Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce

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Abstract: We evaluated the influence of protracted low-level nitrogen (N) fertilization on 29 morphological, physiological, or chemical parameters measured on mature red spruce (*Picea rubens* Sarg.) growing within 10 study plots on Mount Ascutney, Vermont. For 8 consecutive years prior to this study, each plot received one of five treatments: 0, 15.7, 19.8, 25.6, or 31.4 kg $N \cdot ha^{-1} \cdot year^{-1}$. In comparison to plant material from control plots, trees that received N fertilization had fewer but longer and heavier needles, and higher rates of shoot water loss than trees from control plots. N fertilization resulted in increased foliar N concentrations, and reductions in foliar Ca and Mg concentrations to potentially deficient levels. Although no differences in chlorophyll content, chlorophyll fluorescence, or net photosynthesis were evident, N fertilization was associated with increased levels of respiration. Respiration rates were not correlated with foliar N concentrations, suggesting that the influence of N treatment on respiration was indirect. In contrast, respiration levels were negatively correlated with foliar Ca and Mg concentrations. Although we have no direct evidence that they are related, the N-induced alterations in foliar cation and respiration levels that we found may help account for N-induced reductions in tree growth and increases in mortality previously reported for this site.

Résumé : Les auteurs ont évalué l'influence d'une fertilisation prolongée avec de faibles niveaux d'azote (N) sur 29 paramètres morphologiques, physiologiques et chimiques mesurés sur des épinettes rouges (*Picea rubens* Sarg.) matures croissant dans 10 parcelles-échantillons sur le mont Ascutney dans le Vermont. Pendant 8 années consécutives antérieures à cette étude, chaque parcelle a reçu un des cinq traitements suivants : 0, 15,7, 19,8, 25,6 ou 31,4 kg $N \cdot ha^{-1} \cdot an^{-1}$. Comparativement au matériel végétal provenant des parcelles témoins, les arbres qui avaient reçu une fertilisation azotée avaient des aiguilles moins nombreuses mais plus longues et plus lourdes ainsi qu'un taux de perte hydrique plus élevé pour les pousses. La fertilisation azotée a provoqué une augmentation de la concentration foliaire de N et une diminution de la concentration foliaire de Ca et Mg à des niveaux de déficience potentielle. Quoiqu'il n'y avait pas de différence évidente dans le contenu en chlorophylle, dans la fluorescence chlorophyllienne ou la photosynthèse nette, la fertilisation azotée était asociée à une augmentation du niveau de respiration. Le taux de respiration n'était pas corrélé à la concentration d'azote foliaire; ce qui suggère une influence indirecte de la fertilisation azotée sur la respiration. Au contraire, le niveau de respiration était négativement corrélé à la concentration foliaire de Ca et Mg. Quoique nous n'ayons aucune preuve directe qu'ils soient reliés, les altérations causées par l'azote, que nous avons observées dans les cations foliaires, et les niveaux de respiration pourraient aider à expliquer les réductions de croissance des arbres causées par l'azote et l'augmentation de la mortalité déjà rapportée dans ce site.
[Traduit par la Rédaction]

Introduction

Many pollutants have high rates of deposition in the montane forests of the northeastern United States (Mohnen 1992). Declines in the growth and health of forest trees such as red spruce (*Picea rubens* Sarg.) have been linked to these inputs (Peart et al. 1992; DeHayes 1992; McLaughlin and Kohut 1992). Because of these and other potential impacts, the *1990 Amendment to the United States Clean Air Act* targeted the reduction of several pollutant additions to the atmosphere. However, required reductions are not equal for all contaminants. For example, although a 50% decrease in sulfur inputs is anticipated, only a 10% reduction in nitrogen (N) deposition is required and expected. Because levels of N deposition are likely to remain high, the potential consequences of N accumulation have an enhanced importance.

Results of studies conducted along regional N deposition gradients suggest that high N additions can lead to changes in forest floor and foliar chemistry, and may result in N saturation (Aber et al.1989; McNulty et al. 1991). Reductions in tree growth or increases in tree mortality can accompany these changes (Aber et al. 1995). However, because additional factors, including the deposition of other atmospheric contaminants, can follow similar regional gradients, it is difficult to attribute alterations in chemistry and tree vigor solely to changes in N. To isolate the impacts of N accumulation, McNulty and Aber (1993) established a series of low-level N fertilization plots in a high-elevation spruce–fir forest on Mount Ascutney, Vermont. Results from this site indicated that long-term additions of N can lead to N saturation within

