperature and a lack of carbon availability would explain why the two incubation methods yield similar results on the treatment plots.

Red spruce forest floor N concentration below  $14.5 \text{ g N kg}^{-1}$  forest floor had no measured net nitrification potential in a trans-New England sampling study (Fig. 3 from McNulty et al., 1991). However, when forest floor N was  $>14.5 \text{ g N kg}^{-1}$  forest floor, net nitrification was linearly correlated with forest floor N. The control and treatment plots on Mt. Ascutney in 1988 had no measured net nitrification potential. However, the treatment plots on Mt. Ascutney in 2000 had begun to show increased nitrification potential (Fig. 3). Furthermore, we hypothesize that the control plots will also begin to experience an increase in the nitrification potential now that the forest floor N is also above  $14.5 \text{ g N kg}^{-1}$  forest floor. A comparison of net nitrification potential and forest floor C:N revealed that nitrification occurred on the low and high N addition plots at a forest floor C:N threshold of 28 (Fig. 4). McNulty et al. (1991) found that net potential nitrification occurred when forest floor C:N reached 30. Gundersen et al. (1998) also found a similar threshold.

Although the rate of net N mineralization potential has declined over time, the fraction of potential nitrification to mineralization increased from the 1994, 2000 and 2002 sampling for the low and high N

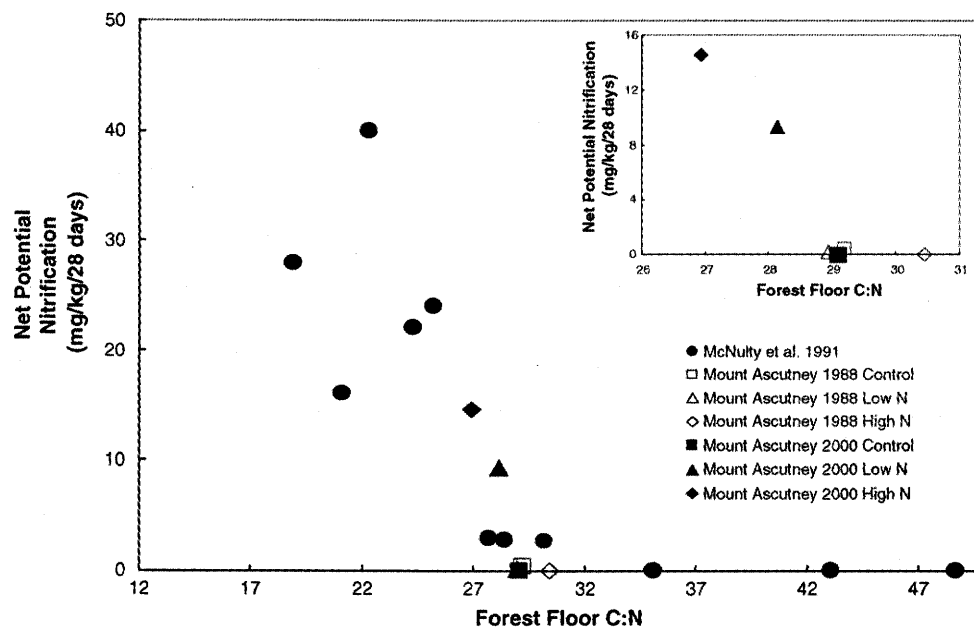


Fig. 4. Comparison of the relationships between forest floor C:N and potential net nitrification from McNulty et al. (1991) and Mt. Ascutney 1988 and 2000. *Inset*: Mt. Ascutney 1988 and 2000 data.

addition plots (Fig. 5). This increase in the fraction of the potential N nitrification to net N mineralization was consistent with latter stages of N saturation (Aber et al., 1989). These relationships support our hypothesis that chronic, low level (i.e., less than  $40 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) amounts of added N could induce N saturation on these plots after several years of treatment.

### 3.6. Ecosystem level relationships

It is difficult to determine what ecosystem level relationships are causal and which are just correlated. For example, total live basal area was highly negatively correlated with the maximum July 2002 forest floor temperature and maximum August 2002 air temperature (Table 3), thus indicating that as live basal area

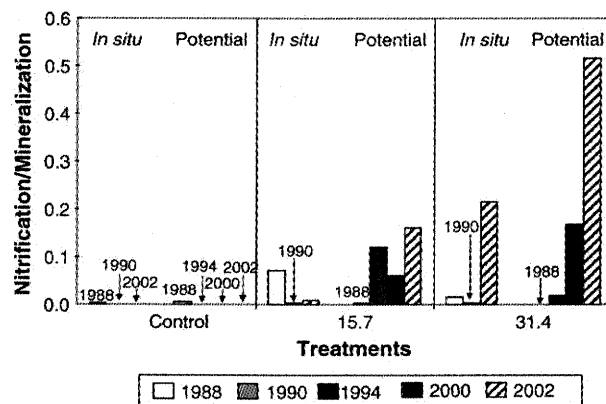


Fig. 5. In situ and potential nitrification/mineralization on six spruce-fir plots (two paired N addition treatments and one paired control) on Mt. Ascutney, Vermont from 1988 to 2002.

