



## Foliar free polyamine and inorganic ion content in relation to soil and soil solution chemistry in two fertilized forest stands at the Harvard Forest, Massachusetts

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Received 24 September 1999. Accepted in revised form 29 February 2000

**Key words:** ammonium nitrate, calcium, Harvard Forest, magnesium, nitrate leaching, polyamines

### Abstract

Polyamines (putrescine, spermidine, and spermine) are low molecular weight, open-chained, organic polycations which are found in all organisms and have been linked with stress responses in plants. The objectives of our study were to investigate the effects of chronic N additions to pine and hardwood stands at Harvard Forest, Petersham, MA on foliar polyamine and inorganic ion contents as well as soil and soil solution chemistry. Four treatment plots were established within each stand in 1988: control, low N (50 kg N ha<sup>-1</sup> yr<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>), low N + sulfur (74 kg S ha<sup>-1</sup> yr<sup>-1</sup> as Na<sub>2</sub>SO<sub>4</sub>), and high N (150 kg N ha<sup>-1</sup> yr<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>). All samples were analyzed for inorganic elements; foliage samples were also analyzed for polyamines and total N. In the pine stand putrescine and total N levels in the foliage were significantly higher for all N treatments as compared to the control plot. Total N content was positively correlated with polyamines in the needles ( $P \leq 0.05$ ). Both putrescine and N contents were also negatively correlated with most exchangeable cations and total elements in organic soil horizons and positively correlated with Ca and Mg in the soil solution ( $P \leq 0.05$ ). In the hardwood stand, putrescine and total N levels in the foliage were significantly higher for the high N treatment only as compared to the control plot. Here also, total foliar N content was positively correlated with polyamines ( $P \leq 0.05$ ). Unlike the case with the pine stand, in the hardwood stand foliar polyamines and N were significantly and negatively correlated with foliar total Ca, Mg, and Mn ( $P \leq 0.05$ ). Additional significant ( $P \leq 0.05$ ) relationships in hardwoods included: negative correlations between foliar polyamines and N content to exchangeable K and P and total P in the organic soil horizon; and positive correlations between foliar polyamines and N content to Mg in soil solution. With few exceptions, low N + S treatment had effects similar to the ones observed with low N alone for both stands. The changes observed in the pine stand for polyamine metabolism, N uptake, and element leaching from the soil into the soil solution in all treatment plots provide additional evidence that the pine stand is more nitrogen saturated than the hardwood stand. These results also indicate that the long-term addition of N to these stands has species specific and/or site specific effects that may in part be explained by the different land use histories of the two stands.

**Abbreviations:** Perchloric acid (PCA), Polyamines (PAs), Zero tension lysimeter (ZTL), hardwoods (HW)

### Introduction

There is increasing concern about the potential adverse effects of elevated rates of N deposition on water

quality and the health of forest ecosystems (Aber et al., 1989, 1998; Rasmussen and Wright, 1998). This concern stems from the fact that in 1990 the United States (US) Clean Air Act targeted a 50% reduction in S deposition but only a 10% reduction in N depos-

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ition (McNulty et al., 1996). Although most temperate forests are N limited, continuous deposition of N from the atmosphere can move them towards nitrogen saturation. Nitrogen saturation has been defined as the availability of ammonium and nitrate in excess of total combined plant and microbial nutritional demand (Aber et al., 1989). It is important to understand how chronic additions of N to ecosystems change the structure and function of forest ecosystems (Asner et al., 1997; Jefferies and Maron, 1997).

Long-term elevated N deposition typically leads to an increase in the concentration of total foliar N, with or without similar changes in the important base elements such as Ca, Mg and K (Aber et al., 1995; Magill et al., 1997, 1999; Rasmussen and Wright, 1998; van Dijk and Roelofs, 1988). This increase in leaf N content also leads to significant shifts in the internal partitioning of N within the leaf. For example in conifers, N deposition significantly increases leaf N present in the form of free amino acids such as arginine (Ericsson et al., 1993, 1995; Näsholm et al., 1997). Little is known about N partitioning for hardwoods under these conditions. Lawlor (1992) suggested that these changes in N partitioning are probably not related to leaf function. This idea, however, has yet to be experimentally tested in terms of whether the alterations in N partitioning due to long-term N deposition actually have a positive or a negative effect on photosynthetic capacity and biomass production. A possible decoupling of the relationship between foliar N and photosynthetic rate may occur under these conditions.

The aliphatic polyamines (putrescine, spermidine, and spermine) play an important role in the growth and development of all living organisms. They are metabolically derived from the amino acids arginine and ornithine, and at cellular pH they carry a net positive charge (Cohen, 1998). Abiotic stress conditions such as low pH, high SO<sub>2</sub>, high salinity, osmotic shock, nutrient stress, low temperature (Flores, 1991 and references therein) and high Al (Minocha et al., 1992, 1996) all result in an increase in cellular putrescine levels. Polyamines show a reverse proportionality to cellular elements such as Ca, Mg, Mn, and K in response to Al treatment in periwinkle (*Catharanthus roseus*) and red spruce (*Picea rubens*) cells (Minocha et al. 1992, 1996; Zhou et al., 1995). A key distinction between the polyamines (organic cations) and the inorganic elements is that, even if the latter (e.g. Ca<sup>2+</sup> and Mg<sup>2+</sup>) undergo re-compartmentalization in response to external stimuli, their cellular levels are derived mainly from uptake across the biological membranes

and this uptake ultimately depends upon their availability in the soil or soil solution. In contrast, polyamines are synthesized within the cell, permitting adjustment of their cellular concentrations to meet physiological needs. Also, cellular polyamine levels can be regulated by conjugation, degradation, and sequestration via enzymatic means (Minocha et al., 1996). Thus polyamine synthesis may play an important role in the survival of plants under stress (Galston, 1989). Recent work examining the concentration of polyamines in plant foliage has been aimed at using foliar polyamine concentrations as indicators of stress (Dohmen et al., 1990; Minocha et al., 1997; Santerre et al., 1990). In the case of mature red spruce trees, an increase in foliar putrescine concentration was associated with a decrease in foliar and soil Ca and Mg concentrations and an increase in the Oa horizon soil Al or Al:Ca ratios (Minocha et al., 1997).

Structurally, polyamines are composed of carbon, hydrogen, and nitrogen. Therefore, we suspect that their levels may also change in response to chronic N additions, thus affecting the internal N partitioning within the leaf, a situation similar to that observed with free amino acids in conifers. The objective of this study was to determine the effects of chronic additions of N on: (1) foliar polyamines (a proposed stress indicator) and inorganic ions; (2) soil and soil solution inorganic ion chemistry, especially Al mobilization; and (3) the relationship between polyamines and soil chemistry in pine and hardwood stands.

## Materials and methods

### Study sites

The study plots are located at Harvard Forest, Petersham, MA (42°30' N, 72°10' W). This site is a part of the National Science Foundation's Long-Term Ecological Research (LTER) program. These plots are a part of the ongoing study on chronic nitrogen additions since 1988 (Aber et al., 1993; Magill et al., 1997, 1999). An even-aged red pine (*P. resinosa*) stand, 74 yr old, and an adjacent mixed hardwood stand, approx. 55 yr old, were chosen for this study. The hardwood stand is dominated by black oak (*Quercus vetulina* Lam.) and red oak (*Q. Rubra* Michx. f., respectively) with significant amounts of black birch (*Belutinaa lenta* L.), red maple (*Acer rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.). Most of the currently forested area at this site was in cultivation or pastureland during the mid-1800's (Foster, 1992). The

dominant soil types are stony sandy loams classified as Typic Dystrochrepts. Mean annual temperature ranges from 19 °C in July to -12 °C in January and mean total annual precipitation is 112 cm. The estimated nitrogen deposition to the forest is about 6 kg ha<sup>-1</sup> yr<sup>-1</sup> wet and 2 kg ha<sup>-1</sup> yr<sup>-1</sup> dry (Aber et al., 1993; Magill et al., 1997; Ollinger et al., 1993).

#### *Treatments*

Four treatment plots were established within each stand: control, low N, low N + sulfur (N+S), and high N. Each plot measured 30 × 30 m (0.09 ha) and was divided into 36 subplots (each 5 × 5 m). Fertilizer additions of NH<sub>4</sub>NO<sub>3</sub> and Na<sub>2</sub>SO<sub>4</sub> began in 1988 as six equal applications over the growing season. The fertilizer was weighed, mixed with 20 L of water (equivalent to 0.002 cm rainfall) and applied using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of fertilizer.

As described in Magill et al. (1997), a partial application was made in year 1 (1988). The total amount of fertilizer applied was 38 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the low nitrogen treatment and the nitrogen portion of the N+S treatment, 113 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the high nitrogen treatment, and 74 kg (S) ha<sup>-1</sup> yr<sup>-1</sup> to the N+S plots. Applications for all following years were at the rate of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the low and N+S plots and 150 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the high N plots. Sulfur additions remained the same as used for year one.

#### *Collection and analyses of needle samples*

Foliage samples were collected during the first week of August each year from mid to upper canopy branches of dominant or co-dominant trees using a shotgun. Early August was chosen as the sampling time because the trees were still physiologically very active at this time, compared to early or late summer. At each sampling time, current-year needle samples were collected from 20 different red pine trees in the pine stand and leaves from 10 different trees of black or red oak and red maple each were collected from the hardwood plots. A sub-sample was taken from each individual tree collection for analyses of polyamine and exchangeable inorganic elements. The remaining pine needle samples from 20 different trees in each plot were pooled into 5 composite samples of 4 trees per sample for total inorganic elements and N analyses. Similarly, the remaining samples from 10–12 trees per species collected from each plot in the hardwood stand

were pooled into 4 composite samples. Red and black oak were treated as a single species in all collections.