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montane spruce stands; although N concentrations within the forest floor were unaltered by treatment, N fertilization resulted in greater N leaching from the forest floor and increased foliar N concentrations relative to control plots (McNulty and Aber 1993; McNulty et al. 1996). Reductions in growth and increases in mortality for trees within N addition plots were also found, suggesting that chronic low-level additions of N can initiate stand decline (McNulty and Aber 1993; McNulty et al. 1996). Although results of correlations of foliar nutrients with net basal area growth suggested that N-induced alterations in stand structure resulted from imbalances among key foliar elements (especially Ca/Al and Mg/N ratios), the physiological basis for observed changes in tree vigor and stand structure was not determined (McNulty and Aber 1993; McNulty et al. 1996). This paper summarizes results of an analysis of the physiological impacts of protracted low-level N fertilization on mature red spruce from the Mount Ascutney study site.

Methods

Study plots and N treatments

In June 1988, ten 15 × 15 m plots were established at an elevation of 762 m on Mount Ascutney, Vermont, in an area where red spruce composes >80% of the total basal area (McNulty and Aber 1993; McNulty et al. 1996). Since establishment, two plots (controls) have received no N fertilizer. Of the other eight plots, two have received yearly applications of each of the four following treatments: (1) 15.7 kg $N \cdot ha^{-1} \cdot year^{-1}$ in the form of NH_4Cl; (2) 19.8 kg $N \cdot ha^{-1} \cdot year^{-1}$ in the form $NaNO_3$; (3) 25.6 kg $N \cdot ha^{-1} \cdot year^{-1}$ from a combination of NH_4Cl and $NaNO_3$; and (4) 31.4 kg $N \cdot ha^{-1} \cdot year^{-1}$ in the form NH_4Cl. Treatments included cationic and anionic forms of N so that potential differences in leaching, uptake, and ecological impact could be assessed. Fertilizers were applied during June, July, and August from 1988 to 1995. Rates of N application were comparable to annual N deposition rates recorded for spruce–fir forests within industrialized regions of the United States (e.g., 16 kg $N \cdot ha^{-1} \cdot year^{-1}$, Friedland et al. 1991) and Germany (e.g., 30 to 40 kg $N \cdot ha^{-1} \cdot year^{-1}$, Grennfelt and Hultberg 1986). In addition to N treatments, bulk precipitation collectors located in open areas adjacent to research plots measured ambient additions of 5.4 kg $N \cdot ha^{-1} \cdot year^{-1}$ (McNulty and Aber 1993).

Foliar sampling

Three randomly selected dominant red spruce trees were tagged on each plot prior to the start of physiological assessment. In August 1995, three randomly oriented, sunlit branches (≤30 cm long) from the mid–upper crown of each tagged tree were removed using a pruning pole. Excised branches were placed in plastic bags and stored in a cooler while transported to laboratory facilities for further processing.

Foliar chemistry

Current-year shoots from each branch were oven-dried (70°C for 96 h) and ground to a No. 10 mesh. Foliar N concentration was measured using near-infrared reflectance spectroscopy (Wessman et al. 1988). After determination of N, cation concentrations were measured using 0.5 g of needles ashed at 450°C for 4 h and added to 10 mL of a buffer consisting of 300 mL HCl, 100 mL HNO_3, 20 mL of 1000 ppm molybdenum standard, and 580 mL of distilled–deionized H_2O (Jones 1988). Buffer extracts were analyzed using inductively coupled argon plasma emission spectroscopy (Jarrell-Ash 965 Atomcomp).

Needle and shoot morphology

The total length of 20 individual current-year shoots from each tree and the length of 10 needles on each shoot were measured using digital calipers. Needles and buds on 10 replicate 10-cm shoots were counted, and the number of needles per centimetre and buds per centimetre shoot were multiplied by the average shoot length to determine the average number of needles and buds per shoot. The fresh and ovendry (70°C for 96 h) mass of these same needles were measured to the nearest 0.0001 g.

Water content and loss rate

Foliar and total shoot water contents were measured for each tree. The fresh weight (FW) of three shoots bearing foliage was individually measured to the nearest 0.0001 g, then allowed to air dry on a laboratory bench for 4 h and reweighed (LW). Foliage was then dried in an oven at 70°C for 96 h and reweighed (DW). In addition, 10 current-year needles were excised from three replicate shoots, and the fresh and ovendry weights were determined. Initial water loss rate was calculated as ((FW – LW)/DW) × 100. Water content was calculated as a percentage of dry mass as follows: ((FW – DW)/DW) × 100. After oven-drying, twigs and shoots were separated and weighed to determine the percentage each component contributed to total dry mass of the shoot.

