EFFECTS OF ACIDIC DEPOSITION ON NUTRIENT UPTAKE, NUTRIENT CYCLING AND GROWTH PROCESSES OF VEGETATION IN THE SPRUCE-FIR ECOSYSTEM

Summary of a Research Project Conducted by Oak Ridge National Laboratory for The Environmental Protection Agency under Interagency Agreement with the Department of Energy

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Draft Report - October 16, 1996
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PROJECT SUMMARY

Three years of field research were conducted to evaluate biological and chemical indicators of the current and future health of the Southern Appalachian Spruce-Fir Ecosystem. The focus of this research has been on identification and understanding mechanisms through which current levels of acidic deposition are impacting ecosystem processes. Principal mechanisms and key biological indicators of change were examined to improve our capabilities to detect, monitor, and assess the effects of air quality regulations and attendant future air quality changes on ecosystem response. The research was organized into three Tasks: I. Nitrogen Deposition and Cycling; II. Soil Chemistry and Plant Nutrition; and III. Physiological Indicators of Plant Function.

Studies on nitrogen deposition and cycling found that the forest canopy can either decrease or increase deposition to the forest floor. At a site below cloud base the canopy acted as a sink intercepting 83% of the incident ammonium and 34% of the incident nitrate. This significantly reduced nitrogen inputs to the forest floor compared to deposition in nearby gaps, which experienced greater hydrologic fluxes. Above cloud base increased canopy interception increased throughfall and deposition. Resultant atmospheric inputs of nitrogen are generally higher above cloud base, however local topography and year to year meteorological conditions can significantly influence total deposition and the ratios of wet to dry deposition. $^{15}$N Tracer studies demonstrated that ammonium and nitrate are retained and incorporated by needles and stems of red spruce and Fraser fir saplings. Canopy retention by current-year needles was similar for NH$_4$ and NO$_3$, similar between spruce and fir, and was found to range between 1 and 9% of the isotopic additions to individual trees. Over 76% of deposited nitrate and 55% of ammonium deposited on in situ soil cores was mobile, indicating a high potential for N deposition to increase cation leaching losses from these poorly buffered soils.

Reduced calcium availability is apparently limiting growth of spruce and possibly fir at high elevation sites and recently analyzed survey data indicate reduced foliar levels of base cations, most notably calcium, at high elevations. Reduced ratios of foliar to soil cation levels indicated a reduced efficiency of cation uptake and/or retention likely related to increased acidic deposition at higher elevation sites. Fertilization of 25 two-tree plots of mature red spruce at Whitetop Mountain Virginia in 1990 and 1991 improved growth (+20%) of foliage, improved foliar Ca:Al ratios (+20-60%), and continued to improved Ca:Al ratios in soil solutions three years after the last calcium application date. Similarly calcium additions to red spruce saplings on Clingman's Dome increased the efficiency of carbon metabolism, increased stem growth, and increased nitrogen uptake. Histochemical analysis of fine roots of Ca fertilized red spruce trees indicated that calcium addition improved the efficiency of nutrient uptake into the root conducting system and increased internal Ca:Al ratios.

Physiological studies with Fraser fir indicated reduced efficiency of carbon metabolism associated with lower Ca:Al ratios in foliage at higher elevation sites in a manner comparable to that previously reported for red spruce. Foliar Ca:Al ratios for fir are approximately 2/3 those of red spruce indicating possible high sensitivity of this species to aluminum-induced Ca limitation. Foliar nutrient levels of both red spruce and Fraser fir are lower for mature trees than for saplings. Dendroclimatic analysis of growth patterns of mature red spruce trees indicate that annual responses to both warmer temperatures and increasing precipitation have shifted negatively in most recent decades compared to preceding decades in this century. Preliminary studies of relationships between wood calcium, wood lignin content, and wood structural characteristics suggest that reduced cation availability may reduce structural integrity of wood formed under these conditions. Collectively these studies provide strong evidence that acidic deposition of nitrogen and strong anions to the high elevation Southern Appalachian Spruce-Fir Ecosystem is a significant stress, which alters the normal processes involved in nutrient cycling and primary production.
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INTRODUCTION

This report summarizes progress in three years of field research designed to evaluate biological and chemical indicators of the current and future health of the Southern Appalachian spruce-fir ecosystem. The emphasis of this research has been on the identification and understanding of mechanisms through which current levels of acidic deposition are impacting ecosystem processes. The identification of these principal mechanisms and key biological indicators of change was designed to improve our capabilities to detect, monitor, and assess the effects of air quality regulations and attendant future air quality changes on ecosystem response. Individual research tasks focused on the following research areas: (1) the significance of foliar uptake of atmospheric sources of nitrogen in relationship to plant utilization of N from available soil reserves; (2) linkages between atmospheric inputs to the soil surface, solution chemistry, and decomposition in the upper organic soil horizons; (3) effects of soil solution chemistry on uptake of cations and aluminum by fine roots; and (4) the effects of varying rates of calcium supply on carbon metabolism of Fraser fir and red spruce, and the relationship between calcium levels in wood cells and integrity of wood formed in bole and branches. Each of the individual tasks was designed to focus upon a mechanism or process that we consider critical to understanding chemical and biological linkages. These linkages will be important determinants in understanding the basis of past and potential future responses of the high elevation Southern Appalachian Forest to acidic deposition and other co-occurring environmental stresses. We provide in this report (1) the background and rationale for the research undertaken during and following a three-year (1992-94) funding interval; (2) a summary of principal research findings; (3) publications derived from this research; and finally (4) characterization of data sets produced by this research which will be the basis of future research, analyses and/or publications.

I. BACKGROUND

The spruce-fir forests of the Southern Appalachians offer unique opportunities to study a natural ecosystem that we know is being influenced by atmospheric deposition and that will be sensitive to further environmental change. The decline in vigor in red spruce at high elevation sites in the Southern Appalachians is now well documented (Eagar and Adams, 1992). Symptoms of this decline include reduced radial growth in mature trees at high elevation sites (McLaughlin et al., 1987), and physiological dysfunctions and altered growth of sapling trees (McLaughlin et al., 1990, 1991, 1993). The concurrent timing of declining radial growth and changing patterns of wood chemistry of mature trees suggest that the soil solution chemistry has changed in the last three decades, reflecting reduced availability of calcium and magnesium and increased availability of aluminum (Bondietti and McLaughlin, 1992).

Levels of atmospheric input of sulfur and nitrogen impinging on the high elevation peaks in the Great Smoky Mountains National Park (GSMNP) are among the highest along the East coast (Lindberg et al., 1992), and both soil solution chemistry (Johnson et al., 1991), and rooting patterns (Joslin et al., 1992) suggest that conditions at some high elevation sites are unfavorable for growth of red spruce roots. If additional perturbations occur (such as predicted increases in soil temperature), the large stores of nitrogen currently present in organic matter pools have the potential to contribute to the release of significant quantities of nitrate and thus further exacerbate the soil changes which are already in progress.

However, the importance of current and potential future changes to the Southern red spruce has not yet received adequate recognition. One reason for this is that the visual evidence of red spruce
decline is much less spectacular than in the North where high levels of mortality are readily apparent at both high and low elevation sites across the region. Additionally, the death of large numbers of Fraser fir in the South in association with the presence of the balsam wooly adelgid, has partially obscured changes in spruce and greatly compromised studies of aspects of fir decline that may not be related to insect attack. Yet the southern spruce-fir ecosystem represents a unique opportunity to study the earlier stages of forest decline and hence a system where a clearer picture of the sequences of changes leading to more serious decline can be developed experimentally.

In the Northern Appalachians serious visual deterioration of the red spruce forests began approximately 35 years ago. By contrast, in the South the early stages of visual deterioration are still apparent and offer excellent opportunities to study "first causes". This picture of the chronosequence of early stage changes in soil chemistry, plant nutrition, tree physiology, and growth is important to exploring the extent to which the southern spruce/fir ecosystem may serve as a model for changes which may be occurring now or in the future in poorly buffered southern pine, or other, ecosystems.

The research conducted by the Spruce-fir Cooperative of the National Forest Response Program made substantial progress toward understanding key ecosystem components that are sensitive to chemical inputs from acid deposition. Many of these same pathways are likely to be affected by anthropogenic disturbances other than direct phytotoxicity from air pollution. For example, increases in air and soil temperature can be expected to have a major impact on this ecosystem by increasing rates of plant tissue respiration, organic matter decomposition, nitrogen mineralization, nitrification, and soil nitrate release. If these expected changes indeed occur, research conducted in this ecosystem will be extremely important in understanding their likely impact in these ecosystems in terms of soil cation leaching, soil acidification, the mobilization of Al, and the effects that additional losses of cations and/or increases in Al will have on tree and stream health.