#### *Total elements and nitrogen analyses*

The composite samples were dried at 70 °C for 48 h and ground using a Wiley mill with a 1 mm mesh screen. The ground samples were dried overnight at 70 °C and analyzed for N content using near-infrared (NIR) spectroscopy (Bolster et al., 1996; McLellan et al., 1991). These samples were also used for extraction of total inorganic ion content by a modification of the method of Isaac and Johnson (1976) as described in Minocha and Shortle (1993). The extracts were analyzed for total Ca, Mg, Mn, K, Al, and P content using a Beckman Spectrospan V ARL DCP (Direct Current Plasma Emission Spectrometer, Beckman Instruments, Inc., Fullerton, CA) using the Environmental Protection Agency's method number 66-AE0029 (1986).

#### *Analysis of polyamines and exchangeable inorganic elements*

The fresh foliage samples were placed in individual pre-weighed microfuge tubes containing 1 ml of 5% perchloric acid (PCA). The tubes were kept on ice during transportation to the laboratory and then stored at -20 °C until they were processed. The samples were weighed, frozen and thawed (3X), and centrifuged at 13,000 rpm in a microfuge for 10 min. Details of the freeze-thawing extraction procedure are described in Minocha et al. (1994). The freeze-thawing method was also chosen for the extraction of exchangeable fraction of inorganic ions. This method extracted a consistent fraction of the total acid extractable inorganic ions from foliage of various species of mature trees and the quantity of this fraction varied for each ion type and tree species (Table 1 and Minocha et al., 1994). The moisture content data for individual exchangeable ion samples was not collected. Therefore, for comparison of total and exchangeable ions on weight basis, an average moisture content of 53%, 62%, and 61% has been used for this site from data collected in 1992 for pine ( $n=19$ ), oak ( $n=33$ ), and maple ( $n=45$ ), respectively to convert fresh weight to dry weight.

Briefly, the samples for both inorganic ions as well as for polyamines were frozen at -20 °C and thawed at room temperature, repeating the process two more times. Duration of the freezing step could vary from 4

*Table 1.* Comparison of effects of nitrogen treatments on total and exchangeable inorganic ion levels in the foliage of pine, oak and maple trees. Data has been averaged over three year period (1995–1997). Data for exchangeable ions are mean  $\pm$  se of  $n=60$  for pine stand and  $n=30$  for hardwoods. Data for total ions are mean  $\pm$  se of  $n=15$  for pine stand and  $n=12$  for hardwoods. The numbers in parenthesis indicate % of total ions extracted as exchangeable. The moisture content data for individual exchangeable ion samples was not collected. Therefore, for comparison of total and exchangeable ions on weight basis, an average moisture content of 53%, 62%, and 61% has been used for this site from data collected in 1992 for pine ( $n=19$ ), oak ( $n=33$ ), and maple ( $n=45$ ), respectively to convert FW to DW

Treatment	Ion	Inorganic ions	Pine ( $\mu\text{mol g}^{-1}$ DW)	Oak ( $\mu\text{mol g}^{-1}$ DW)	Maple ( $\mu\text{mol g}^{-1}$ DW)
Control	Ca	Total	62.5 $\pm$ 4.8	121.5 $\pm$ 5.9	156.0 $\pm$ 6.8
Low N			72.3 $\pm$ 5.1	113.5 $\pm$ 8.1	137.0 $\pm$ 6.7
High N			60.0 $\pm$ 5.5	71.8 $\pm$ 6.2	116.4 $\pm$ 6.1
Control		Exchangeable	11.4 $\pm$ 0.6 (18.3%)	102.1 $\pm$ 5.9 (84.0%)	79.2 $\pm$ 4.7 (50.8%)
Low N			11.9 $\pm$ 0.7 (16.5%)	87.1 $\pm$ 4.7 (76.7%)	69.7 $\pm$ 2.3 (50.9%)
High N			9.9 $\pm$ 0.9 (16.5%)	57.3 $\pm$ 5.0 (79.9%)	59.2 $\pm$ 3.4 (50.9%)
Control	Mg	Total	32.4 $\pm$ 1.5	62.8 $\pm$ 2.1	64.7 $\pm$ 3.3
Low N			35.5 $\pm$ 1.3	63.1 $\pm$ 2.3	59.9 $\pm$ 1.3
High N			35.4 $\pm$ 1.3	54.1 $\pm$ 3.3	55.7 $\pm$ 2.9
Control		Exchangeable	13.6 $\pm$ 0.5 (41.9%)	56.1 $\pm$ 3.4 (89.3%)	43.7 $\pm$ 2.3 (67.5%)
Low N			14.7 $\pm$ 0.4 (41.3%)	58.0 $\pm$ 3.4 (91.9%)	43.5 $\pm$ 1.4 (72.7%)
High N			13.5 $\pm$ 0.4 (38.1%)	51.0 $\pm$ 3.0 (94.2%)	38.5 $\pm$ 1.9 (69.1%)
Control	Mn	Total	16.7 $\pm$ 1.1	41.6 $\pm$ 2.3	37.4 $\pm$ 2.3
Low N			22.4 $\pm$ 2.1	27.3 $\pm$ 2.0	23.6 $\pm$ 0.9
High N			15.8 $\pm$ 1.6	15.3 $\pm$ 0.9	22.5 $\pm$ 1.9
Control		Exchangeable	3.6 $\pm$ 0.2 (21.3%)	50.8 $\pm$ 3.5 (122.1%)*	21.1 $\pm$ 1.5 (56.6%)
Low N			3.9 $\pm$ 0.3 (17.3%)	33.4 $\pm$ 2.4 (122.4%)*	14.5 $\pm$ 1.0 (61.6%)
High N			3.2 $\pm$ 0.4 (20.5%)	17.4 $\pm$ 1.5 (113.4%)*	11.1 $\pm$ 0.9 (49.5%)
Control	K	Total	120.1 $\pm$ 6.7	218.7 $\pm$ 9.5	184.6 $\pm$ 11.8
Low N			125.5 $\pm$ 7.2	265.7 $\pm$ 12.0	171.6 $\pm$ 10.5
High N			135.3 $\pm$ 7.4	202.2 $\pm$ 9.4	176.0 $\pm$ 9.7
Control		Exchangeable	72.8 $\pm$ 3.8 (60.6%)	158.0 $\pm$ 6.8 (72.3%)	132.2 $\pm$ 6.0 (71.6%)
Low N			79.6 $\pm$ 3.6 (63.4%)	165.1 $\pm$ 7.2 (62.2%)	138.8 $\pm$ 5.9 (80.9%)
High N			84.0 $\pm$ 3.2 (62.1%)	157.5 $\pm$ 6.7 (77.9%)	147.0 $\pm$ 5.8 (83.5%)

\* The calculation of greater than 100% extraction for Mn in the case of oak was possibly caused by slight overestimation of the mean moisture content for 1995–1997.

h to a few days. Samples were allowed to thaw completely (approximate time 1–1.5 h) before refreezing. After freeze-thawing, the samples were centrifuged at  $13,000 \times g$ . This supernatant was used directly for free polyamine analysis without further dilution and for inorganic-ion analysis after proper dilution with distilled, deionized water (final concentration of PCA 0.01 or 0.02 N) by the procedures described below. The diluted fractions were analyzed for inorganic ion content with a Beckman Spectrospan V ARL DCP as described above. For quantitation of polyamines, heptanediamine was added as an internal standard to aliquots of the above extracts prior to dansylation. Fifty or one hundred  $\mu\text{L}$  of the extract were dansylated according to the procedure described in Minocha et al. (1990). Dansylated polyamines were separated by reversed phase HPLC (Perkin-Elmer Corp., Norwalk,

CT) using a gradient of acetonitrile and heptane-sulfonate, and quantified by a fluorescence detector (Minocha et al., 1990).

#### *Collection and analyses of soil and soil solution samples*

Three sets of two adjacent soil cores (<30 cm apart) were taken to a depth of 10 cm in the mineral soil in each of the three designated subplots (nine samples per plot). Cores were split into organic (Oe+Oa) and mineral horizons (top 10 cm) and placed in gas-permeable polyethylene bags. The soils were air-dried, sieved (<2 mm size), and stored at room temperature prior to analyses. Before analyses, the mineral soil and organic soil samples were oven-dried at 105 °C and 70 °C, respectively.

Exchangeable inorganic elements were determined from a sub-fraction of the above samples using a modification of the procedure of Taylor (1987). Briefly, either 6 g of mineral soil or 1 g of organic soil was added to 30 ml of extraction solution (0.05 N HCl and 0.025 N H<sub>2</sub>SO<sub>4</sub>) and placed on a gyratory shaker at 90 rpm for 15 min. The extract was filtered with a glass fiber syringe filter (Gelman A/E, Gelman Sciences, Ann Arbor, MI) and stored at 4 °C until quantitation by DCP.

Prior to digestion for total elemental analysis, a sub-sample of air-dried and sieved soil was processed for 1 min in a Shatter Box Laboratory Mill (Spex Industries, Inc., Edison, NJ) to powder the sample. Microwave digestion (Hallett and Hornbeck, 1997) was used for obtaining inorganic elements. Briefly, for mineral soils, 0.1 g sample was digested with 5 ml of concentrated HNO<sub>3</sub>, 2 ml of concentrated HCl, 2 ml of fluoroboric acid (HBF<sub>4</sub>) and 2 ml of H<sub>2</sub>O<sub>2</sub>. For organic soils, 0.1 g sample was digested the same way with acids but without the presence of H<sub>2</sub>O<sub>2</sub>. The following microwave programs were used. Given in pairs are: Time (min)–Power (Watts). For mineral soil, 1–250, 1–0, 5–250, 5–400, 5–500, and 5–600. For organic soil, 1–250, 1–0, 5–250, 5–400, 1–0, 5–500, 1–0, 5–600, 1–0, and 2.5–650. Total running time was 27.5 min.