Chlorophyll concentration and fluorescence

Three replicate samples of foliar pigments were measured for each sample tree. Approximately 0.1 g fresh weight of needle tissue was homogenized in test tubes containing 10 mL of chilled 80% acetone. Tubes were centrifuged at 2000 rpm for 10 min, and supernatants were decanted and placed in quartz tubes for analysis. Spectrophotometric scans (from 400 to 700 nm at 0.5-nm bandwidths) were made using a Shimadzu scanning spectrophotometer. Concentrations of chlorophyll *a* and *b* were calculated using coefficients given by Lichtenthaler and Wellburn (1983).

Fast and slow chlorophyll fluorescence kinetic parameters were measured using a PK Morgan CF1000 fluorometer using an actinic light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 5 s. Foliage was dark-equilibrated for 15 min prior to measurement. Five replicates of foliage from each sample tree were measured. The ratio of variable (F_v) to maximal (F_m) chlorophyll fluorescence was assessed as a quantitative measure of photochemical efficiency (Mohammed et al. 1995).

Gas exchange

In early September 1995, net photosynthesis and dark respiration were measured on current-year shoots from two of the three trees per plot sampled in August. Gas exchange was assessed on sunlit branches cut using a pole pruner. Numerous studies using a variety of coniferous species, including red spruce, have shown that measurements of gas exchange from detached branches are comparable to those for intact shoots provided measurements are made soon after excision (Lange et al. 1986; Ginn et al. 1992; Gage and DeHayes 1992; Zhang et al. 1993). Measurements for this study were all made within 5 min of harvest. For each branch, gas exchange was measured on one current-year, 5 cm long shoot using a LI-6200 portable photosynthesis instrument (LI-COR Inc., Lincoln, Nebr.) with a 0.25-L cuvette. Net photosynthesis was measured under saturating (>1000 $mmol \cdot m^{-2} \cdot s^{-1}$) natural light conditions. Respiration measurements were made on the same shoot approximately 1 min after darkening the cuvette with a double-layered cloth (McLaughlin et al. 1990; Schaberg et al. 1996). For each photosynthesis and respiration measurement, three 15-s assessments of gas exchange were averaged to obtain one rate per shoot. Gas exchange measurements were expressed on a dry-weight basis. For each sample, the instantaneous photosynthetic nitrogen-use efficiency (PNUE) was calculated by dividing the photosynthetic rate ($\mu mol \cdot g^{-1} \cdot s^{-1}$) by the N concentration ($g \cdot g^{-1}$).

Statistical analyses

Analyses of variance were used to test for differences among N treatment groups. Previous studies at the Mount Ascutney site have shown that the mineral nutrition and health of red spruce were influenced

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Table 1. Means and standard errors (±1 SE) of morphological, physiological, and nutritional measurements for red spruce trees from the Mount Ascutney, Vermont, study site.

	Nitrogen addition (kg N·ha^{-1}·year^{-1})				
	0	15.7	19.8	25.6	31.4
K (g·kg^{-1})	5.42±0.09	5.17±1.01	4.97±0.16	5.03±0.53	4.81±0.34
Al (g·kg^{-1})	0.04±0.00	0.03±3.33(×10^{-3})	0.03±1.67(×10^{-3})	0.03±0.01	0.04±3.33(×10^{-3})
P (g·kg^{-1})	1.23±0.10	1.17±0.01	1.00±1.67(×10^{-3})	1.14±0.10	1.09±0.02
Ca/Al	48.37±12.37	40.27±6.67	44.33±12.23	39.64±20.61	24.88±0.82
Shoot length (mm)	47.48±0.46	55.74±7.75	64.22±0.19	59.77±6.93	49.92±3.21
Shoot fresh wt.(g)	0.39±0.00	0.34±0.00	0.40±0.00	0.42±0.04	0.38±0.07
Shoot dry wt. (g)	0.16±0.00	0.14±0.00	0.17±0.00	0.17±0.02	0.16±0.03
Stem dry wt. (%)	21.72±2.18	23.13±0.33	23.80±0.50	22.23±4.33	24.25±0.38
Needle wt. (%)	78.28±2.18	76.87±0.33	76.20±0.50	77.77±4.33	75.75±0.38
Buds/cm	0.98±0.07	0.93±0.01	0.84±0.03	1.08±0.16	1.00±0.07
Water content (% dry wt.)	138.37±1.03	144.72±6.42	140.50±4.17	140.95±4.98	140.57±4.77
Chlorophyll (mg·g^{-1})					
Fresh wt.	0.05±0.00	0.04±0.00	0.05±0.00	0.05±0.01	0.04±0.01
Dry wt.	0.03±0.00	0.03±0.00	0.03±0.93(×10^{-3})	0.03±0.45(×10^{-3})	0.03±0.13(×10^{-3})
F_v/F_m	0.79±0.02	0.82±1.83(×10^{-3})	0.76±0.03	0.81±0.01	0.78±4.00(×10^{-3})
Photosynthesis (μmol·g^{-1}·s^{-1})	0.02±0.00	0.02±0.97(×10^{-3})	0.02±0.95(×10^{-3})	0.02±1.93(×10^{-3})	0.02±1.92(×10^{-3})
Photosynthesis/ respiration	3.89±0.92	3.33±0.08	2.94±0.01	2.35±0.52	2.61±0.33