Table 3

Correlations between N cycling, total live basal area and forest floor and air temperature

	1988		2002	
	$R^2$	$p$	$R^2$	$p$
Total live basal area vs.				
Maximum July forest floor temperature			0.99 <sup>a</sup>	0.06
Maximum August air temperature			0.99 <sup>a</sup>	0.06
July in situ net N mineralization vs.				
Maximum July forest floor temperature			0.99	0.02
Potential net N mineralization (mg/kg/28 days) vs.				
Maximum July forest floor temperature			0.81	0.29
July in situ nitrification/mineralization vs.				
Maximum July forest floor temperature			0.99	0.06
In situ net N mineralization ( $\text{kg ha}^{-1} \text{ year}^{-1}$ ) vs.				
Foliar N	0.99	0.01		

<sup>a</sup> Negatively correlated.

decreased, temperature increased. The high N addition plots had lost 43% of their original live basal area, versus a 20% increase in live basal area on the control plots over same 14 year time period. We (subjectively) observed that direct exposure of the forest floor to sunlight was rare in the control plots (<5% of the forest floor directly lit at any given point in time). However, we (subjectively) observed that more than 50% of the forest floor was directly impacted by sunlight on the high N addition plots at any point in time. The dark color of the forest floor readily absorbed incoming direct solar radiation, and was likely the cause for the significant temperature differences between the control and high N addition plots. Other studies have shown that soil temperature does influence net N mineralization (Emmer and Tietema, 1990; Reich et al., 1997). We also observed the influence of elevated soil temperature on net N mineralization rates on Mt. Ascutney. The maximum July 2002 forest floor temperature was significantly correlated (Table 3) with the 2002 July in situ net N mineralization rate. The increased in situ net N mineralization rates in the high N addition plots were likely a direct result of the forest floor warming because in 2002, there was no significant relationship between maximum July forest floor temperature and potential net N mineralization (Table 3). The in situ nitrification/mineralization ratio (July 2002) also increased linearly with maximum July 2002 forest floor temperature (Table 3). This relationship could be a function of increased  $\text{NH}_4\text{-N}$  availability for nitrification on the

high N addition plots due to reduced biological N demand (because of high red spruce mortality).

There was a strong relationship between foliar N concentration and in situ net N mineralization after the first year of the treatments in 1988 (Table 3). However, this relationship rapidly declined and was no longer significant by 1990. The loss of this relationship could be expected because red spruce foliar N% on the treatment plots was no longer limited by N supplied by direct N deposition and net N mineralization. Additional process level tree and soil measurements will be required to test which of these relationships were causal, and which were simply correlated.

#### 4. Conclusion

The result of 14 years (1988–2002) of observation and N fertilization to spruce-fir stands on Mt. Ascutney Vermont, suggest that the early stage N saturation may be naturally occurring on the control plots, and that mid to late stage N saturation may have been induced on low and high N addition plots, respectively. Most noticeably, the high N addition plots exhibited a 40% loss in live basal area during the course of this study. Inter-treatment spruce foliar N concentration, live spruce basal area growth, and forest floor N concentration have changed significantly after 14 years. We previously predicted that forest floor and vegetative changes are only the beginning in a series of ecosystem alterations

that could eventually lead to a shift in species composition (McNulty et al., 1996). We hypothesized that if N additions continue, experimental high elevations spruce forests on Mt. Ascutney (largely through N fertilization), and across New England (from anthropogenic N deposition) could eventually be converted into stands of birch and maple. We also observed that anthropogenic N deposition inputs are impacting the control plots through increased forest floor N concentration and a doubling of the net N mineralization potential. To date, N deposition does not appear to be having any negative impacts on live basal area on the control plots. However, it is only through the continued long-term monitoring of these plots that we will be able to track ecosystem response to continued N deposition and fertilizer impacts on stand structure and function. We will also continue to observe the low and high N addition plots for continued signs of forest floor N cycling shifts. Increases in forest floor net nitrification and the nitrification:N mineralization ratio and forest floor N% and decreases in C:N ratio would be indicators of reduced N immobilization, and a shift to a faster N cycling ecosystem.

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