Our current identification of some of the key ecosystem processes and pathways which are sensitive to disturbance, coupled with the extensive data bases on a host of environmental parameters collected as a part of research and monitoring activities in the Great Smoky Mountains National Park represented an excellent opportunity to make additional advances in understanding the likely rate and direction of future change. However, we have lacked the detailed understanding of the mechanisms involved in changing process rates, and such understanding is critically important to our capacity to predict future change. This research was designed to provide an integrated and systematic combination of studies to address a series of processes involved in chemical and biological reactions of acid deposition as it moves through the plant-soil system. Not only are these responses critical to predicting future forest responses, but they will also help define interrelationships between forest and stream processes as this sensitive system experiences longer term stresses from atmospheric deposition and possible global climate change. Such information is essential to efforts to monitor and understand changes in this system and for assessing responses which are related to the need and effects of air quality regulations.

IDENTIFICATION OF KEY SCIENTIFIC ISSUES

The research addressed five basic research issues with important implications for the health and function of the spruce fir ecosystem.

ISSUE I. What is the significance of atmospheric sources of nitrogen to foliar nutrition and relative rates of nitrogen uptake from the soil environment?
ISSUE II. How is soil chemistry, including mineralization of cations and mobilization of aluminum of the upper organic soil horizons where fine roots are most prolific, influenced by short term inputs of high anionic and acidic content?

ISSUE III. How is fine root uptake of calcium and magnesium influenced by the high levels of aluminum mobilized in upper organic and underlying mineral soil by acidic deposition?

ISSUE IV. How does reduced calcium uptake and incorporation into woody tissues influence the structural integrity and strength of wood formed in high elevation spruce and fir.

ISSUE V. What are the physiological limitations placed upon Fraser fir growing in an environment in which red spruce has experienced physiological dysfunction associated with acid deposition-induced limitations on cation availability?

We will return to these issues at the end of this report as a means of summarizing enhancement of our current understanding by the research we describe below. That research will be described under three task headings:

I. Nitrogen Deposition and Cycling

II. Soil Chemistry and Plant Nutrition

III. Physiological Indicators of Plant Function

Each of the tasks is introduced by a research summary that quickly overviews the objectives, experimental components, and principal findings from each task. These are followed by discussions of the rationale and background for each task, an overview of experimental protocols, and a summary of research results.
TASK I. NITROGEN DEPOSITION AND CYCLING

RESEARCH SUMMARY

Objectives: To evaluate principal factors contributing to nitrogen deposition, retention, and nitrogen saturation, in high elevation spruce-fir forests.

Experiment 1. Throughfall N Inputs Beneath the Canopy and in a Gap

Findings: The forest canopy acts as a sink to significantly reduce direct nitrogen deposition to the forest floor. Based on comparisons with deposition in gaps, the forest canopy intercepted 83% of the incident ammonium and 34% of the incident nitrate. The greater fluxes of both water and nitrate in canopy gaps are expected to increase nitrate leaching losses in gaps relative to soil below an intact forest canopy. The combination of increased hydrologic and chemical fluxes in canopy gaps may increase nutrient limitations on the regenerating vegetation in these gaps.

Experiment 2. Comparison of Wet and Dry N Inputs Above and Below Cloud Base.

Findings: Atmospheric inputs of nitrogen are generally higher above cloud base, however local topography and year to year meteorological conditions can significantly influence total deposition and the ratios of wet to dry deposition. Canopy interception of clouds and subsequent throughfall can increase N deposition levels below the canopy by 2-3 fold compared to open areas.

Experiment 3. Quantifying retention of wet deposition of N by red spruce and Fraser fir saplings.

Findings: $^{15}$N Tracer studies provided the first direct evidence that ammonium and nitrate in wet deposition are retained and incorporated by needles and stems of red spruce and Fraser fir saplings. Canopy retention by current-year needles were found to be higher at the upper site, to be similar for NH$_4$ and NO$_3$ and between spruce and fir, and to range between 1 and 9% of the isotopic additions to individual trees. Direct uptake of atmospheric sources of nitrogen by the canopy may reduce N uptake from the soil and contribute to N saturation of these high elevation soils.

Experiment 4. Retention of N Inputs by the Forest Floor.

Findings: Ammonium inputs to surface soil are more strongly retained than nitrate, but large losses of nitrogen inputs can be expected due to high rates of nitrification, nitrate leaching, and or denitrification in the high elevation spruce-fir forest soils. Approximately 34-45% of ammonium deposition was retained by soil cores, whereas NO$_3$ retention ranged from 8-26%. The high mobility of nitrate in soil ($\geq$ 74% loss) indicates that atmospheric N deposition will be an important contributor to soil nitrate leaching and aluminum mobilization at high elevation Southern Appalachian sites.
RESEARCH TASK I. RETENTION OF ATMOSPHERIC N Inputs TO A HIGH ELEVATION SPRUCE-FIR FOREST

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RATIONALE

Red spruce decline in high elevation forests of the Southern Appalachian Mountains is well documented, but the causal linkages between atmospheric deposition, soil chemistry, plant nutrition, and forest decline are not fully understood. High elevation, spruce-fir forests in the Great Smoky Mountains National Park are characterized by both high rates of atmospheric N deposition and high rates of nitrate leaching from soil. High rates of soil nitrate leaching in association with elevated soil aluminum levels suggest that these forests are under stress due to changes in soil chemistry which can be directly or indirectly attributed to high rates of atmospheric N deposition.

Atmospheric N deposition may be an important determinant of soil nitrate leaching in high elevation spruce-fir forests. The relative importance of internal and external processes that contribute to nitrate leaching from these forests is unclear. On the one hand, input-output budgets suggest that N deposition is a major cause of high soil solution nitrate concentrations and nitrate leaching. On the other hand, nitrification is an important component of soil N mineralization in high elevation forests suggesting that endogenous soil N dynamics also play an important role in the rate and periodicity of nitrate leaching.

We conducted four experiments over a period of several years (1992 to 1994) at two sites in a spruce-fir forest in the Great Smoky Mountains National Park. Prior work indicated that N exports from this ecosystem exceed N inputs. The current study focused on the behavior of atmospheric N inputs at two sites with different elevations and with different amounts of cloudwater deposition. Collectively, these studies shared a common purpose which was to better understand and monitor the N dynamics of high elevation spruce-fir forests in the Southern Appalachian Mountains.

SITE DESCRIPTIONS

Two study sites were selected near Clingman's Dome (elevation 2025 m) in the Great Smoky Mountains National Park, North Carolina. Both sites were situated on slopes of 15 to 20° and have been described in detail by Lindberg and Owens (1993) and McLaughlin et al. (1990). The upper study site (1940 m) was located above the cloud base and had a greater hydrologic flux and a greater frequency of forest immersion in clouds than the lower study site (1720 m). Despite greater hydrologic inputs at the upper site, throughfall measurements indicate approx. 30% more nitrate deposition occurs at the lower site due to its location on a ridgetop and a greater penetration of windborne nitric acid vapor (i.e., dry deposition) into the forest canopy. Dry deposition contributes approx. 50% of the total atmospheric N deposition to forest stands near the lower study site.
Overstory cover at both sites was patchy and consisted mostly of old-growth red spruce (23 to 32 m tall). The mean height of understory red spruce and Fraser fir at both sites was approximately 2.4 m. Other common understory plants included yellow birch (*Betula alleghaniensis*) and *Viburnum*. Soils at both sites are umbric dystrochrepts derived from Thunderhead sandstone. They are characterized by a deep O-horizon (4 to 8 cm thick) over a dark brown, organically-rich A horizon. Prior studies indicate that 42 to 66% of the fine root (<0.5 cm) biomass is found in the O horizon. These forests are described in more detail by Johnson et al. (1991) and Johnson and Lindberg (1992).

**METHODS**

**Experiment 1. Throughfall N Inputs Beneath the Canopy and in a Gap**

The purpose of the first experiment was to test how the presence or absence of the red spruce overstory influenced both throughfall N inputs to the forest floor and the leaching of nitrate from the surface soil horizons.

In 1992, throughfall was sampled at the lower study site beneath artificial Christmas trees similar to those described by Joslin et al (1993). The artificial trees provided an inert surface for the interception of cloud water, dry deposition, and wet deposition. Precipitation, cloudwater, and the washoff of dry N deposition from plastic needles all contributed to N measured in solution samples collected beneath artificial trees.

One tree was placed in a canopy gap where the red spruce overstory was absent because of tree mortality. A second tree was placed beneath a mature red spruce overstory. Each tree was 1.5 m tall with a 1.1 m base diameter and had a gross morphology similar to live Fraser fir saplings that were prevalent at the upper and lower study sites. Three polyethylene funnels (19.5 cm diameter) were placed equidistant around the center pole beneath the lower branches of each artificial tree. The distance from the center pole to the center of each funnel was approx. 15 cm. The funnels drained through a glass wool filter into a polyethylene carboy (12 L). The volume of throughfall at the end of each sampling period, in combination with measured concentrations, was used to calculate the throughfall flux of ammonium and nitrate to the forest floor.

Fritted glass plate lysimeters (6-8 cm diameter) were used to sample soil water from the rooting zone at the lower site in 1992. Five lysimeters were placed in soil beneath the forest canopy and 5 were placed in soil at the canopy gap. Three of the 5 lysimeters at each location had been installed in prior years. Newly installed lysimeters were allowed to equilibrate for 3 months (July to September) prior to sample collection and analysis.