Note: Only parameters that showed no differences attributable to N treatment are included.

more by the amount of N added than by the form of N applied (McNulty and Aber 1993; McNulty et al. 1996). To further assess the impact of N additions, differences between treatment means were evaluated using four mutually exclusive orthogonal contrasts: 0 kg N·ha^{-1}·year^{-1} versus all N additions, 15.7 and 19.8 versus 25.6 and 31.4 kg N·ha^{-1}·year^{-1}, 15.7 versus 19.8 kg N·ha^{-1}·year^{-1}, and 25.6 vs. 31.4 kg N·ha^{-1}·year^{-1}. These contrasts maximized the statistical power for evaluating the issues that we felt were of greatest scientific concern: (1) the influence of long-term N fertilization relative to a control and (2) the comparative impact of low versus high levels of N addition. Contrasts within low and high N fertilization groups provided additional resolution concerning the potential impacts of increased N fertilization. Correlation analyses were used to evaluate relationships among morphological or physiological parameters that showed a significant response to N treatment and foliar nutrient concentrations. The low level of replication (two plots per N treatment) provided limited power for detecting treatment impacts. Despite this, differences were considered statistically significant if $P \leq 0.05$.

Results

Treatment differences

Foliar nutrient status

N fertilization significantly increased foliar N concentrations: foliage from trees on N-fertilization plots had significantly higher levels of N than foliage from trees on control plots (Fig. 1). In addition, foliage from plots receiving higher doses of N (25.6 or 31.4 kg N·ha^{-1}·year^{-1}) had greater N concentrations than that from plots receiving lower N inputs (15.7 or 19.8 kg N·ha^{-1}·year^{-1}). Differences in foliar N levels were also detected within low and high N addition groups. However, these differences did not follow overall trends: foliage from plots that received 15.7 kg N·ha^{-1}·year^{-1} had higher concentrations of N than foliage from plots supplemented with 19.8 kg N·ha^{-1}·year^{-1}. Similarly, foliage from plots that received 25.6 kg N·ha^{-1}·year^{-1} had higher N concentrations than samples from plots treated with 31.4 kg N·ha^{-1}·year^{-1}. Although it is conceivable that some of the elevation in foliar N within sample plots that received 25.6 kg N·ha^{-1}·year^{-1} resulted from the combined application of NO_3 and NH_4, it is also possible that variations in atmospheric N inputs contributed to observed differences in foliar N incorporation. Plots that received the 25.6 kg N·ha^{-1}·year^{-1} treatment had slightly higher rates of N deposition (14 kg N·ha^{-1}·year^{-1}) compared with other treatment and control plots (12 kg N·ha^{-1}·year^{-1}) (McNulty and Aber 1993).

N fertilization also had an impact on the concentrations of other foliar nutrients. In comparison to foliage from control plots, N fertilization resulted in a significant reduction in Ca and Mg concentrations within needles (Fig. 1). However, no differences were detected in the concentrations of these elements among samples from the four N treatments.

N fertilization had no apparent impact on the foliar incorporation of potassium (K), phosphorus (P), or aluminum (Al) (Table 1). These results are consistent with the findings of Joslin and Wolfe (1994) for mature montane red spruce, which received 2 years of N fertilization. Although not significantly different, there was a tendency for trees within high N addition plots to have lower foliar Ca/Al ratios than trees from control and low N addition plots (Table 1). The mean foliar Ca/Al ratio for trees from the 31.4 kg N·ha^{-1}·year^{-1} treatment (24.88 ± 0.82) was approximately 2× higher than values associated with reductions in tree growth (Cronan and Gridal 1995).