The details on installation of zero tension lysimeters (ZTL) and soil solution sample collection are described in Currie et al. (1996) and McDowell et al. (1998). Briefly, 5 polyethylene ZTL's were installed per plot except for N+S plots. Solutions were collected after major rain events and all 5 samples per plot were pooled prior to analyses. Over the course of three years (1995–1997), the collections were made approx. 50 times from each plot. Samples were transported on ice to the laboratory and filtered through pre-combusted Whatman GF/F glass fiber filters (Whatman Inc., Clifton, NJ) within 36 h of collection before freezing. These solutions were analyzed for inorganic elements using DCP as described earlier.

#### *Statistical methods*

Linear regression analyses were performed to establish the strength and significance of relationships between two different variables ( $n=12$  except for soil samples where  $n=8$ ) using Excel 5.0 for Windows (Microsoft Corporation, Roselle, IL). Data for each variable (e.g., foliar or soil Ca, Mg and Al) were analyzed as a series of one-way analysis of variance (ANOVA)

to determine whether statistically significant differences occurred between control and treatment plots for each individual variable. When F values for one-way ANOVA were significant, differences in treatment means were tested by Tukey's multiple comparisons test. ANOVA and Tukey's tests were performed with Systat for Windows, version 7.01 (SYSTAT Inc., Evanston, IL) and a probability level of 0.05 was used for tests unless specified otherwise.

## **Results**

With few exceptions, the low N + S treatment showed effects similar to the ones observed for low N treatment alone for both stands. For this reason, results for low N + S will not be discussed separately. Also, in foliage exchangeable ions always represented a consistent fraction of the total ions. Even though the quantity of this fraction varied for each ion type and tree species, nitrogen treatments had similar effects on both exchangeable and total Ca, Mg, Mn, and K levels in each case for all three species (Table 1). Thus due to this similarity of trends between exchangeable and total ions, only total inorganic ion data are used for further comparison of foliar results with soil and soil solution data.

#### *Foliar polyamines and N content*

*Pine:* There was a significant increase in the level of putrescine in the needles of trees growing on all three treatment plots as compared to the control plot (Figure 1A–D). A small but statistically significant increase was also observed in spermidine levels in response to high N treatment. Spermine, which was a relatively small proportion of free polyamines in red pine, increased significantly in response to low and high N additions. In spite of year to year variations in the total amounts of polyamines (possibly due to different growth conditions resulting from variable weather patterns), similar trends were observed for each of the three years of data collection.

*Hardwoods:* Putrescine levels in oak leaves were three- to four-fold higher in response to high N treatment for each of the three years of this study (Figure 2A–D). For other treatments no significant change was observed. The level of spermidine and spermine did not change significantly in response to low and high N treatments. While there were annual variations

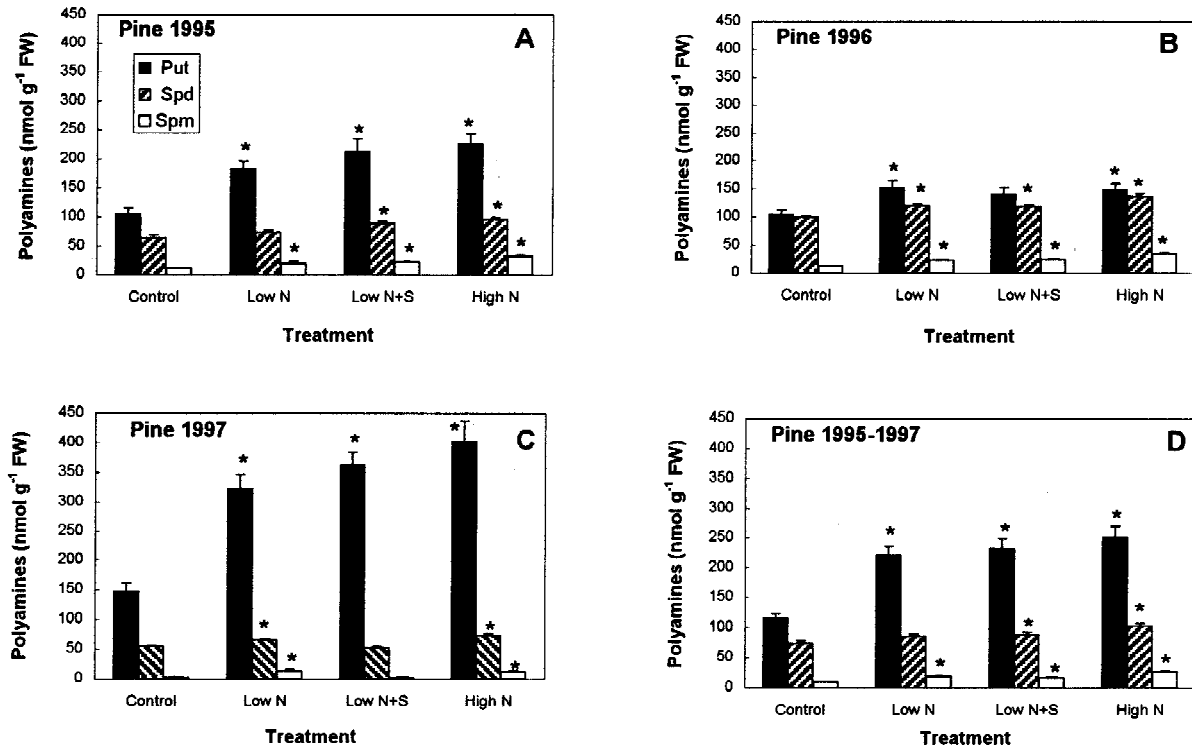


Figure 1. The effect of chronic N additions on foliar polyamines in pine. Data presented for each treatment are mean  $\pm$  SE of  $n=20$  for A–C. Figure 1D presents cumulative data for three years ( $n=60$ ). The asterisks denote the significant differences from control.

in the total amount of polyamines, the trends were the same for each year.

High N treatment caused a significant increase in putrescine levels in maple leaves (Figure 3A–D) for two of the three years. Spermidine and spermine levels were also present in quantities comparable to putrescine in maple leaves. For 1995, a parallel increase in spermidine was also observed (Figure 3A). No change in spermine content was observed for any of the three years. As with pine and oak, year to year variation in the three polyamines was also observed in maple.

The total N content of pine needles and maple leaves increased in response to all N treatments (Figure 4A and 4C). The total N content in oak foliage also rose significantly but only in response to the high N treatment (Figure 4B). The changes in N were always positively and significantly correlated with changes in foliar polyamines for all three species (Figure 4A–C).

#### Foliar inorganic elements

**Pine:** No significant changes in total foliar Ca, Mg, Mn, and K were observed in response to chronic N additions to the soil. However, there was a decrease in total Al concentration in response to all N treatments

and an increase in total P with high N treatment only (Figure 5).

**Hardwoods:** Total Ca and Mn levels showed a significant decrease in response to high N treatment in both maple and oak leaves (Figure 6). Changes in total Mn were also significant for low N treatment in both species. The only other statistically significant change observed was a decrease in P with all N treatments in maple. Mg showed a statistically insignificant decrease with high N addition for both species.

#### Foliar Al:Ca and Mg:N ratios

A significant decrease in foliar total Mg:N and Al:Ca ratios was observed with all N treatments in case of pine (Figure 7A–B). In contrast, an increase in the Al:Ca ratio (though statistically insignificant for maple) accompanied by a decrease in total Mg:N ratio was observed in response to high N treatment for maple and oak (Figure 7C–F).

#### Inorganic elements in the organic horizon of soil

**Pine:** A significant decrease in exchangeable Ca, Mg, Mn, and K in the organic soil was observed in

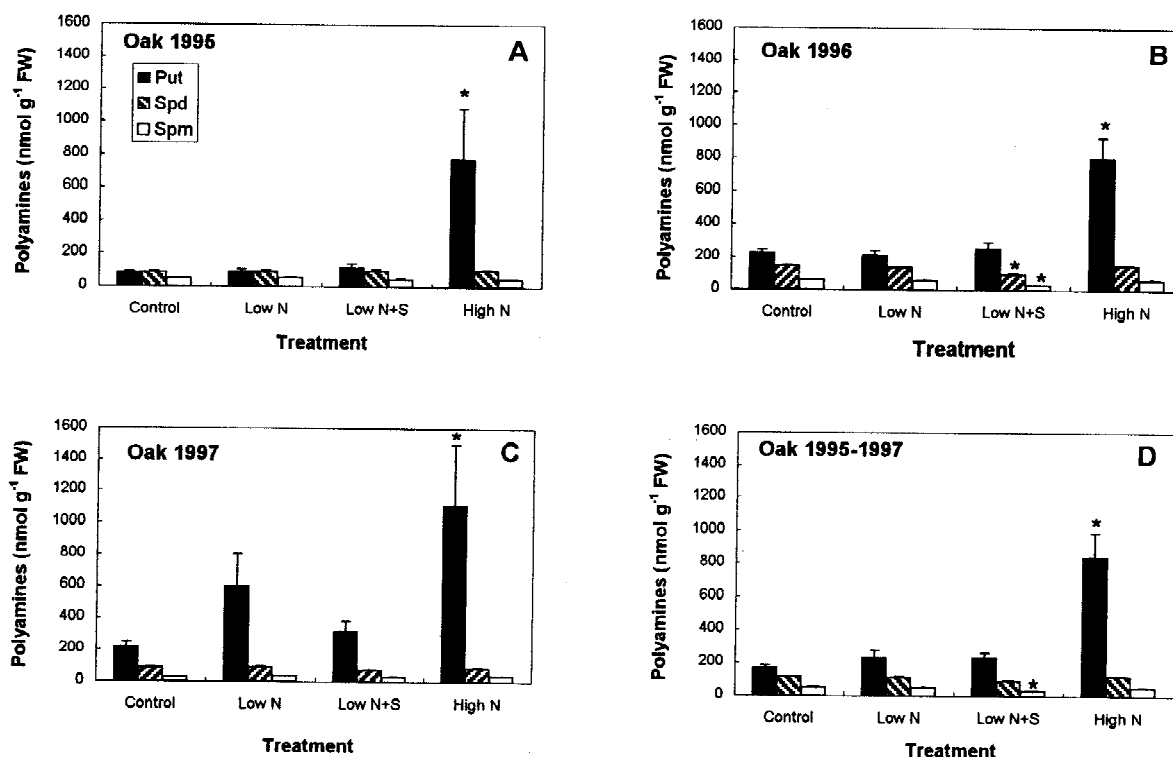


Figure 2. The effect of chronic N additions on foliar polyamines in oak. Data presented for each treatment are mean $\pm$ SE of  $n=10$  for A–B and  $n=5$  for C. Figure 2D presents cumulative data for three years ( $n=25$ ). The asterisks denote the significant differences from control.

response to all N treatments. There was an increase in exchangeable P in response to the low N treatment only (Figure 8: Pine). However, there was no significant effect of these treatments on exchangeable Al levels. Whereas total Ca decreased in response to high N treatment only, total Mn and P decreased in response to all N treatments with no significant change in K in the organic soil. A slight but statistically insignificant decrease in the level of total Mg and Al with N treatments was also observed (Figure 9: Pine).