A tension of approx. 10 kPa was applied to each lysimeter by a hanging column method and was sufficient to capture nitrate leached with the gravimetric drainage of free soil water and some loosely bound capillary water. Extracted soil solutions were kept cool with minimum exposure to sunlight by using polyethylene collection bottles (7 L) set in a shallow pit. Lysimeters were checked weekly for tension, and solution samples were collected every other week from 10 Sep through 9 Nov 1992. Rain gauges were installed at both the gap and the canopy location to measure relative differences in the hydrologic flux.

Upon return to the laboratory, throughfall or lysimeter water samples were immediately sampled for analysis of ammonium and nitrate. Ammonium and nitrate concentrations were determined at Oak
Ridge National Laboratory (ORNL) by colorimetric methods or were determined by the U. S. Geological Survey-Water Resources Laboratory (Atlanta, GA). Quality assurance checks showed that the results from colorimetric methods at ORNL were highly correlated \( r = 0.99 \) with analytical results from the USGS Laboratory.

**Experiment 2. Comparison N Inputs Above and Below the Cloud Base**

The purpose of the second experiment was to test for differences in N inputs to spruce-fir stands located above and below the cloud base.

Throughfall fluxes beneath an artificial tree at the upper and lower study site were sampled during 1993 and 1994. The artificial tree (see Experiment 1 for details) at each site was placed in a canopy gap to avoid effects of the red spruce overstory on throughfall chemistry. In 1994, bulk deposition was also sampled next to each artificial tree, over the same time interval, using three open funnels in an arrangement like that used beneath artificial trees. Solution samples were analyzed for ammonium and nitrate concentrations as described in Experiment 1.

**Experiment 3. Retention of N in Wet Deposition by Saplings**

The purpose of the third experiment was to test retention of ammonium and nitrate in wet deposition by red spruce and Fraser fir saplings at locations above and below the cloud base.

During the 1993 growing season, ammonium-\(^{15}\)N or nitrate-\(^{15}\)N (99 atom %) was applied directly to Fraser fir and red spruce saplings to test for uptake of N from wet deposition. Four Fraser fir and 4 red spruce trees (0.7 to 1.4 m tall) were selected as experimental units at both the upper and lower study site. Initial measurements of tree height, number of branches per tree, and total branch length were used to assign trees to chemical treatments so that treatment effects were not biased by tree size.

Spray applications of the \(^{15}\)N tracers were made to each sapling on 4 occasions: June 23, July 7, July 21, and July 23, 1993. Each addition consisted of 3.5 mg \(^{15}\)NH\(_4\)NO\(_3\) or NH\(_4\)^{15}\)NO\(_3\) in 100 ml of deionized water. Each sapling received 43 ± 1 \(\mu\)mol of ammonium-\(^{15}\)N or nitrate-\(^{15}\)N per application. The cumulative tracer application was 172 ± 5 \(\mu\)mol \(^{15}\)N per tree. During applications we attempted to wet each sapling uniformly and tried to minimize the loss of isotope solution via drifting spray.

On 16 Aug 93 (24 days after the last tracer application), we harvested the new needles and stems from each study tree. Old needles and old stems (prior year's growth) were also sampled. Tissues were oven dried (70 °C), weighed, homogenized in a Waring blender, and analyzed for N concentrations and N isotope ratios. The number of moles of N in new needles and stems was calculated as the product of dry mass (g DM) and N concentration (g N/g DM). The fraction of the tracer applied to each tree that was recovered in new needles and stems was calculated using equations presented by Cabrera and Kissel (1989).

Plant tissue N concentrations were determined by combustion methods using a Carlo-Erba NA 1500 Analyzer. Stable N isotopes were measured using a VG SIRA II dual inlet stable isotope ratio mass spectrometer in the Environmental Sciences Division at ORNL. The methods of stable isotope analysis are described elsewhere. All measurements from tracer experiments were expressed in units of atom percent (atom %) or atom percent excess (at. % ex.):
where,

\[
\text{atom } \% = \left(\frac{\text{number of } ^{15}\text{N atoms} + \text{number of } ^{14}\text{N atoms}}{2}\right) \times 100.
\]

Compressed nitrogen gas served as a secondary standard for measurements of N isotopes. The secondary standard was calibrated against atmospheric nitrogen and reference materials from the National Institute of Standards and Technology (ammonium sulfate standards N-1 and N-2).

**Experiment 4. Retention of N Inputs to the Forest Floor**

The purpose of the fourth experiment was to test for differences in both soil N mineralization and the retention of ammonium and nitrate inputs to the forest floor at study sites above and below the cloud base.

Net N mineralization in the surface soil (O + A horizon) was measured by *in situ* buried bag incubations at the upper and lower study site during the 1993 growing season. Soil cores (10 cm deep) were sealed in plastic bags and placed in the soil in mid-June. Additional soil cores were taken to determine the initial extractable soil ammonium and nitrate (2 M potassium chloride extraction in the field) as well as gravimetric soil moisture.

The buried bags were recovered after 47 days and the soil in each bag was immediately extracted in the field with a solution of 2 M potassium chloride. Both the initial and final extracts were filtered (Whatman #42) prior to chemical analysis. Nitrification potential was calculated as the final nitrate concentration minus the initial nitrate concentration. Net N mineralization was calculated as the sum of the final nitrate and ammonium concentrations minus the sum of the initial nitrate and ammonium concentrations (all units are \(\mu g\) N/g dry soil; based on moisture content of the initial sample).

The retention of N inputs to the forest floor was measured by recovery of ammonium-\(^{15}\text{N}\) and nitrate-\(^{15}\text{N}\) additions (99 atom \% \(^{15}\text{N}\)). Four PVC tubes (17 cm long and 4 cm diameter) were driven into the forest floor at both the upper and lower study site to isolate surface soil cores (approx. 15 cm deep) that included both O and A horizons. Half of the soil cores received additions of \(^{15}\text{NH}_4\text{NO}_3\) and half received additions of \(\text{NH}_4\text{NO}_3\) (6.2 \(\mu\)mol \(^{15}\text{N}\) per core on each application). Tracer additions to the top of each core were made in 50 mL of deionized water on four occasions: June 23, July 7, July 21, and July 23, 1993. The cumulative tracer additions were 25 \(\mu\)mol \(^{15}\text{N}\) per soil core. Two control soil cores were collected at each site to determine the natural abundance of nitrogen-15 in total soil N.

The \(^{15}\text{N}\)-labelled soil cores were removed from the field and returned to the laboratory on 3 Aug 93. They were frozen prior to processing. Soil columns were thawed, extruded from the PVC tubing, and measured. Each core was cut into two parts 7.5 cm in length. The top and bottom portions of each core were dried at 100 °C, and sieved (2 mm) to remove rocks and gravel. The < 2 mm fraction was weighed and analyzed for N concentrations and N isotope ratios (see Experiment 3 for details of these analyses). The fractional retention of the nitrogen-15 applied to each soil core was calculated using equations presented by Cabrera and Kissel (1989).
RESULTS AND DISCUSSION

Experiment 1. Throughfall N Inputs Beneath the Canopy and in a Gap

The purpose of the first experiment was to test how the presence or absence of the red spruce overstory influenced both throughfall N inputs to the forest floor and the leaching of nitrate from surface soil horizons at a site below cloud base.

Comparison of the ammonium and nitrate flux in throughfall sampled at the gap and canopy location (Table 1) indicated important interactions between atmospheric N deposition and the forest canopy. Over a 56 day sampling period, the flux of ammonium in throughfall was 4.5 and 27 mg N/m² at the canopy and gap location, respectively. This difference indicated an 83% reduction in ammonium inputs to the forest floor due to the presence of the red spruce overstory. The total flux of nitrate in throughfall was also reduced (by 34%) at the canopy location relative to gap location. These findings are different from previous studies where water and ion fluxes were greater beneath the forest edge bordering canopy gaps. Our canopy location was set in the interior of the spruce-fir forest.

Artificial trees placed in the canopy gap were more exposed to direct cloud impaction and dry deposition than those shielded beneath the mature red spruce canopy despite their location near the ground. Consequently, differences between throughfall N inputs at the two sites could be attributed to either greater interception of wet and dry N deposition by artificial trees at the canopy gap or retention of atmospheric N deposition by the overstory red spruce trees at the canopy site. This comparison of throughfall N fluxes in the first experiment indicated that the net effect of the red spruce canopy was to reduce both throughfall ammonium and nitrate inputs to the forest floor. Nitrogen retained by the forest canopy is presumably later returned to the forest floor as organic-N in throughfall or litter fall.

There were no apparent differences in lysimeter water nitrate concentrations between the two sampling sites (Table 2), but the concentrations in lysimeter water were approximately 10 times greater than nitrate concentrations measured in throughfall inputs to the forest floor (Table 1). The hydrologic balance for the spruce-fir forest near the lower study site has been previously described. Evapotranspiration (ET) alone might increase the concentration of lysimeter water nitrate 4 times over concentrations measured in forest throughfall. The increase in soil solution nitrate levels beyond that expected to result from ET alone must originate from the transport of loosely held water in the surface soil where nitrate accumulates as a result of soil nitrification.