Gross morphology

N fertilization had no discernible impact on the lengths or weights of shoots, on the dry weights of stems and needles, or on the number of buds per centimetre of stem (Table 1). However, when compared with plant material from control plots,

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Fig. 1. Treatment means and standard errors (±1 SE) of morphological, physiological, and nutritional measurements for red spruce trees from the Mount Ascutney, Vermont, study site. Where no standard errors are visible, error bars are too small to depict at the given scale. Only those parameters that showed a significant response to N treatment are included. Means with the same letter are not different ($P \leq 0.05$) based on the following orthogonal contrasts: 0 kg N·ha^{-1}·year^{-1} versus all N additions, 15.7 and 19.8 versus 25.6 and 31.4 kg N·ha^{-1}·year^{-1}, 15.7 versus 19.8 kg N·ha^{-1}·year^{-1}, and 25.6 versus 31.4 kg N·ha^{-1}·year^{-1}.

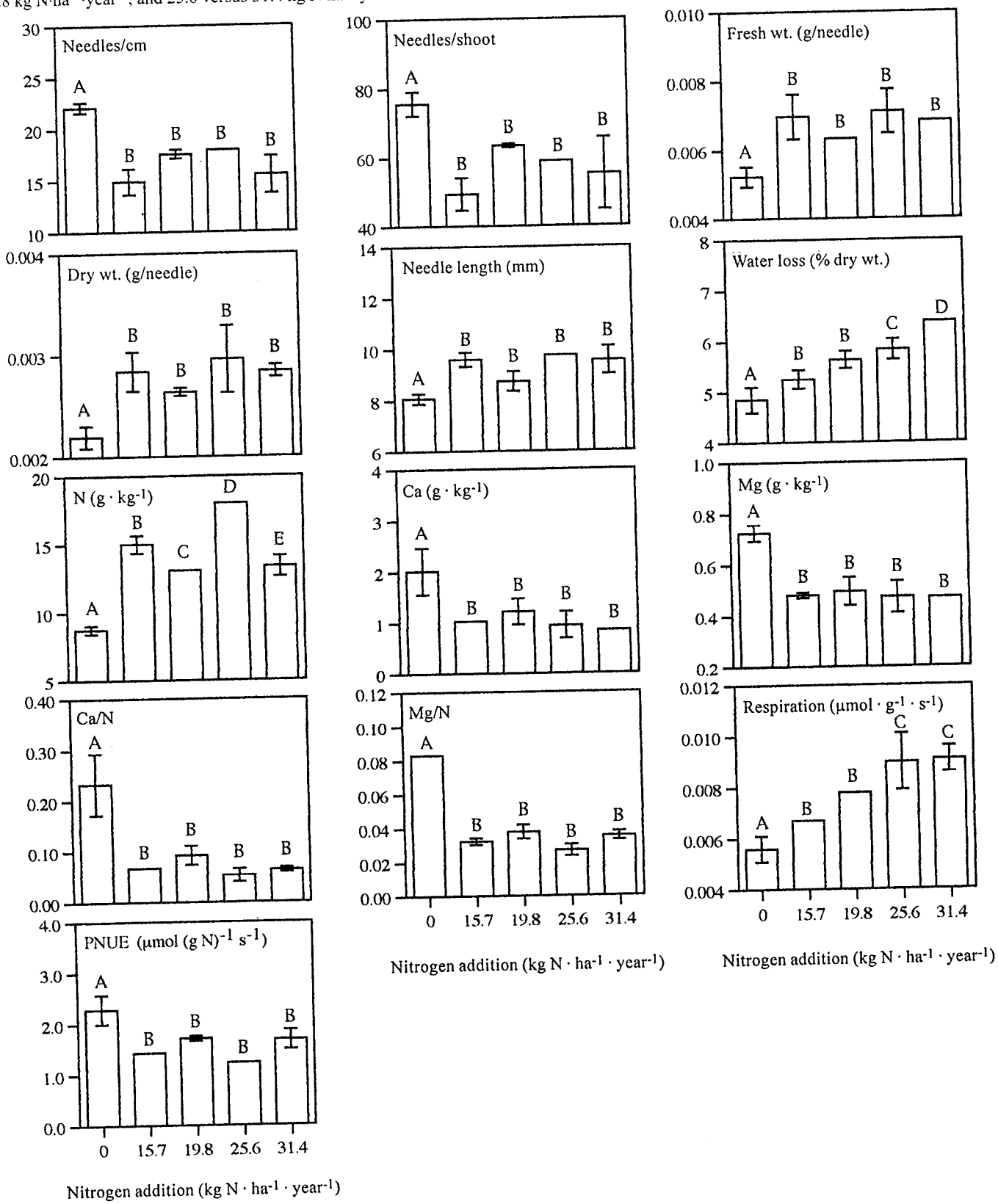

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Table 2. Correlation coefficients (*P*-values) for relationships among morphological or physiological parameters and foliar nutrient concentrations for red spruce trees from the Mount Ascutney, Vermont, study site.