*Hardwoods:* N additions alone had no significant effect on exchangeable or total Ca levels of the organic horizon. However, exchangeable Mg, K, and P concentrations were significantly lower in high N plots with Mn being low in all N treatment plots (Figure 8: HW). Total Mg, Mn, and P levels decreased in response to all N treatments in this soil horizon (Figure 9: HW).

#### *Inorganic elements in the mineral horizon of soil*

*Pine:* Whereas changes in the exchangeable Ca, Mg, Mn, and K did not show any specific and statistically

significant patterns, P increased significantly in relation to all N additions in the mineral soil. Both low and high N treatments decreased the exchangeable Al concentrations significantly in this soil layer (Figure 10: Pine).

*Hardwoods:* The exchangeable ion chemistry of the mineral horizon showed decreases in the levels of some inorganic elements, but the changes were not statistically significant except for Al in the high N plot (Figure 10: HW).

#### *Inorganic elements in zero tension lysimeter (ZTL) solution*

*Pine:* A significant increase was observed in the levels of all inorganic elements tested in response to high N addition. Levels of P and Al were high even for low N treatment (Figure 11: Pine).

*Hardwoods:* There was typically a small increase in the levels of most elements in the ZTL solution samples in response to N treatments, but the only significant increase that was observed was in Al levels in response to high N treatment (Figure 11: HW).

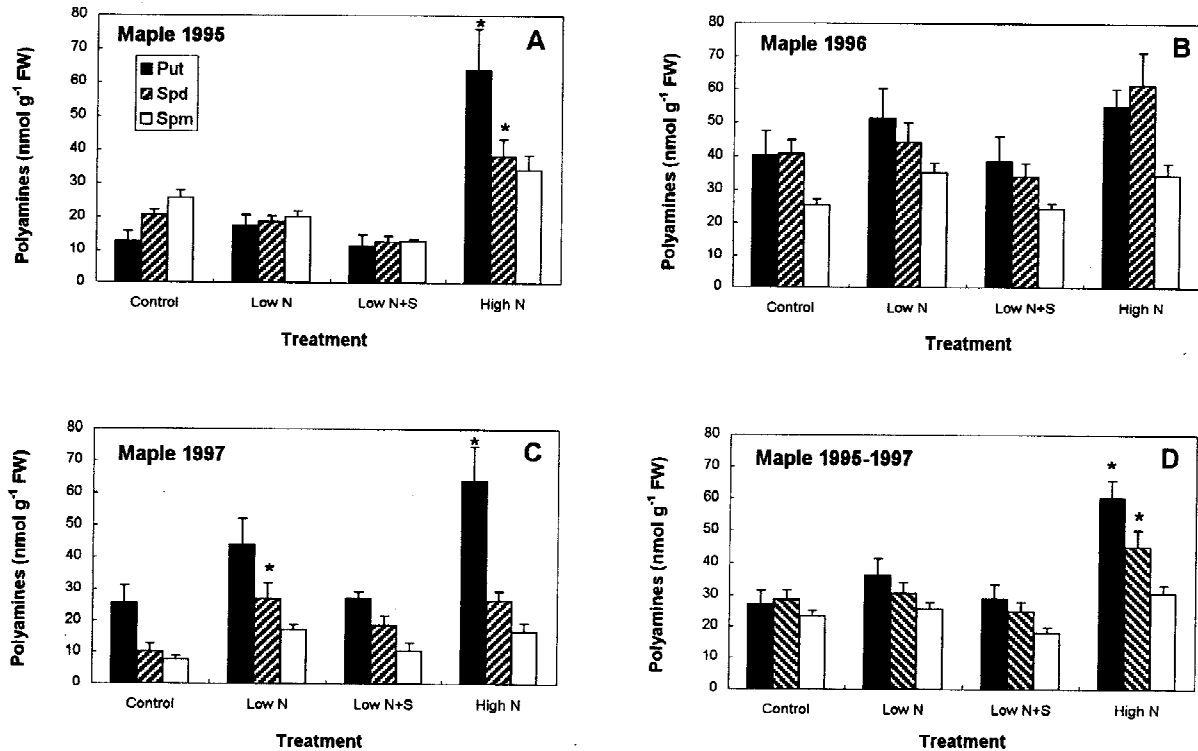


Figure 3. The effect of chronic N additions on foliar polyamines in maple. Data presented for each treatment are mean $\pm$ SE of  $n=10$  for A–B with one exception and  $n=4$  for C. Figure 3D presents cumulative data for three years ( $n=24$ ). The asterisks denote the significant differences from control.

#### Relationship between foliar and soil chemistry

**Pine:** Putrescine levels did not correlate with most inorganic elements in the pine needles. However, putrescine was negatively correlated with all but P of the exchangeable and all of the total inorganic ions in the organic soil horizon (Table 2). There was no correlation between putrescine or total polyamines and exchangeable elements in the mineral soil except for P and Al. In contrast to the results for organic soil horizon, there was a positive correlation between Ca, Mg, and Al in soil solution and foliar putrescine as well as total polyamines (Table 2).

N content in the pine needles was positively correlated with foliar Mg and P only. There was also a strong negative correlation between foliar N and exchangeable Ca, Mg, Mn, and K in the organic soil horizon. Similar to the situation with total polyamines, foliar N showed a significant negative correlation only with total Mn and P in the organic soil horizon. N content, however, was positively correlated with all elements in the soil solution (Table 2).

**Hardwoods:** Unlike the situation with pine, putrescine and total PAs in oak and maple leaves showed a

strong negative correlation with foliar Ca, Mg, Mn, and P. In the organic soil horizon, putrescine, total polyamines as well as N were significantly and inversely related with exchangeable K and total P in both oak and maple. In the mineral soil, putrescine in oak was negatively correlated only with Al but in maple it had a negative correlation with several elements. Foliar putrescine and N were also positively correlated with soil solution Mg and/or Al. For more details on individual correlations refer to Table 2.

#### Discussion

Biochemical changes that occur in response to exposure to a particular stress can be measured before any visible symptoms appear at the level of the organism and may even be used as early indicators of change(s) in the vitality of trees within a stand (Baur et al., 1998; Minocha et al., 1997). Among the physiological and molecular responses of plants to high N deposition, is the increase in cellular content of leaf N. Nitrogen has been shown to be stored in the leaf in the form of nitrate and/or specific free amino acids such as arginine (Aber et al., 1995; Ericsson et al., 1993, 1995;



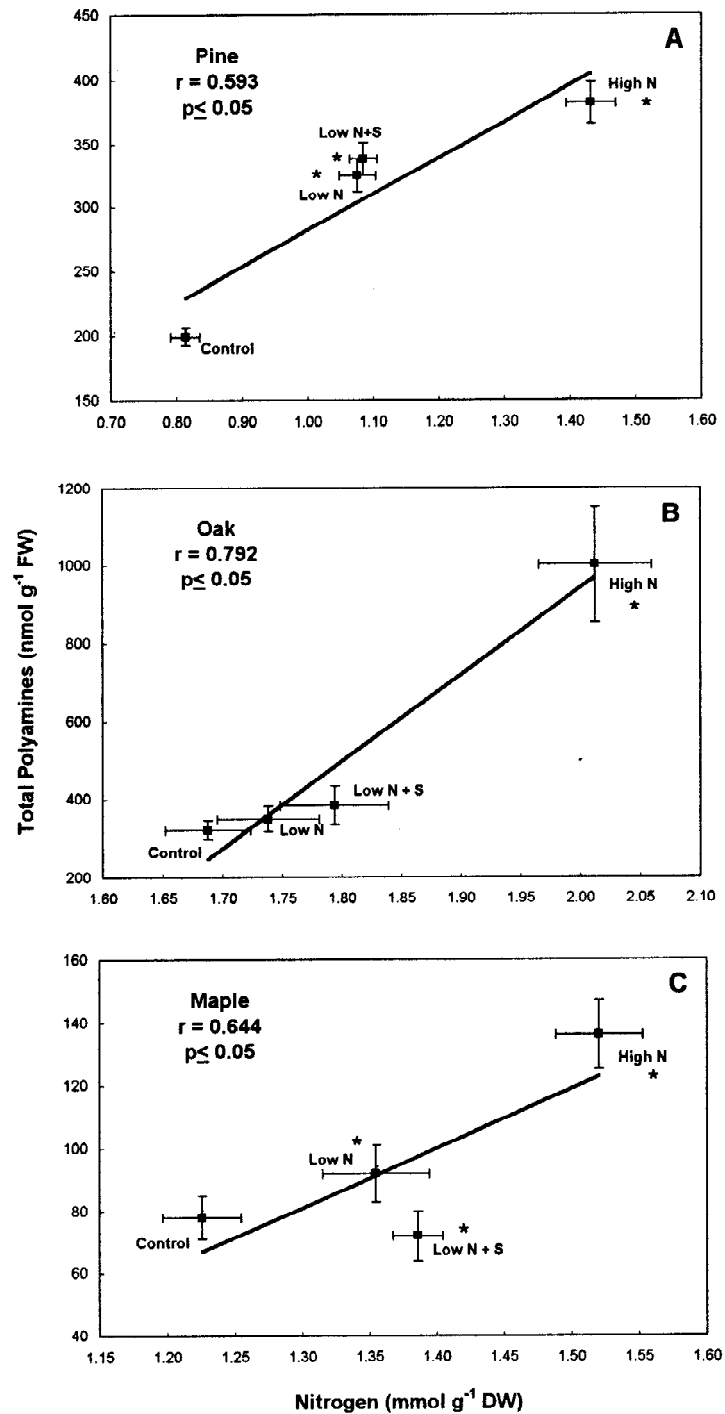


Figure 4. Correlations between total foliar polyamines (putrescine+spermidine+spermine) and nitrogen. Three years data was pooled for each treatment and represented as mean $\pm$ SE of  $n=60$  for polyamines,  $n=15$  for nitrogen in pine; and  $n=20-25$  for polyamines,  $n=10-15$  for nitrogen in hardwoods. The asterisks denote the significant differences from control.