Soil nitrate leaching is a product of the soil water flux and soil water nitrate concentrations. Prior hydrologic studies indicate that precipitation inputs and soil water outputs are significantly correlated in the spruce-fir forest near the lower study site ($r = 0.89; p < 0.001$). Therefore, soil nitrate leaching losses were likely greater at the canopy gap than beneath the forest canopy because of the greater precipitation inputs at the former location (Table 2). It appears that soil nitrate leaching losses will be greater in forest canopy gaps as a direct result of increased precipitation inputs and a greater soil water flux.
Table I-1: Ammonium and nitrate inputs to the forest floor at the lower study site. The throughfall flux was measured beneath an artificial Christmas tree placed beneath a mature red spruce canopy and in a canopy gap.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Sampling Period (1992)</th>
<th>Sampling Duration (days)</th>
<th>Throughfall Flux (mg N/m²)</th>
<th>Throughfall Conc (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Canopy</td>
<td>Gap</td>
</tr>
<tr>
<td>Ammonium</td>
<td>20 Aug - 31 Aug</td>
<td>11</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>31 Aug - 09 Sep</td>
<td>9</td>
<td>2</td>
<td>65</td>
</tr>
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<td></td>
<td>09 Sep - 24 Sep</td>
<td>15</td>
<td>46</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>24 Sep - 06 Oct</td>
<td>1</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>06 Oct - 15 Oct</td>
<td>9</td>
<td>65</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Sum</td>
<td>56</td>
<td>4.5</td>
<td>27</td>
</tr>
<tr>
<td>Nitrate</td>
<td>20 Aug - 31 Aug</td>
<td>11</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>31 Aug - 09 Sep</td>
<td>9</td>
<td>64</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>09 Sep - 24 Sep</td>
<td>15</td>
<td>60</td>
<td>110</td>
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<td>24 Sep - 06 Oct</td>
<td>12</td>
<td>80</td>
<td>80</td>
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<td></td>
<td>06 Oct - 15 Oct</td>
<td>9</td>
<td>230</td>
<td>100</td>
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<tr>
<td></td>
<td>Sum</td>
<td>56</td>
<td>23</td>
<td>35</td>
</tr>
</tbody>
</table>
Table I-2. Precipitation amounts and mean (± SE) concentrations of nitrate in lysimeter water collected from soils beneath a red spruce canopy and in a canopy gap. Sample numbers are shown in parentheses.

<table>
<thead>
<tr>
<th>Sampling Period (1992)</th>
<th>PTN Sampling Duration (days)</th>
<th>Lysimeter Water Nitrate (μg N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sampling (cm)</td>
<td>Gap</td>
</tr>
<tr>
<td>10 Sep - 17 Sep</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>17 Sep - 24 Sep</td>
<td>7</td>
<td>5.6</td>
</tr>
<tr>
<td>24 Sep - 06 Oct</td>
<td>12</td>
<td>10.8</td>
</tr>
<tr>
<td>06 Oct - 15 Oct</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>15 Oct - 22 Oct</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>22 Oct - 09 Nov</td>
<td>18</td>
<td>15.2</td>
</tr>
</tbody>
</table>
Experiment 2: Comparisons of N Inputs Above and Below the Cloud Base

The purpose of the second experiment was to test for differences in atmospheric N inputs to spruce-fir stands at study sites located above and below the cloud base.

In 1993 and 1994, throughfall ammonium fluxes beneath the artificial tree were greater at the upper study site than at the lower study site (Table 3). Differences in bulk ammonium deposition in 1994 followed the same trend but not as dramatically as differences in throughfall fluxes beneath the artificial trees. The artificial trees intercepted more cloudwater and more dry deposition than the bulk collectors. Cloudwater typically has higher concentrations of ammonium than other forms of wet deposition. The comparisons in the second experiment indicated that ammonium inputs are greater for spruce-fir sites located above the cloud base. This difference can be partly explained by the greater importance of cloudwater inputs at the upper site.

Contradictory results were observed for nitrate fluxes beneath the artificial trees in 1993 and 1994. In the first year, the throughfall nitrate flux beneath the artificial tree was greater at the lower site than at the upper site (Table 3). The greater throughfall nitrate flux at the lower site in 1993, a very dry year, can be attributed to its location on a ridgetop and a greater penetration of windborne dry deposition into the forest canopy. A more limited sampling in the second year indicated the opposite result. A comparison of nitrate flux in throughfall and bulk deposition at the upper site indicated more interception of nitrate by the artificial tree. As for ammonium, the higher flux of nitrate beneath the artificial tree at the upper site in 1994 is attributed to the greater importance of cloudwater inputs during this relatively wet year.

Overall, the results from the second experiment indicated that the N inputs are greater for spruce-fir forests above the cloud base than below the cloud base. However, there may be variations from this finding (especially for nitrate) depending upon how the local effects of topography influence spatial patterns of cloudwater and dry deposition.

Experiment 3: Retention of N in Wet Deposition by Saplings

The purpose of the third experiment was to test retention of ammonium and nitrate in wet deposition by red spruce and Fraser fir saplings at locations above and below the cloud base.

Tracer applications to the red spruce and Fraser fir saplings increased the $^{15}$N content of new needles and stems, old needles, and old stems relative to natural abundance levels. Table 4 shows the atom % excess nitrogen-15 in different plant tissues following tracer applications. In most cases, new needles and stems contained more of the tracer than old needles. In all cases, old stems contained more nitrogen-15 than old needles or new needles and stems, probably because old stems are more wettable and therefore more absorbent than needles. The results of the third experiment demonstrated that needles and stems of spruce and fir saplings can retain N from wet deposition.

Although retention of ammonium and nitrate in wet deposition by old stem tissues may be very significant in the spruce-fir forest (Table 4), we only calculated retention of the tracers by new needles and stems. Retention of the tracers by old stems cannot be calculated because the mass of old stem tissue per tree was not measured.
Table 1-3. Ammonium and nitrate inputs to the forest floor at study sites located above and below the cloud base. The throughfall flux was measured beneath an artificial Christmas tree placed in a forest canopy gap at a study site above (upper site) and below (lower site) the cloud base.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Year</th>
<th>Sampling Period</th>
<th>Sampling Duration (days)</th>
<th>Throughfall Bulk Flux (mg N/m²)</th>
<th>Deposition (mg N/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper Site</td>
<td>Lower Site</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper Site</td>
<td>Lower Site</td>
</tr>
<tr>
<td>Ammonium</td>
<td>1993</td>
<td>23 Jun - 01 Jul</td>
<td>8</td>
<td>15</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>01 Jul - 15 Jul</td>
<td>14</td>
<td>3.6</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 Jul - 21 Jul</td>
<td>6</td>
<td>6.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 Jul - 03 Aug</td>
<td>13</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>03 Aug - 12 Aug</td>
<td>9</td>
<td>16</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Aug - 16 Aug</td>
<td>4</td>
<td>7.9</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 Aug - 30 Aug</td>
<td>14</td>
<td>31</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 Aug - 10 Sep</td>
<td>11</td>
<td>16</td>
<td>0.16</td>
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<tr>
<td></td>
<td></td>
<td>10 Sep - 29 Sep</td>
<td>19</td>
<td>22</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum</td>
<td>98</td>
<td>130</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>30 Aug - 13 Sep</td>
<td>14</td>
<td>17</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 Sep - 27 Sep</td>
<td>14</td>
<td>18</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 Sep - 18 Oct</td>
<td>21</td>
<td>18</td>
<td>0.22</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1993</td>
<td>23 Jun - 01 Jul</td>
<td>8</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>01 Jul - 15 Jul</td>
<td>14</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 Jul - 21 Jul</td>
<td>6</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
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<td>21 Jul - 03 Aug</td>
<td>13</td>
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<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>03 Aug - 12 Aug</td>
<td>9</td>
<td>23</td>
<td>39</td>
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<td>12 Aug - 16 Aug</td>
<td>4</td>
<td>9.1</td>
<td>11</td>
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<td></td>
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<td>16 Aug - 30 Aug</td>
<td>14</td>
<td>36</td>
<td>39</td>
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<td></td>
<td></td>
<td>30 Aug - 10 Sep</td>
<td>11</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
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<td></td>
<td>10 Sep - 29 Sep</td>
<td>19</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sum</td>
<td>98</td>
<td>180</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>30 Aug - 13 Sep</td>
<td>14</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 Sep - 27 Sep</td>
<td>14</td>
<td>18</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27 Sep - 18 Oct</td>
<td>21</td>
<td>7.9</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>
Table I-4. Atom percent excess nitrogen-15 in different plant tissues following spray applications of $^{15}\text{N}$-labelled ammonium or $^{15}\text{N}$-labelled nitrate to red spruce and Fraser fir saplings at a study site above (upper site) and below (lower site) the cloud base.