	Foliar nutrient concentration							
	Ca	Mg	Al	P	N	Ca/Al	Ca/N	Mg/N
Needles/cm	0.422 (0.023)*	0.351 (0.058)	0.254 (0.183)	0.156 (0.410)	–0.408 (0.025)*	0.182 (0.344)	0.538 (0.002)**	0.492 (0.006)
Needles/shoot	0.303 (0.110)	0.218 (0.246)	0.218 (0.257)	0.044 (0.817)	–0.385 (0.036)*	0.101 (0.604)	0.412 (0.026)*	0.369 (0.045)*
Needle fresh wt. (g)	–0.462 (0.012)*	–0.508 (0.004)**	0.098 (0.615)	–0.299 (0.109)	0.540 (0.002)**	–0.448 (0.015)*	–0.574 (0.001)**	–0.621 (0.0003)**
Needle dry wt. (g)	–0.448 (0.015)*	–0.529 (0.003)**	0.156 (0.420)	–0.355 (0.054)	0.527 (0.003)**	–0.465 (0.011)*	–0.548 (0.002)**	–0.608 (0.0004)**
Needle length (mm)	–0.395 (0.034)*	–0.419 (0.021)*	–0.035 (0.857)	0.178 (0.346)	0.608 (0.0004)**	–0.247 (0.196)	–0.495 (0.006)**	–0.587 (0.001)**
Water loss (% dry wt.)	–0.231 (0.228)	–0.057 (0.765)	0.161 (0.404)	0.062 (0.745)	0.462 (0.010)*	–0.178 (0.357)	–0.284 (0.136)	–0.263 (0.160)
Respiration ($\mu mol \cdot g^{-1} \cdot s^{-1}$)	–0.492 (0.038)*	–0.688 (0.001)**	–0.046 (0.855)	–0.539 (0.017)*	0.288 (0.232)	–0.467 (0.050)*	–0.490 (0.039)*	–0.597 (0.007)**
PNUE ($\mu mol \cdot (gN)^{-1} \cdot s^{-1}$)	0.091 (0.721)	0.308 (0.200)	0.511 (0.030)*	0.105 (0.670)	–0.685 (0.001)**	–0.265 (0.289)	0.325 (0.188)	0.546 (0.016)*

Note: Only those parameters that showed a significant response to N treatment are included. *, **, correlation significant at the 0.05 or 0.01 level, respectively.

trees from N-fertilization plots had fewer needles (per centimetre of stem and per shoot) and existing needles were longer and heavier (on both a fresh- and dry-weight basis) (Fig. 1). No differences in gross morphology were detected among samples from treatments receiving fertilizer N.

Shoot water loss

Shoots from N-fertilization plots had significantly higher rates of water loss than shoots from control plots. Among N treatments, shoots from plots that received 15.7 or 19.8 kg $N \cdot ha^{-1} \cdot year^{-1}$ had lower rates of water loss than shoots from plots supplemented with 25.6 or 31.4 kg $N \cdot ha^{-1} \cdot year^{-1}$. No differences in shoot water content attributable to N treatment were detected (Table 1).

Chlorophyll concentration, chlorophyll fluorescence, and gas exchange

No differences in chlorophyll content, chlorophyll fluorescence, or net photosynthesis associated with N treatment were evident (Table 1). However, the mean photosynthetic rate per unit foliar N (PNUE) was greater in foliage from control plots (Fig. 1). No differences in PNUE were detected among samples from the various N treatments.

In general, N fertilization resulted in an increase in respiration. In comparison to trees on control plots, foliage from N-fertilized plots had higher rates of respiration (Fig. 1). Differences in respiration levels were also evident among the N treatments: foliage from plots receiving higher doses of N (25.6 or 31.4 kg $N \cdot ha^{-1} \cdot year^{-1}$) had greater rates of respiration than those from plots receiving lower N inputs (15.7 or 19.8 kg $N \cdot ha^{-1} \cdot year^{-1}$). No differences in respiration rates were detected for trees within low and high N addition groups. Although there was a trend for photosynthesis/respiration ratios to decrease with increasing N fertilization, treatment means were not significantly different (Table 1).

Correlations

Increases in environmental N availability can also influence the availability and uptake of other nutrients (Aber et al. 1995). To evaluate the possibility that N-treatment-induced alterations in morphology and physiology were influenced by alterations in the presence of other nutrients, we conducted a series of correlation analyses. Using data from individual trees, we evaluated the relationship between the nutrient status of foliage and those morphological or physiological parameters that showed a significant response to N fertilization (Table 2).