Näsholm et al., 1997; van Dijk and Roelofs, 1988). Little is known about the molecular events which eventually lead to inhibition of growth due to chronic N deposition. It is, therefore, essential to study in more

detail the changes in various physiological and biochemical processes associated with exposure of plants to high N deposition using not only cell cultures and seedlings but also intensive site-level manipulations.

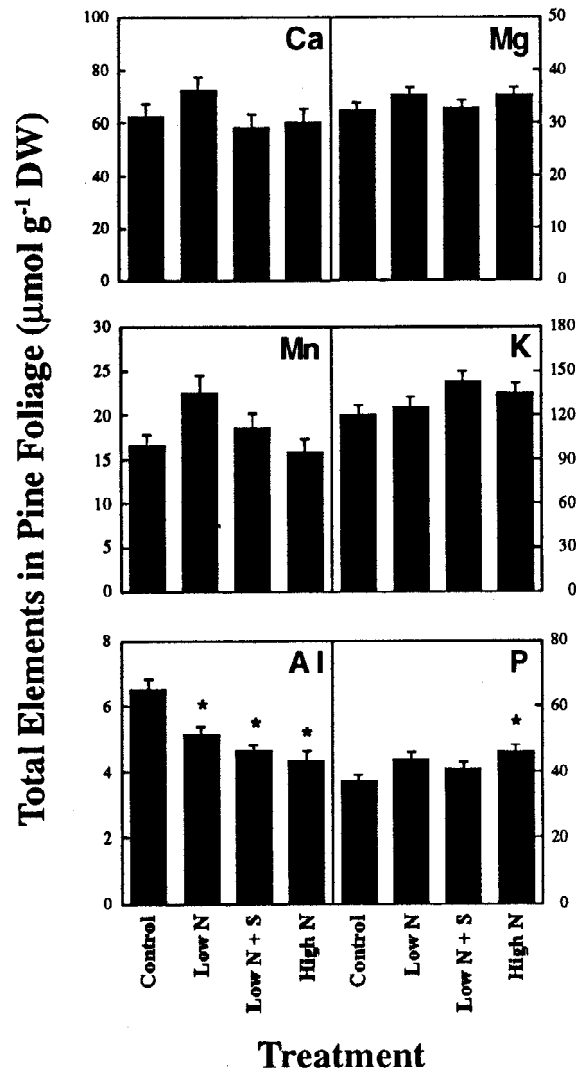


Figure 5. The effect of chronic N additions on foliar total inorganic elements in pine trees. Data presented for each treatment are mean $\pm$ SE of  $n=15$  for 1995–97. The asterisks denote the significant differences from control.

Because land use history and species specificity may also affect the response of trees to a particular stress (Aber and Driscoll, 1997; Aber et al., 1998), these factors should be considered when initiating regional and/or global level studies.

An increase in polyamines in response to nutrient deficiencies (e.g. Ca and Mg) and other stress-causing factors (pathogens, ozone, salt, and water) has been previously documented (Dohmen et al., 1990; Flores, 1991; Minocha et al., 1992, 1996, 1997; Santerre et al., 1990). In the present study, an increase in foliar polyamine content was observed in response to chronic nitrogen additions to soil and this increase correlated significantly with an increase in total foliar N content. Arginine, a precursor of putrescine,

has also been shown to be present in higher levels in spruce and pine needles in response to long-term high N deposition (Ericsson et al., 1993, 1995; Näsholm et al., 1997; van Dijk and Roelofs, 1988). This increase in free polyamines and arginine may be indicative of the significance of their role in detoxification of ammonia within the cells. Unlike arginine, changes in polyamines, however, do not account for a significant portion of total foliar N in the foliage under conditions of long-term N deposition. Findings from this study (Table 2 and Figure 11) were similar to the results obtained from red spruce sites across Northern New England (Minocha et al., 1997), where the increase in putrescine was also positively correlated with Al

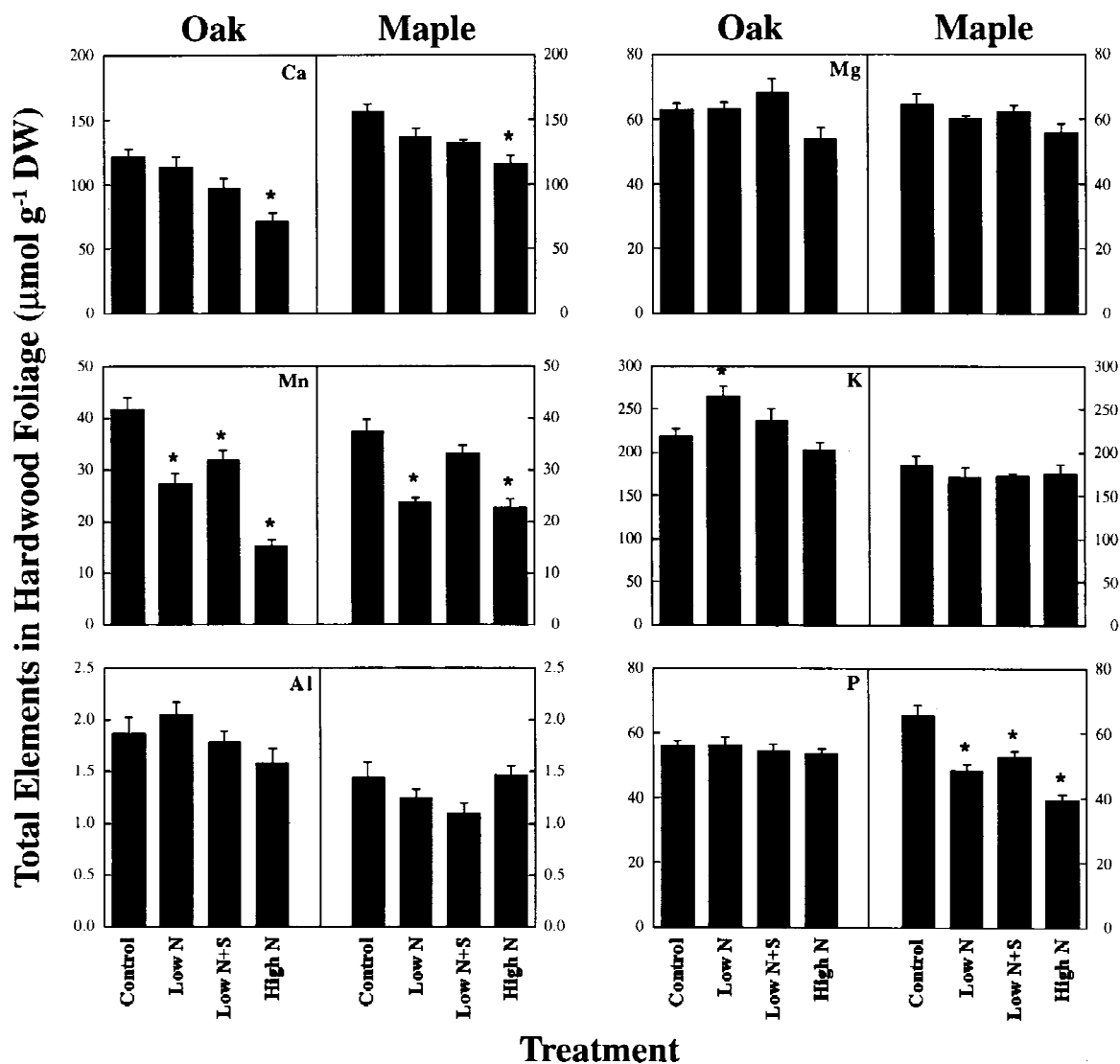


Figure 6. The effect of chronic N additions on foliar total inorganic elements in oak and maple trees. Data presented for each treatment are mean  $\pm$  SE of  $n=15$  for oak and  $n=10-15$  for maple for 1995-97. The asterisks denote the significant differences from control.

mobilization in the soil as evidenced by high Al levels in the soil solution.

The year-to-year variations in polyamine content may in part be related to additional stress imposed on trees due to relatively low levels of precipitation in 1995 and 1997 (John Aber, unpublished data). Foliar putrescine, a proposed indicator of general stress, showed a much higher increase during this period of low precipitation as compared to 1996. Thus the effects of chronic N additions and possible water stress may have had additive effects on polyamine accumulation in 1995 and 1997.