<table>
<thead>
<tr>
<th>Nitrogen-15 Applied</th>
<th>Plant Tissue</th>
<th>Atom % Excess Upper Site</th>
<th>Atom % Excess Lower Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spruce</td>
<td>Fir</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Old Needles</td>
<td>0.0210</td>
<td>0.0141</td>
</tr>
<tr>
<td></td>
<td>New Needles and Stems</td>
<td>0.0288</td>
<td>0.0084</td>
</tr>
<tr>
<td></td>
<td>Old Stems</td>
<td>0.2394</td>
<td>0.0726</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Old Needles</td>
<td>0.0241</td>
<td>0.0084</td>
</tr>
<tr>
<td></td>
<td>New Needles and Stems</td>
<td>0.0604</td>
<td>0.0242</td>
</tr>
<tr>
<td></td>
<td>Old Stems</td>
<td>0.1854</td>
<td>0.0525</td>
</tr>
</tbody>
</table>
At the end of the experiment, the measured dry mass of new needles and stems was significantly different between sites ($F_{1,8} = 9.32, p = 0.02$) and between species ($F_{1,8} = 7.17, p = 0.03$). Mean ($\pm$ SD) dry mass of new needles and stems on Fraser fir was 137 $\pm$ 69 g/tree at the upper site and 23 $\pm$ 11 g/tree at the lower site. Mean ($\pm$ SD) dry mass of new needles and stems on red spruce was 32 $\pm$ 14 and 11 $\pm$ 2 g/tree at the upper and lower sites, respectively. These differences in the growth of new needles and stems confounded the site comparisons of N retention following spray applications of nitrogen-15 tracers.

Recovery of the nitrogen-15 tracer in plant tissues is assumed to represent either absorption or adsorption of ammonium-$^{15}$N or nitrate-$^{15}$N by the saplings. Retention of the tracer by new needles and stems ranged from 0.9 to 9.4% of the nitrogen-15 applied to each tree (Table 5). Analysis of variance indicated no significant difference in tracer retention attributable to chemical form (ammonium vs nitrate) or tree species. Retention of ammonium-$^{15}$N and nitrate-$^{15}$N at the upper study site (5.9%) was significantly greater than that at the lower study site (2.9%) ($F_{1,8} = 7.41; p < 0.05$). The greater retention of tracer by new needles and stems from saplings at the upper site could be attributed to the greater amount of new needle and stem growth at that site.

Results from the third experiment are direct proof that part of the ammonium and nitrate in wet deposition is retained by needles and stems on red spruce and Fraser fir saplings. Retention of atmospheric N deposition by high elevation spruce-fir canopies has been previously inferred from measured differences between N inputs to the forest canopy and throughfall N fluxes to the forest floor. There were no differences in the retention of ammonium-$^{15}$N and nitrate-$^{15}$N. Aside from the greater retention of the $^{15}$N tracer by old stems on red spruce saplings, there were no strong differences between the two species in N retention. The third experiment also indicated that, for reasons which are not currently known, the growth of new needles and stems on red spruce and Fraser fir saplings in 1993 was greater above the cloud base (upper study site) than below the cloud base (lower study site).

Experiment 4. Retention of N Inputs to the Forest Floor

The purpose of the fourth experiment was to test for differences in both soil N mineralization and the retention of ammonium and nitrate inputs to the forest floor at study sites above and below the cloud base.

In situ incubations indicated different patterns in soil N mineralization at the upper and lower study site (Table 6). Nitrogen mineralization was significantly greater at the lower study site during the 1993 growing season. However, net nitrification (as a percentage of total net N mineralization) was more important in surface soils at the upper site.

Ammonium-$^{15}$N inputs to the soil were more strongly retained than nitrate-$^{15}$N inputs at both the upper and lower site. The retention of ammonium-$^{15}$N ranged from 34 to 45% with most of the tracer found in the upper part of each soil core (Table 7). Retention of nitrate-$^{15}$N ranged from 8 to 26% with most of the tracer found in the lower part of each soil core.
Table I-5. Dry mass, N amounts, initial and final nitrogen-15 contents, and nitrogen-15 tracer recovery in new needles and stems from 16 understory saplings located at study sites above (upper site) and below (lower site) the cloud base. The mass of nitrogen-15 (99 atom %) applied to each tree was 172 μmoles.

<table>
<thead>
<tr>
<th>Sapling</th>
<th>$^{15}$N Tracer</th>
<th>Study Site</th>
<th>Dry Mass (g)</th>
<th>N in Needles and Stems (moles)</th>
<th>Initial $^{15}$N Content (atom %)</th>
<th>Final $^{15}$N Content (atom %)</th>
<th>Tracer Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fir</td>
<td>NH$_4$-N</td>
<td>Upper</td>
<td>199.1</td>
<td>0.169</td>
<td>0.3665</td>
<td>0.3744</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>73.8</td>
<td>0.063</td>
<td>0.3659</td>
<td>0.3743</td>
<td>3.0</td>
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<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12.2</td>
<td>0.014</td>
<td>0.3658</td>
<td>0.4013</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>33.8</td>
<td>0.039</td>
<td>0.3661</td>
<td>0.3887</td>
<td>5.1</td>
</tr>
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<td></td>
<td>NO$_3$-N</td>
<td>Upper</td>
<td>79.6</td>
<td>0.067</td>
<td>0.3663</td>
<td>0.3905</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>194.8</td>
<td>0.165</td>
<td>0.3666</td>
<td>0.3741</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>15.0</td>
<td>0.017</td>
<td>0.3660</td>
<td>0.4050</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32.2</td>
<td>0.037</td>
<td>0.3658</td>
<td>0.3846</td>
<td>4.1</td>
</tr>
<tr>
<td>Spruce</td>
<td>NH$_4$-N</td>
<td>Upper</td>
<td>50.2</td>
<td>0.041</td>
<td>0.3659</td>
<td>0.3947</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.0</td>
<td>0.015</td>
<td>0.3662</td>
<td>0.3870</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>12.6</td>
<td>0.010</td>
<td>0.3656</td>
<td>0.3866</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.9</td>
<td>0.009</td>
<td>0.3655</td>
<td>0.3832</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>NO$_3$-N</td>
<td>Upper</td>
<td>31.3</td>
<td>0.026</td>
<td>0.3663</td>
<td>0.3892</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26.5</td>
<td>0.022</td>
<td>0.3655</td>
<td>0.4259</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>8.6</td>
<td>0.007</td>
<td>0.3661</td>
<td>0.4116</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.5</td>
<td>0.009</td>
<td>0.3656</td>
<td>0.4258</td>
<td>3.1</td>
</tr>
</tbody>
</table>
Table I-6. Mean ± SE net N mineralization, net nitrification, and relative nitrification in surface soils (O + A horizon) above (upper site) and below (lower site) the cloud base. Eight soil cores (10 cm deep) were incubated for 47 days in buried bags placed at each site.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Upper Site</th>
<th>Lower Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net nitrogen mineralization (µg N/g soil)</td>
<td>81 ± 26</td>
<td>139 ± 26</td>
</tr>
<tr>
<td>Net nitrification (µg N/g soil)</td>
<td>73 ± 20</td>
<td>72 ± 17</td>
</tr>
<tr>
<td>Relative nitrification</td>
<td>90%</td>
<td>52%</td>
</tr>
</tbody>
</table>

Relative nitrification = 100 (Net nitrification/Net nitrogen mineralization)
Table I-7. Dry Mass, N content, initial $^{15}$N content, final $^{15}$N content, and $^{15}$N tracer recovery in the top (0-7 cm) and bottom (7-15 cm) parts of soil cores that received cumulative additions of 25 $\mu$mol ammonium-$^{15}$N or nitrate-$^{15}$N.

<table>
<thead>
<tr>
<th>$^{15}$N Applied to Soil Core</th>
<th>Study Site</th>
<th>Core Number</th>
<th>Core Part</th>
<th>Dry Mass (g)</th>
<th>Initial $^{15}$N Content (moles)</th>
<th>Final $^{15}$N Content (atom %)</th>
<th>$^{15}$N Tracer Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4$-N</td>
<td>Upper 1</td>
<td>Top</td>
<td>23.6</td>
<td>0.023</td>
<td>0.3674</td>
<td>0.3920</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>44.0</td>
<td>0.022</td>
<td>0.3680</td>
<td>0.3929</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Upper 2</td>
<td>Top</td>
<td>23.3</td>
<td>0.021</td>
<td>0.3674</td>
<td>0.4044</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>41.5</td>
<td>0.022</td>
<td>0.3680</td>
<td>0.3748</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Lower 1</td>
<td>Top</td>
<td>34.1</td>
<td>0.029</td>
<td>0.3663</td>
<td>0.3886</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>57.3</td>
<td>0.018</td>
<td>0.3669</td>
<td>0.3763</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Lower 2</td>
<td>Top</td>
<td>23.9</td>
<td>0.027</td>
<td>0.3663</td>
<td>0.3926</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>46.2</td>
<td>0.023</td>
<td>0.3669</td>
<td>0.3779</td>
<td>10</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>Upper 1</td>
<td>Top</td>
<td>34.6</td>
<td>0.016</td>
<td>0.3674</td>
<td>0.3735</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>30.7</td>
<td>0.021</td>
<td>0.3680</td>
<td>0.3773</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Upper 2</td>
<td>Top</td>
<td>20.3</td>
<td>0.016</td>
<td>0.3674</td>
<td>0.3759</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>53.0</td>
<td>0.045</td>
<td>0.3680</td>
<td>0.3786</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Lower 1</td>
<td>Top</td>
<td>11.4</td>
<td>0.013</td>
<td>0.3663</td>
<td>0.3709</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>25.2</td>
<td>0.022</td>
<td>0.3669</td>
<td>0.3709</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Lower 2</td>
<td>Top</td>
<td>16.7</td>
<td>0.018</td>
<td>0.3663</td>
<td>0.3720</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bottom</td>
<td>24.9</td>
<td>0.026</td>
<td>0.3669</td>
<td>0.3747</td>
<td>8.1</td>
</tr>
</tbody>
</table>
Results of the fourth experiment indicated that there are marked differences in the mobility of ammonium and nitrate inputs to the spruce-fir forest soils. Ammonium inputs are more strongly retained by the surface soil than nitrate, but more than half of the cumulative ammonium-N input to soil cores at both study sites was lost during the experiment. The losses of ammonium can be attributed entirely to nitrification because the soil cores excluded nitrogen-15 uptake by plant roots. More than 74% of the nitrate-N added to the soil cores was not recovered. The losses of nitrate-N can be attributed to nitrate leaching and/or denitrification.