Measurements of gross morphology that showed a significant response to N fertilization were also significantly correlated with foliar N levels. However, these morphological measurements were also correlated with the foliar concentrations of one or more base cations. The number of needles per centimetre of shoot was positively correlated with Ca and Ca/N measurements, and the total number of needles per shoot was positively correlated with Ca/N and Mg/N levels. Needle weights (fresh and dry) were negatively correlated with Ca, Mg, Ca/Al, Ca/N, and Mg/N measurements, and needle length was negatively correlated with Ca, Mg, Ca/N, and Mg/N levels. In contrast, shoot water loss was positively correlated with foliar N concentrations, but not with any other nutrient.

Although differences in respiration attributable to N fertilization were detected, respiration rates were not significantly correlated with foliar N concentrations. However, respiration rates were negatively correlated with foliar concentrations of Ca, Mg, P, Ca/Al, Ca/N, and Mg/N. PNUE measurements were negatively correlated with needle N levels, but PNUE values were positively correlated with Al and Mg/N concentrations.

Discussion

Our results show that low-level but protracted N additions can significantly alter foliar chemistry, morphology, and physiology

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within montane red spruce. Although foliar N concentrations for trees from control plots were low relative to levels typical for the Northeast (Joslin et al. 1992), N treatment resulted in a significant increase in foliar N concentrations (Fig. 1). In fact, mean foliar N concentrations for trees from the two highest N treatments were at least 40% higher than those reported for mature red spruce in New England (Friedland et al. 1988; Fernandez et al. 1990; Huntington et al. 1990; McNulty et al. 1991).

N fertilization was also associated with a reduction in foliar Ca, Mg, Ca/N, and Mg/N levels (Fig. 1). Several processes probably contributed to treatment-associated impacts on foliar Ca and Mg concentrations. First, NH_4+ uptake by spruce roots may have had an antagonistic effect on the uptake of base cations (Schulze 1989). In addition, the anionic components of N treatments (NO_3– and Cl^-) likely leached cations from soils and reduced potentials for Ca^{2+} and Mg^{2+} uptake (Reuss and Johnson 1986). It is also plausible that anionic additions increased the proportion of Al^{3+} in the soil solution and that increased competition with Al^{3+} acted to limit Ca^{2+} and Mg^{2+} uptake (Reuss and Johnson 1986). However, foliar analyses showed no evidence of increased Al uptake with N treatment (Table 1).

Whatever the mechanism for treatment-induced reductions in foliar Ca and Mg levels, resulting concentrations were close to or below levels associated with deficiencies within red spruce. Foliar Ca concentrations of trees from N-treatment plots averaged 1.00 $g \cdot kg^{-1}$, which is within the 0.8–1.2 $g \cdot kg^{-1}$ transition zone from deficiency to sufficiency reported for greenhouse-grown seedlings (Swan 1971), and below the 1.7 $g \cdot kg^{-1}$ deficiency threshold established for saplings (Van Miegroet et al. 1993) and mature trees (Joslin and Wolfe 1994). Similarly, foliar Mg concentrations in N-treatment plots averaged 0.48 $g \cdot kg^{-1}$, which is within the 0.4–0.6 $g \cdot kg^{-1}$ range for the development of moderate Mg deficiencies determined for red spruce seedlings (Swan 1971).

N treatment was associated with a reduction in the number of needles produced, but the impact of this reduction was at least partially compensated for by concomitant increases in needle weight and length. Consequently, no treatment-induced differences in the total dry or fresh weights of needles per shoot were found (Table 1). No other alterations in morphology were found (Table 1).

N treatment was associated with increases in shoot water loss. However, because no treatment differences in water content were found (Table 1), the physiological importance of N-induced differences in water loss is uncertain.

No treatment-associated alterations in light capture (as indicated by chlorophyll content or chlorophyll fluorescence) or photosynthesis were evident (Table 1). However, N fertilization resulted in an increase in respiration, and the extent of this increase differed among N treatments. Respiration levels were highest for trees receiving 25.6 or 31.4 kg $N \cdot ha^{-1} \cdot year^{-1}$; the same treatments for which previously reported reductions in growth and levels of mortality were greatest (McNulty et al. 1996). Although we have no direct evidence that N-induced increases in respiration were causally related to the decline of trees on Mount Ascutney, elevated rates of respiration have been linked to the decline of red spruce exposed to other chemical inputs (McLaughlin et al. 1990, 1991; McLaughlin and Tjoelker 1992; McLaughlin et al. 1993). High levels of respiration may contribute to decline through a number of mechanisms (McLaughlin and Kohut 1992). For example, high respiration rates could deplete carbon reserves and reduce the availability of carbohydrates used to support root growth and nutrient uptake. Reduced carbon storage may also diminish carbohydrate support of cellular defense and repair mechanisms and, thereby, lower overall stress resistance. And, increases in respiration could be particularly disruptive to the carbon relations of mature trees (like the ones in our study) that have a higher proportion of respiring stem and root tissues and, thus, a less favorable net carbon balance than young trees.