The observed responses to N additions were species-specific and also may be dependent upon the

land use history of each site. In the beginning of this study, the pine stand was less nitrogen limited than the hardwood stand. The hardwood site was last harvested around 1945 (Aber et al., 1993; Magill et al., 1997) and may have been more depleted of N (Aber et al., 1998 and references therein). In the pine stand, the application of both low and high levels of ammonium nitrate resulted in significant changes in foliar and soil chemistry. So far, in the hardwood stand, similar responses have been observed only with the high N treatment. These results support the previously proposed notion that the pine stand is closer to becoming fully nitrogen saturated (Magill et al., 1999). The effect of land use and N deposition on N cycling and

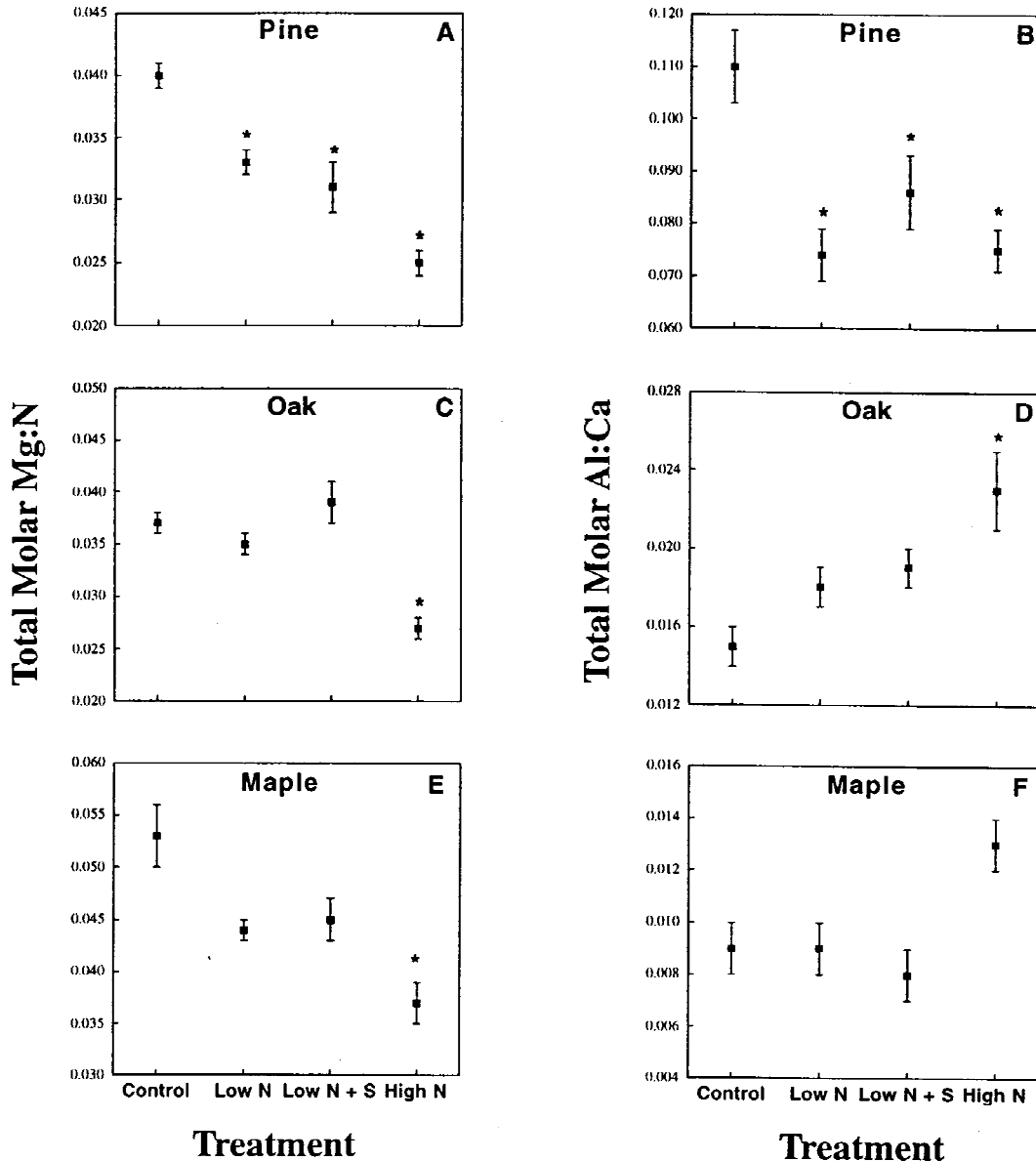


Figure 7. The effect of chronic N additions on foliar total Mg:N and Al:Ca ratios. Data are mean  $\pm$  SE of  $n=18$  samples collected per treatment during 1995–1997. The asterisks denote the significant differences from control.

C storage has been described in detail by Aber and Driscoll (1997) and Aber et al. (1998).

The inorganic elements, in general, are present in relatively low concentrations in the foliage of red pine as compared to red maple, sugar maple and red oak species (Richard Hallett, unpublished data). One possible explanation for this may be that red pine has a low demand for nutritional elements such as Ca and Mg and thus can survive on nutritionally poor soils. In the red pine stand, even though there was an increase in foliar putrescine and a corresponding decrease in inorganic elements in the organic horizon in response

to all N treatments, there were no significant changes in foliar elements with the exception of Al. The soil inorganic elements may have to decline even more substantially in the red pine stand for the trees to experience any foliar nutrient deficiencies because of its low nutritional requirements.

In the case of hardwoods, however, the increases in foliar polyamines corresponded with a lowering of most inorganic elements in the foliage as well as K and P in the organic horizon of the soil of the high N plots. The magnitude of the changes in polyamine levels for the high N treatment was much greater in

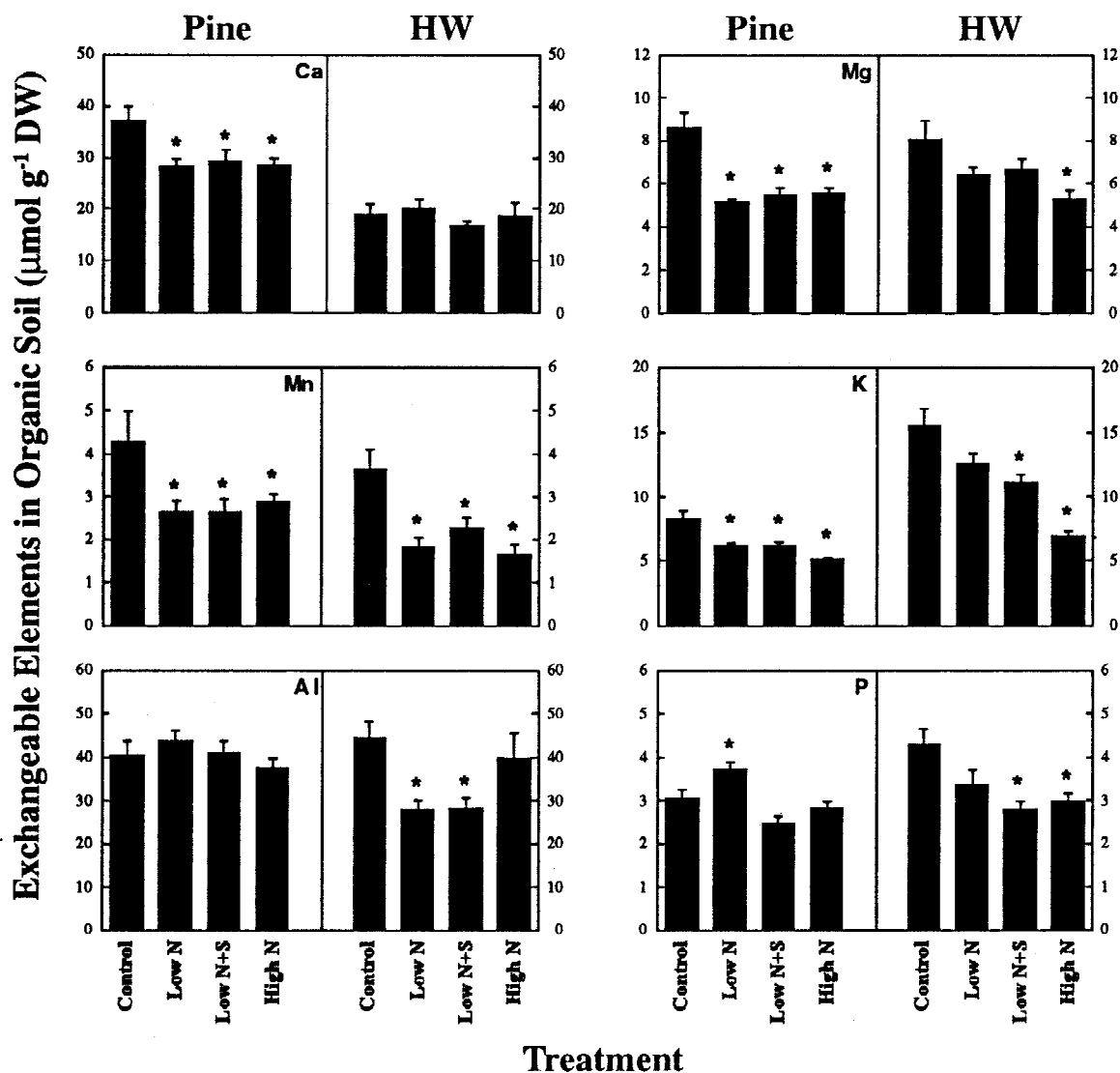


Figure 8. The effect of chronic N additions on the exchangeable element chemistry of the organic soil horizon in pine and hardwood stands. Data are mean  $\pm$  SE of 18 samples collected per treatment during 1995–1996. The asterisks denote the significant differences from control.

the oak leaves than in the maple and pine. One possible explanation for the observed differences between pine and hardwood in terms of polyamine accumulation and changes in inorganic element could be that in pine needles, polyamine accumulation is important in sequestering excess N but that in hardwood leaves, it may be serving a dual function of sequestering excess N and substituting for nutrient (such as Ca and Mg) deficiencies.

Long-term additions of nitrogen to these stands is acidifying the system as evidenced by leaching of  $\text{H}^+$  and inorganic elements into soil solution (Currie et al., 1999). Lowering of the Mg:N ratio in the pine foliage

resulted mainly from a significant increase in foliar N. In oak and maple leaves, this change in Mg:N resulted from a significant increase in N accompanied by statistically nonsignificant decrease in Mg. The foliar Al:Ca ratio for high N hardwood plots increased because of a significant decrease in Ca along with or without a change in Al depending upon the species. Decreases in Mg:N and Ca:Al have also been reported previously as indicators of nutrient imbalances and soil acidification (Aber et al., 1995; Boxman et al., 1998; McNulty et al., 1996; Minocha et al., 1997; Schultze, 1989).