CONCLUSIONS

Conclusions about the retention of nitrogen inputs to high elevation spruce-fir forests near Clingman's Dome in the Great Smoky Mountains National Park can be summarized as follows:

A comparison of throughfall N fluxes beneath artificial trees placed in a canopy gap and beneath the spruce overstory indicated that the net effect of the forest canopy is to reduce forest floor N INPUTS below cloud base. Although soil solution nitrate concentrations were similar at the canopy gap and beneath the forest canopy, it appears that soil nitrate leaching losses will be greater in forest canopy gaps as a direct result of increased precipitation inputs and a greater soil water flux.

Atmospheric nitrogen inputs are greater to spruce-fir forests above the cloud base than below the cloud base. However, there may be variations, especially for nitrate, depending upon how the local effects of topography influence spatial patterns of cloudwater and dry deposition.

Tracer studies demonstrated that ammonium and nitrate in wet deposition is retained by needles and stems on red spruce and Fraser fir saplings. Following tracer additions to foliage, old stems on red spruce saplings had the highest concentrations of nitrogen-15. Retention of N in wet deposition by mature red spruce is a likely cause of lower throughfall N inputs beneath the forest canopy.

For reasons which are not currently known, the growth of new needles and stems on red spruce and Fraser fir saplings in 1993 was greater above the cloud base (upper study site) than below the cloud base (lower study site). The differences in new growth contributed to site differences in the retention of nitrogen-15 tracers applied to saplings.

Ammonium inputs to surface soil are more strongly retained than nitrate, but large losses of nitrogen inputs can be expected due to high rates of nitrification, nitrate leaching, and/or denitrification in the high elevation spruce-fir forest soils. The high mobility of nitrate in soil indicates that atmospheric N deposition may be an important contributor to soil nitrate leaching at high elevations.

ACKNOWLEDGMENTS

We thank our student assistants, Alison B. Schwab and Terri L. Shirshac, for their helpful contributions to the laboratory and the field components of this project. We also thank Bonnie Lu (ORNL) for analyzing ammonium and nitrate concentrations in solution samples. Steve Lindberg (ORNL), Pat Mulholland (ORNL), and Mark Mitch (University of Virginia) provided comments and suggestions for improvement of the draft manuscript.
TASK II. SOIL CHEMISTRY AND PLANT NUTRITION

RESEARCH SUMMARY

OBJECTIVES: To evaluate the influence of soil solution chemistry on nutrient availability, root function, and plant nutrition in poorly buffered high elevation soils with and without fertilization with base cations.

Experiment 1. Effects of Calcium Fertilization on Soil Solution Chemistry

Findings: Fertilization of 25 two-tree plots of mature red spruce at Whitetop Mountain Virginia in 1990 and 1991 improved growth (+20%) of foliage, improved foliar Ca:Al ratios (+20-60%), and continued to improve Ca:Al ratios in soil solutions three years after the last calcium application date. This substantiates the existence of calcium deficiency in mature red spruce and the importance of calcium for improved foliar nutrient uptake.

Experiment 2. Effects of Soil Solution Chemistry on Nutrient Uptake by Roots

Findings: Increased concentrations of Ca, Mg, and K and increased Ca/Al ratios in the parenchyma cells of the central stele of roots from Ca fertilized plots of mature red spruce at Whitetop Mountain indicated that Ca fertilization improved physiological function and improved cation uptake of fine roots in the field. Higher concentrations of Al, Mn, and Fe in cortical walls of unlimed roots is consistent with the hypothesis that these elements, which are mobilized by acidic deposition, act antagonistically to block cation uptake.

Experiment 3. Survey of Soil and Foliar Nutrient Status as a Part of Forest Health Monitoring and Analysis

Findings: Initial analyses of soil and plant tissue chemistry data collected in connection with a routine forest health survey on Clingman’s Dome indicate that the most significant trends in soil chemistry occur in the surface organic (Oa) soil horizon and include decreasing Ca/Al ratios and strong decreases in organic matter content with increasing elevation. Less significant cation and organic matter trends in the A horizon indicate that the chemistry and nutrient dynamics of the Oa horizon may have undergone recent changes in chemistry and decomposition rates which would lead to reduced plant uptake potential. Reduced foliar levels of base cations, most notably calcium, at high elevations and reduced ratios of foliar to soil cation levels indicated a reduced efficiency of cation uptake and/or retention likely related to increased acidic deposition at higher elevation sites.
TASK II. SOIL CHEMISTRY AND PLANT NUTRITION

Principal Task Scientists: J. D. Joslin and Mark Wolfe, Tennessee Valley Authority

RATIONALE

Nutrient input - output budgets conducted as part of the IFS research indicated that the high elevation spruce-fir forests in the Southern Appalachians showed little retention of atmospheric nitrogen and sulfur inputs and that soil solutions are largely dominated by nitrate and sulfate anions (Johnson and Lindberg, 1992). Because high elevation soils have typically low base saturation, these mobile anions mobilize Al in the upper mineral soil (Johnson et al., 1991) and may cause Al peaks above reported toxicity thresholds for red spruce seedlings (Raynal et al., 1990), which in turn may affect root distribution and function. Internal processes, such as organic matter decomposition, also seem to play an important role in soil solution chemistry especially for nitrogen in these nitrogen saturated systems (Van Miegroet et al., 1992a and b). Global changes in climatic conditions are most likely to affect these decomposition processes, particularly nitrogen mineralization and nitrification, which may have a direct impact on belowground growth conditions for these forests. Soil solution chemistry (and stream water chemistry lower in the watershed) will therefore reflect a combination of external and internal sources of nitrogen and sulfur.

The introduction of strong acid anions, such as sulfate and nitrate, to forest ecosystems may result in both the alteration of forest cation availability and increased availability of toxic elements such as Al. The resultant alteration in nutrient availability can result in forest ecosystem stress and may, at the same time, leave some important chemical fingerprints that warrant examination as useful indicators of incipient ecosystem stress. Root tissue ratios between Al and various base cations have been demonstrated to be sensitive indicators of exposure of plant roots to soil solution Al levels that are high enough to be limiting to the uptake and transport of nutrients (Godbold et al., 1988). The detection of exposures to such Al levels is an indication that the forest ecosystem in question has reached a level of acidification at which cation deficiencies are probably growth-limiting and may result in increased mortality.

The fertilization study on mature red spruce trees at Whitetop Mt. demonstrated a significant and substantial (20%) growth response in treatments receiving added Ca. Foliar chemistry has been characterized and the results provide evidence that low Ca and/or high Al is interfering with the uptake of a number of nutrients. Previous X-ray microanalysis research by Dr. Schlegel from the University of Gottingen confirmed this hypothesis, with particular support that low root Ca levels are restricting base cation uptake.

This Whitetop fertilization study provided an excellent opportunity to evaluate potential changes in soil solution chemistry and associated root histochemistry of mature red spruce in a field environment.

Experiments 1 and 2 were based on soil solution and root tissue samples collected from this Whitetop Mountain fertilization study.

Experiment 3 was undertaken as an opportunistic response which enabled us to couple soil and foliar characterization of red spruce and Fraser fir with a 1995 resurvey of forest health monitoring plots on Clingman's Dome. The focus of this work was to provide increased understanding of potential role that nutrient deficiency may play in the deteriorating condition of the high elevation forest canopy.
An additional experiment was initiated with primary support from the US Forest Service, Southern Global Change Program, to evaluate the potential effects of global warming on soil decomposition processes, particularly nitrogen mineralization. A manuscript from this work is cited in Appendix B, but we will not discuss this experiment here.