Kawahara et al. (1976) reported that high foliar N concentrations may themselves result in enhanced respiration rates for conifers. This relationship presumably occurs because most of the organic N in plants exists within proteins and because approximately 60% of maintenance respiration supports protein repair and replacement (Penning de Vries 1975). However, in N-rich environments such as those resulting from N fertilization, much of the N in plant tissues may exist as unassociated amino acids rather than proteins (van Dijk and Roelofs 1988). Because of this, there is often no clear relationship between respiration and N levels in fertilized trees (Sprugel et al. 1995). We found that N treatment resulted in higher foliar N concentrations, but that foliar N and respiration levels were not significantly correlated. The lack of relationship between foliar respiration and N levels suggests that the influence of N treatment on respiration was indirect.

N treatment was also associated with a significant reduction in the foliar concentrations of Ca, Mg, Ca/N and Mg/N. And, in contrast with foliar N levels, concentrations of Ca, Mg, Ca/N and Mg/N were significantly and negatively correlated with respiration rates. These negative relationships between Ca or Mg concentrations and respiration levels raise the possibility that N treatment-induced increases in respiration may be mediated through alterations in base cation availability. For example, high respiration rates could result from reductions in membrane integrity associated with a deficiency in Ca (Bangerth 1979).

Considerable research has now linked acidic inputs (which include N) to reductions in foliar cation levels and alterations in carbon metabolism (McLaughlin et al. 1990, 1991; McLaughlin and Tjoelker 1992; McLaughlin et al. 1993). Field studies have shown that red spruce in high-elevation forests that receive large inputs of acid deposition have higher respiration rates (McLaughlin et al. 1990, 1991), lower ratios of photosynthesis to respiration, and lower foliar Ca and Mg concentrations (McLaughlin et al. 1990; McLaughlin and Tjoelker 1992) than spruce at lower elevations. Controlled exposures of red spruce to acidic inputs have provided more direct evidence of a link between acid-induced changes in gas exchange and foliar cation concentrations: seedlings that received pH 3.0 and 3.8 mist treatments had lower foliar Ca and Mg concentrations, lower photosynthesis to respiration ratios, and higher respiration rates than seedlings treated with pH 5.0 mists (McLaughlin and Tjoelker 1992; McLaughlin et al. 1993). Significant correlations between foliar cation concentrations and carbon exchange measurements have raised the possibility of a causal relationship between these processes (McLaughlin et al. 1991, 1993; McLaughlin and Tjoelker 1992). And, experimental additions of base cations to red spruce exposed to acid mists have helped to support the hypothesis of a

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causal link: saplings that received Ca and (or) Mg supplements experienced a partial reversal of acid-mist-induced increases in respiration (McLaughlin and Tjoelker 1992). Based on these and other findings, McLaughlin and Kohut (1992) concluded that acid-deposition-induced disruptions of mineral nutrition and carbon relations are important and interrelated components that can contribute to the decline of red spruce.

Previous work at the Mount Ascutney study site showed that protracted N fertilization was associated with the reduced growth and increased mortality of red spruce (McNulty et al. 1996). In the current study, we documented that N application was also associated with increased rates of respiration and reduced foliar Ca and Mg concentrations. Similar patterns of N-treatment-induced increases in respiration (Fig. 1) and decline (McNulty et al. 1996) may indicate a link between these processes. And, significant correlations between respiration rates and foliar Ca and Mg levels raise the possibility of a causal relationship between N-induced increases in respiration and alterations in base cation nutrition. Parallels between these findings and results from studies of the impacts of acid precipitation suggest that disruptions in cation nutrition and carbon relations similar to those attributed to acidic inputs may also occur via N additions alone. If our results from Mount Ascutney reflect the impacts of protracted N deposition on montane spruce–fir forests, then important questions regarding the adequacy of current provisions for reducing N deposition must be addressed. Given the economic costs of pollution control and the preliminary nature of existing data, further study of the environmental impacts of N accumulation is needed.

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