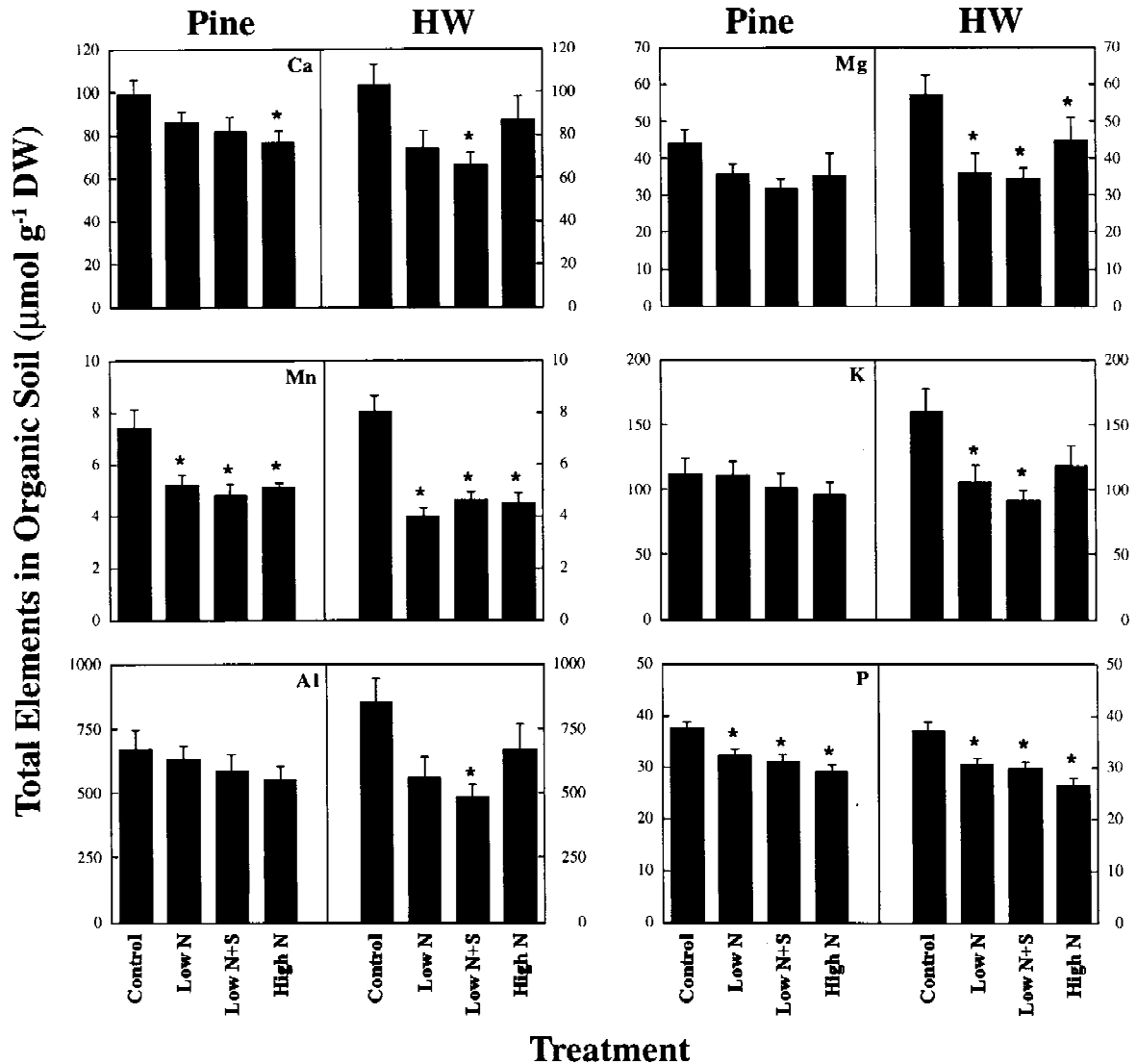


Figure 9. The effect of chronic N additions on the total element chemistry of the organic soil horizon in pine and hardwood stands. Data are mean  $\pm$  SE of 18 samples collected per treatment during 1995–1996. The asterisks denote the significant differences from control.

Joslin and Wolfe (1992) demonstrated the absence of red spruce roots in the mineral horizons. Also in the case of Norway spruce, root length and root growth have been shown to decrease sharply with soil depth (Hahn and Marschner, 1998). The addition of nitrogen or acid irrigation have been shown to mainly affect the vitality of roots in the humus layer in this species as shown by the decrease in root biomass, increase in amount of dead fine roots and decrease in specific root length (Clemensson-Lindell and Persson, 1995; Majdi and Persson, 1995). Douglas-fir seedlings have also been shown to respond to ammonium sulfate addition in terms of negative effects on root length and root growth (Olsthoorn et al., 1991). Only

under drought conditions the roots of Norway spruce were shown to redistribute themselves to deeper mineral soil (Persson et al., 1995). Therefore, even in the absence of root data for this site, we suspect that the lack of a significant correlation between inorganic element concentrations in the mineral soil horizon and foliar chemistry of the pine stand may be due to the paucity of pine roots in the mineral soil. Conversely, in the case of maple, the significant negative correlation between foliar polyamines and several elements in mineral soil confirms the earlier findings that roots are abundant in the mineral soil at this site (Lyford and Wilson, 1964). The increase in inorganic elements in soil solution which we observed was also reported pre-

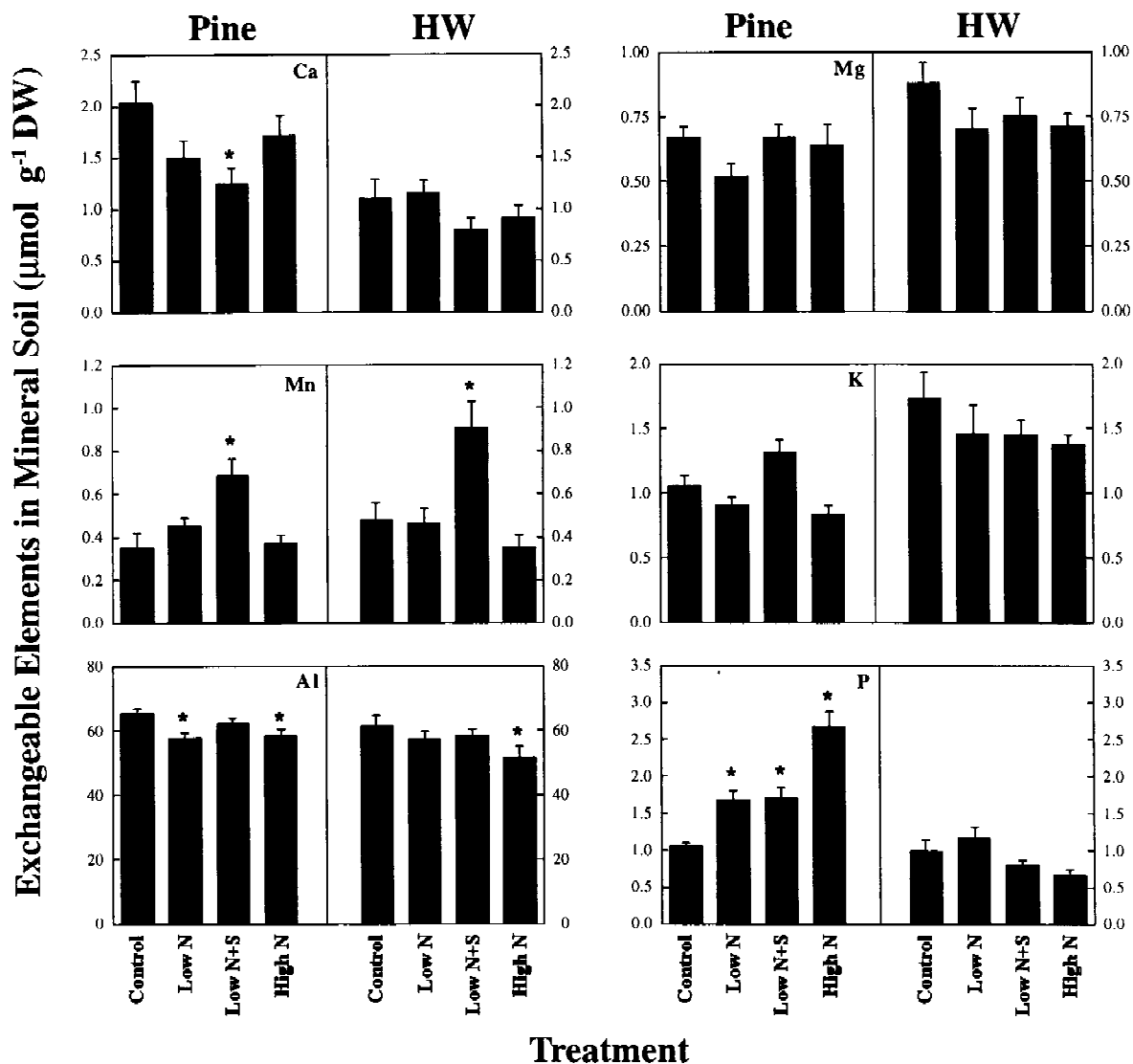


Figure 10. The effect of chronic N additions on the exchangeable element chemistry of the mineral soil horizon in pine and hardwood stands. Data are mean  $\pm$  SE of 18 samples collected per treatment during 1995–1996. The asterisks denote the significant differences from control.

viously at the same study site for 1992–1994 (Currie et al., 1999). The authors found a significant correlation between nitrate and element levels in the soil solution. In the present study, the pine stand has shown greater overall loss of inorganic elements from the forest floor as compared to the hardwood stand. Similar observations were made for nitrate leaching at this site (Aber et al., 1993; Magill et al., 1996, 1999; Nohrstedt et al., 1996).