DESCRIPTION OF RESEARCH ACTIVITIES

Experiments 1 and 2. Fertilization Effects On Solution Chemistry and Root Function in a High Elevation, Poorly Buffered Spruce/Fir Soil

Experiment 1. Effects of Liming on Soil Solution Chemistry

In the early growing season of both 1990 and 1991 fertilizer treatments were applied to 25 plots of paired mature healthy red spruce on Whitetop mountain Virginia. Five treatments (each replicated five times) consisting of the following amounts on an annual basis were applied: (i) 200 Kg/ha Calcium as CaCl₂ (ii) 100 Kg/ha Magnesium as MgCl₂ (iii) Calcium and Magnesium at 200 and 100 Kg/ha respectively (iv) 400 Kg/ha Nitrogen as NH₄NO₃ and, (v) a Control receiving no fertilizer. In may of 1993 low tension hanging column lysimeters were installed in each of the 25 plots. Two lysimeters were placed into each of the Oa/A and Bw horizons at 10 cm and 50 cm respectively in all plots. Lysimeters were sampled periodically through the growing season (May-Oct.) in 1993 and 1994. Lysimeter samples were analyzed for pH as well as for common mineral cations and anions. The data from the last year of lysimeter collection from these plots (1994) focused on the Calcium, Calcium&Magnesium and Control plots.

The results of these analyses (Table 1) show that the effects of the fertilizer application are still strongly evident in the soil solution. In the Oa/A horizon Calcium concentrations in soil solution are 3-5 times greater in the Ca and Ca&Mg treatments than in Control. Soil solution levels of calcium in the Bw horizon show the same pattern although the concentrations are lower overall. The concentrations of Magnesium in soil solution are two fold greater in the Ca&Mg treatment than in the control in both the Oa/A and Bw horizons. Soil solution magnesium levels in the Calcium only treatment were not substantially different from the control in either horizon. The application of additional nitrogen to this system as NH₄NO₃ increased levels of both base cations and aluminum in soil solutions with the effect being strongest in the mineral soil. The effects of increased Ca in soil solution were apparently more than offset by increases in strong anions and aluminum as needle weights were lower in the N addition treatment (Figure 1).
Table II-1. Mean concentrations (mg/l) of cations and anions in soil solution from fertilized plots.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Treatment</th>
<th>Mg</th>
<th>Ca</th>
<th>Al</th>
<th>Mn</th>
<th>K</th>
<th>NH4</th>
<th>NO3</th>
<th>SO4</th>
<th>Cl</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oa/A</td>
<td>Ca</td>
<td>0.41</td>
<td>4.02</td>
<td>1.04</td>
<td>0.10</td>
<td>0.71</td>
<td>0.07</td>
<td>1.36</td>
<td>13.80</td>
<td>0.85</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td>Ca&amp;Mg</td>
<td>0.74</td>
<td>2.30</td>
<td>0.74</td>
<td>0.08</td>
<td>0.82</td>
<td>0.08</td>
<td>0.65</td>
<td>11.51</td>
<td>1.03</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.35</td>
<td>0.68</td>
<td>1.29</td>
<td>0.09</td>
<td>0.98</td>
<td>0.07</td>
<td>0.65</td>
<td>12.60</td>
<td>0.77</td>
<td>3.61</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.89</td>
<td>2.44</td>
<td>1.28</td>
<td>0.42</td>
<td>1.43</td>
<td>0.05</td>
<td>1.43</td>
<td>23.54</td>
<td>1.81</td>
<td>3.38</td>
</tr>
<tr>
<td>Bw</td>
<td>Ca</td>
<td>0.17</td>
<td>1.53</td>
<td>1.52</td>
<td>0.05</td>
<td>0.33</td>
<td>0.05</td>
<td>0.34</td>
<td>12.06</td>
<td>0.88</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td>Ca&amp;Mg</td>
<td>0.57</td>
<td>1.64</td>
<td>1.12</td>
<td>0.07</td>
<td>0.55</td>
<td>0.05</td>
<td>0.43</td>
<td>11.91</td>
<td>0.96</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.21</td>
<td>0.32</td>
<td>2.19</td>
<td>0.06</td>
<td>0.38</td>
<td>0.05</td>
<td>0.75</td>
<td>9.70</td>
<td>0.77</td>
<td>4.19</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.47</td>
<td>0.87</td>
<td>2.65</td>
<td>0.31</td>
<td>0.54</td>
<td>0.03</td>
<td>3.00</td>
<td>11.21</td>
<td>1.22</td>
<td>4.18</td>
</tr>
</tbody>
</table>
Fig. II-1. Mean relative needle weights for 4 fertilization treatments and control. Relative weights are the ratios of current-year weights (post-fertilization) to two-year-old needle weight (pre-fertilization), with control treatment ratios set to 1. Means with no common letter are different at 0.05 level.
Experiment 2. Effects of Soil Solution Chemistry on Root Function—Calcium Fertilization Effects upon Cation Uptake and Distribution in Red Spruce Fine Roots

Reproduction of a Poster presented at the 85th Meeting of the American Society of Agronomy, Cincinnati, OH, Nov. 7-12, 1993

J. D. Joslin and H. Schlegel, TVA, and University of Goettingen, Germany

ABSTRACT

A fertilization study of mature high elevation red spruce, growing in soil of low base saturation and high soil solution Al, produced significant positive growth responses (20%) to fertilization with Ca, accompanied by large (20-60%) and significant (p < 0.0001) increases in foliar concentrations of Ca, Mg, Zn, and Mn. We hypothesized that improvements in root Ca nutrition resulted in improved root uptake of base cations in general. To test this hypothesis, fine root samples were collected from CaCl₂-fertilized, MgCl₂-fertilized, and control plots, quick-frozen, freeze-dried, embedded in styrolbutylmethacrylate, and analyzed by X-ray microanalysis to determine the element distribution across sections of fine roots. The results indicate that Ca:Al ratios in root cortical tissue and the hyphal mantle increased with Ca fertilization. In these Ca-fertilized roots, concentrations of Ca, Mg, and K all increased in the parenchyma cells of the central stele, implying improved base cation uptake.

INTRODUCTION

Previous research indicates that red spruce foliar levels of Ca and Zn in the high elevations of the southern Appalachians are the lowest throughout the species' range.

Base cationic nutritional deficiencies may be related to growth decline at highest elevations. Growth responses in recent fertilization research point to Ca deficiency (McLaughlin et al. 1991, Van Miegroet et al. 1993).

Our own research (Joslin and Wolfe, in press) also indicated that Ca fertilization significantly improves foliar growth (Fig. 1), as well as Mg and Zn foliar concentrations. Vector analysis indicated that Zn was the primary nutrient involved in growth response, with Ca and Mg secondary (Fig. 2).

Survey of foliage across the mountaintop also indicated strong correlation of needle length with needle Zn concentration.

OBJECTIVES

In Joslin and Wolfe (in press), we hypothesized that improvements in root Ca nutrition resulted in improved uptake of base cations in general, especially for Ca, Mg and Zn. The addition of Ca may act by countering the antagonistic action of several elements towards base cation uptake:

Aluminum is well known to be antagonistic to the uptake of base cations, especially divalent ones such as Ca, Mg and Zn (Foy 1984, Schlegel et al. 1992). The addition of Ca has been demonstrated to improve Ca:Al ratios in root tissues and to improve cation uptake (Sucoff et al. 1990). Recent
Fig. II-2. Vector diagram depicting effects of +Ca, +Mg, and +(Ca and Mg) fertilization treatments on current year foliar concentration and content, expressed as relative values with control = 1. Solid lines connect the control level (1, 1) to the most responsive nutrient (Zn or Ca). Values for a given treatment fall along a dotted line.
work (Langheinrich et al. 1992) with red spruce demonstrates that excess Mn can have a similar effect. Antagonisms between Al and Zn (Suthipradit 1988), between Mn and Zn, and between Fe and Zn (Giordano et al. 1974, Haldar and Mandal 1981) have been also demonstrated with agricultural crops.

The objectives of this research were to determine:

1. If a positive relationship existed between Ca levels in the cortex and the quantity of base cations (Ca, Mg, Zn) (i) entering the stele and (ii) being incorporated in foliar tissues, and
2. If a negative relationship existed between Al, Fe, and/or Mn levels in the root cortex and base cations entering (i) the stele and (ii) foliar tissues.

METHODS

To test these hypothesis, we sampled red spruce fine root tissues from plots previously fertilized with CaCl₂ and from control plots (no fertilization).

Separate stands of apparently healthy mature trees (80-120 years old, 50 m² ha⁻¹ basal area) near the summit of Whitetop Mt., VA (1670 m elevation) were fertilized with 200 kg/ha Ca as CaCl₂ in late spring of 1990 and again in 1991. The results of previous foliar sampling (1991) growth and nutritional analysis are presented above in Figs. 1 and 2.

Fine root samples (< 1 mm diameter, within 1 cm of root tip) were collected in August 1992 from 5 control plots (no fertilization) and 5 Ca-fertilized plots. Duplicate samples from each plot were taken from the 0 horizon (approx. 5 cm below the surface of the forest floor) and from the mineral A horizon (5-10 cm into mineral horizon, 12-20 cm below surface).

In order to prevent post-sampling diffusion of cations in root tissues, roots were immediately quick-frozen on site in a liquid propane-isopentane (3:1, v/v) mixture which was cooled in liquid N. Samples were maintained in a frozen state (-79°C) until they could be freeze-dried in the laboratory, infiltrated with ether, embedded in styrol-butylmethacrylate and cut into 1 micron thick sections (Fritz 1989).