Using seedlings and roots of mature spruce and beech, Gessler et al. (1998) demonstrated the preferential uptake of ammonium over nitrate. In the Netherlands, forest decline has been correlated with excess ammonium deposition. Regional variations in forest

decline matched more closely with regional variations in ammonium level than  $\text{SO}_2$  and  $\text{NO}_x$  (Wilson and Skeffington, 1994). Even though ammonium nitrate was applied in the present study no inferences have been drawn so far on the relative uptake of ammonium vs. nitrate by trees. Additional work will be required at this site in order to delineate the effects of chronic N addition on foliar N partitioning and in turn its effect on photosynthesis and N relationship. This will involve construction of canopy access towers to enable us to collect data on various parameters (A max, free amino acids, conjugated polyamines, N content, inorganic ions on the same bunch of needles). Along with

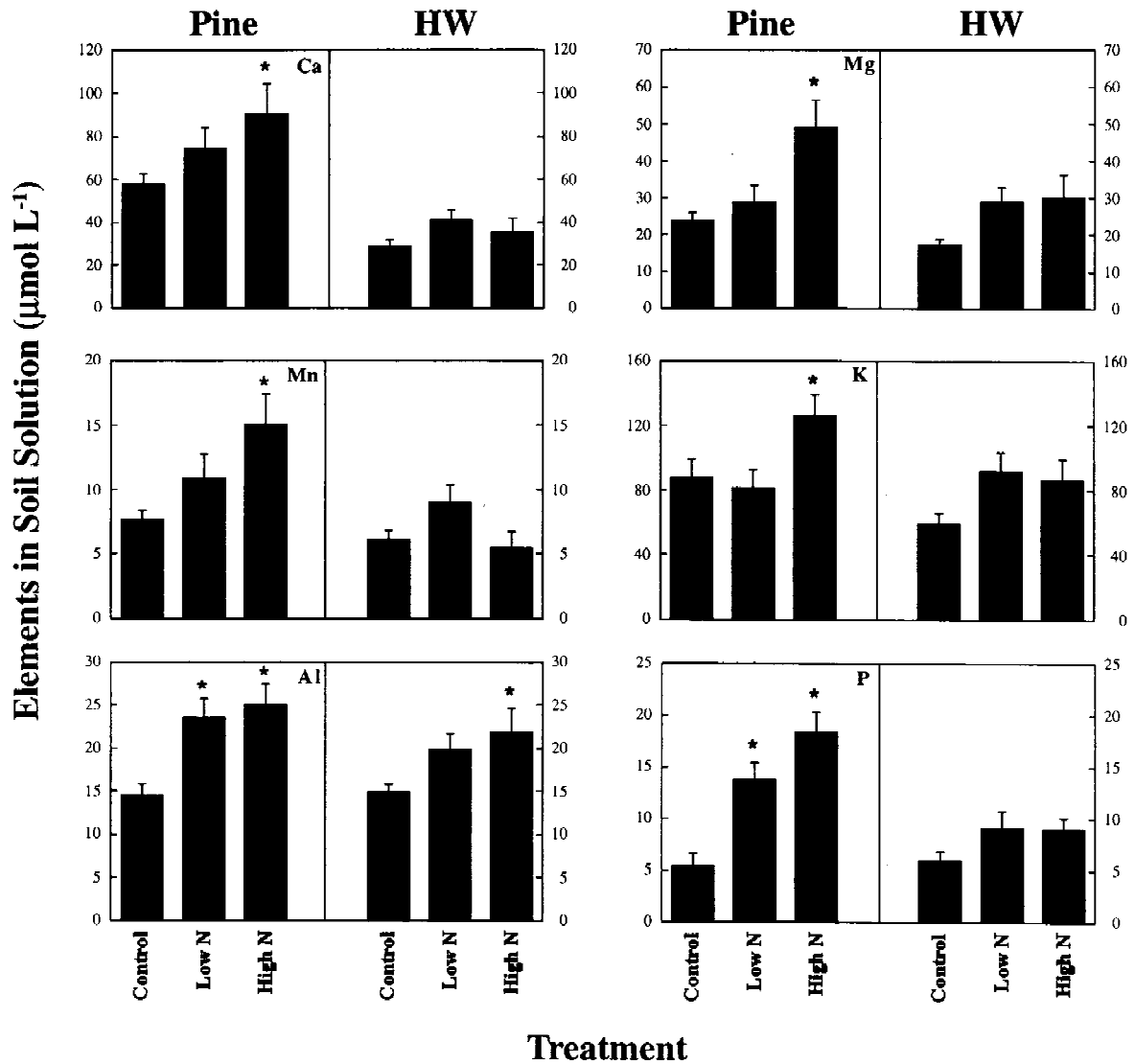


Figure 11. The effect of chronic N additions on the chemistry of the soil solutions collected from zero tension lysimeters in pine and hardwood stands. Data are mean $\pm$ SE of 50 samples collected per treatment during 1995–1997. The asterisks denote the significant differences from control.

this the data on foliage turnover rates, canopy structure and carbon gain etc. will also be required.

In conclusion, N additions resulted in an increase in foliar free putrescine, a potential indicator of general stress in plants, which in turn was positively correlated with foliar total N content. N additions acidified soils as evidenced by an increase in Al mobilization and leaching of other inorganic ions from organic soil horizon into the soil solution and may be responsible for these physiological changes in the foliage. To date significant changes in foliar and soil chemistry have been observed in the case of all N treated pine plots and high N hardwood plots as compared to control plots. The data reported here is especially valuable

because it directly relates the changes occurring in the soil chemistry to the foliar physiology of the trees growing at the same site.

#### Acknowledgements

The authors acknowledge the help of Dr Glenn Bernston, Dr Scott Bailey, Dr Walter Shortle, Dr Kevin Smith, and Prof Subhash Minocha for reviewing the manuscript and Dr Chris Neefus for statistical analyses. Thanks are also extended to Jennifer Heinrich-Pontius, Jeff Merriam, Steve Newman, and Scott



Table 2. Correlation coefficients among various mean data for soil and foliar chemical measurements. Mean data for polyamines in the foliage samples are computed based on  $n=20$  for pine stand and  $n=10$  for hardwood stand. For total ions in the foliage, mean data was computed based on  $n=5$  for pine stand and  $n=4$  for hardwood stand. Mean data for soil samples are computed based on  $n=9$  and for soil solution,  $n=15-20$ . Blank spaces = No significant correlations. Total PAs = putrescine+spermidine+spermine. \*\* $p \leq 0.05$ , \* $p \leq 0.1$

	Pine			Oak			Maple		
	Foliar putrescine	Foliar total PAs	Foliar N	Foliar putrescine	Foliar total PAs	Foliar N	Foliar putrescine	Foliar total PAs	Foliar N
Foliar Total Ions ( $n=12$ )									
Ca	–	–	–	–0.80**	–0.74**	–	–0.65**	–0.60**	–0.91**
Mg	–	–	0.52**	–0.63**	–0.60**	–	–0.69**	–0.65**	–0.59**
Mn	–	–	–	–0.81**	–0.78**	–0.45*	–0.75**	–0.62**	–0.74**
K	0.70**	0.63**	–	–0.44*	–0.47*	–0.63**	–	–	–
P	–	–	0.47*	–	–	0.51**	–0.57**	–0.47*	–0.86**
Al	–0.64**	–0.75**	–0.58**	–0.45*	–	–	–	0.65**	–
Organic (Oe+Oa) Soil: Exch. ( $n=8$ )									
Ca	–0.77**	–0.92**	–0.69**	–	–	–	–	–	–
Mg	–0.84**	–0.89**	–0.58**	–0.58*	–0.54*	–	–	–	–0.69**
Mn	–0.62**	–0.83**	–0.56*	–	–	–	–	–	–0.73**
K	–0.90**	–	–0.78**	–0.80**	–0.77**	–0.61*	–0.61**	–0.56*	–0.87**
P	–	–0.97**	–	–	–	–0.71**	–0.64**	–0.59*	–0.76**
Al	–0.52*	–	–	–	–	–	–	–	–
Organic (Oe+Oa) Soil: total ( $n=8$ )									
Ca	–0.76**	–	–	–	–	–	–	–	–
Mg	–0.77**	–	–	–	–	–	–	–	–
Mn	–0.84**	–0.92**	–0.59*	–	–	–	–	–	–0.61*
K	–0.52*	–	–	–	–	–	–	–	–
P	–0.83**	–0.79**	–0.64**	–0.64**	–0.65**	–0.72**	–0.59*	–0.60*	–0.90**
Al	–0.57*	–	–	–	–	–	–	–	–
Mineral (top 10cm) Soil: Exch. ( $n=8$ )									
Ca	–	–	–	–	–	–	–	–	–
Mg	–	–	–	–	–	–0.70**	–0.57*	–0.65**	–0.58*
Mn	–	–	–	–	–0.54*	–	–0.60*	–0.61*	–
K	–	–	–	–	–	–0.67**	–0.69**	–0.66**	–0.58*
P	0.69**	0.87**	0.90**	–	–	–	–	–	–
Al	–0.68**	–0.50*	–	–0.55*	–0.51*	–	–0.67**	–0.52*	–0.60*
Soil Solution: ZTL ( $n=9$ )									
Ca	0.77**	0.79**	0.49*	–	–	–	–	–	–
Mg	0.72**	0.80**	0.67**	0.71**	0.69**	0.55*	0.64**	–	0.70**
Mn	–	–	0.65**	–	–	–	–	–	–
K	–	–	0.55*	–	–	–	–	–	–
P	–	–	0.65**	0.56*	0.55*	–	–	–	–
Al	0.89**	0.95**	0.54*	0.50*	0.52*	0.51*	–	–	0.59**

Ollinger for their help with aspects of sample collection and/or preparation.

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Section editor: P Ryan