Ion distributions of Mg, Al, Si, P, S, K, Ca, Mn, Fe, and Zn across root sections were analyzed using energy-dispersive X-ray microanalysis in three regions:

(i) mycorrhizal hyphal mantle,
(ii) cortex cell walls, and
(iii) parenchymatic cell walls of the central cylinder (stele).

Only results from the cortex and stele of roots from the organic horizon are reported because few treatment differences were observed in the mineral soil or in mycorrhizae.

RESULTS

The correspondence of treatment differences in Root Uptake Efficiency Indices (stele:cortex) for Ca, Mg, and Zn with differences in foliar concentrations of Ca, Mg, and Zn indicate that the RUE Index
reflects alterations in the uptake process caused by fertilization. For each of the 3 cations, the RUE Index was increased at least 2-fold by the Ca-fertilization treatment (Fig. 4).

Patterns in cortical concentrations of Al, Mn, and Fe indicate that all 3 were reduced by the Ca-fertilization treatment. For Al, the reduction was approximately 50%, whereas for Mn and Fe, it was approximately 90% (Fig. 5).

The much higher concentrations of Al, Mn, and Fe in the cortical cell walls of the control, compared to Ca-fertilized, roots is consistent with the hypothesis that these 3 elements are acting antagonistically toward the uptake of divalent base cations in the control roots.

For all three divalent base cations of interest (Ca, Mg, and Zn), concentrations were higher in the stele of Ca-fertilized roots from the 0 horizon compared to control roots from the 0 horizon (Fig. 3). This was the expected result and is consistent with the finding that levels of all 3 nutrients were also significantly higher in the foliage of Ca-fertilized trees.

In contrast, levels of all three cations - including Ca - were lower in the root cortical cells of Ca-fertilized roots compared to control roots (Fig. 3).

Since the critical step in root uptake of cations is their movement across the Casparian strip into the stele, we propose that, at any one point in time, the ratio of the concentration of a given cation in the stele to its concentration in the cortex is a good index of the efficiency of the uptake process for that cation (Root Uptake Efficiency Index), as shown in Figure 6.

**DISCUSSION**

In acid soils, the Ca:Al ratio of either (or both) soil solutions and root tissues has often been used as an index of rhizosphere conditions favorable to root vitality and cation uptake. For our purposes we have expanded this concept and examined the ratio or \((\text{Ca + Mg}) / (\text{Al + Fe + Mn})\) in the root cortex as an index of favorable conditions for root uptake.

In the case of the critical element Zn in this study, the usefulness of such a ratio is supported by the close positive relationship observed between the \((\text{Ca + Mg}) / (\text{Al + Fe + Mn})\) ratio in the root cortex and both (i) the Root Uptake Efficiency for Zn and (ii) the concentration of Zn in foliage.

Similar relationships were observed for Ca and Mg.

**SUMMARY**

It has been hypothesized that cationic nutritional deficiencies may be related to growth declines in red spruce at the highest elevations of the Southern Appalachians. Red spruce foliar growth responses to Ca fertilization point to Zn as the primary nutrient involved in the response, with Ca and Mg secondary.

We hypothesized that (1) improvements in root Ca nutrition had improved the efficiency of root uptake of base cations in general, especially of Ca, Mg and Zn and (2) these improvements were partially the result of Ca counter-acting the antagonistic action of Al, Mn, and/or Fe towards divalent base cation uptake.
Fig. II-3. Concentrations of Zn, Ca, and Mg in the fine root cortex and the fine root stele of control and Ca-fertilized roots from the 0 horizon.
Root Uptake Efficiency Index (stele:cortex ratio) and foliar concentrations (current year needles) for Zn, Ca and Mg of control and Ca-fertilized trees.
Fig. II-5. Concentrations of Fe, Al, and Mn in the fine root cortex of control and Ca-fertilized roots from the 0 horizon.
Fig. II-6. Relationship between the ratio of (Ca + Mg) to (Fe + Al + Mn) in the fine root cortex (0 horizon) to the RUE Index for Zn and to the foliar concentration of Zn across 3 fertilization treatments.
Red spruce fine root tissues were sampled from the 0 horizon of plots previously fertilized with CaCl₂ and from control plots. Thin sections of roots were prepared under water-free conditions, and ion distributions across the sections were analyzed using X-ray microanalysis.

* For all three divalent base cations of interest (Ca, Mg, and Zn), concentrations were higher in the stele of Ca-fertilized roots from the 0 horizon compared to control roots from the 0 horizon.

* For each of the three cations, the Root Uptake Efficiency Index was increased at least two-fold by the Ca-fertilization treatment.

* Higher concentrations of Al, Mn, and Fe in the cortical cell walls of control roots compared to the Ca-fertilized roots is consistent with the hypothesis that these elements are acting antagonistically toward the uptake of divalent base cations in control roots.

* A strong positive relationship was observed between the (Ca + Mg):(Al + Fe + Mn) ratio in the root cortex and both (i) the Root Uptake Efficiency Index for Zn and (ii) the concentration of Zn in foliage.

**Experiment 3. Survey of Soil and Foliar Nutrient Status as a Part of Forest Health Monitoring and Analysis**

Soil and foliar sampling was carried out on 35 plots of spruce-fir in the Smoky Mountains during the summer of 1995 to evaluate any elevational relationship in soil nitrogen content and other soil nutrients to stand health. The plots sampled were part of a long term monitoring study of stand growth and mortality dynamics and were located between 5000 to 6600 feet elevation. Long term forest health plots were also re-measured in 1995. At each plot the Oa and A horizon were sampled at 4-6 locations within the plot and composited. Oa and A horizon samples were fresh extracted with 0.01 m SrCl₂ on a 2:1 ratio with the calculated dry weight of the sample. Solution extracts were analyzed for common cations and anions. Organic matter content was also determined on all samples by dry ashing at 500°C for 24 hours. Foliar sampling of red-spruce and fir saplings and some mature trees of these species were done in the fall of 1995 on the same plots from which soil samples were collected. The most notable preliminary results of soil and foliar analysis are shown below.

**Soil results (Figures 7, 8, and 9)**

1. Oa horizon Ca/Al ratios decline with elevation, A horizon Ca/Al ratios were roughly the same across elevation

2. Percent OM shows a strong decline with elevation in the Oa horizon, but no change across elevation in the A horizon.

3. Ca levels in both the Oa and A horizon did not exhibit any elevational trend, but Ca levels were higher in the Oa than in the A horizon at all elevations.

4. NH₄ in the Oa horizon declined with elevation, however, no differences were observed across elevation in the A horizon.
Fig. II-7. Nutrient content in the Oa soil horizon from 36 survey plots distributed across 4 elevations in the spruce-fir zone of Clingman’s Dome, GSMNP, in the 1995 survey.
Nutrient content in the A soil horizon from red 36 survey plots distributed across 4 elevations in the spruce-fir zone of Clingman’s Dome, GSMNP, in the 1995 survey.
% O.M. (dry ashing) of Oa and A horizons at four elevations

Fig. II-9. Organic matter from the Oa and A soil horizons from 4 elevations on Clingman’s Dome, GSMNP, in the 1995 survey.
5. NO₃ concentrations were lowest at the 5000 foot elevation in both the Oa and A horizon, but no difference was seen in elevation above 5000 in either horizon. Oa horizon concentrations were higher than those of the A horizon at all elevations.

6. Aluminum levels of the Oa horizon showed no consistent elevation pattern, A horizon levels declined with elevation.

Foliar results (Figures 10-17)

1. Foliar concentrations of nitrogen, calcium, magnesium and aluminum and in red-spruce saplings decreased with increasing elevation.

2. In mature red-spruce foliar concentrations of nitrogen and calcium decreased with elevation. Aluminum increased with elevation. Magnesium concentrations were similar across elevations.

3. In fir saplings nitrogen and aluminum exhibited no clear elevational trend. Calcium and magnesium concentrations were highest at the lowest elevation, but showed no consistent trend across elevations ≥ 5500 ft.

4. Other nutrients (P, Zn, Mn, K, and Fe) measured in both red-spruce and fir either did not change across elevation or showed no clear trend with elevation.

DISCUSSION

Our initial analyses of these data indicate that spruce and fir growing in higher elevation soils experience reduced uptake of base cations, particularly calcium. One measure of the potential to take up and retain nutrients can be calculated as a ratio of foliar calcium to soil-available calcium. The foliar:soil Ca values for mature red spruce, red spruce saplings, and Fraser fir saplings comparing lowest elevation sites (5000 ft) to highest elevation sites (6500 ft) were 0.79 vs 0.36, 0.60 vs 0.39, and 1.2 vs 0.65 respectively for the Oa horizon. Similar differences were noted for the mineral A horizon. These data emphasize the decreased capability of trees to take up and retain nutrients at higher elevation sites where leaching from both soils and foliage is accelerated.

In addition, sapling trees of both spruce and fir have relatively higher foliar levels of base cations than do mature trees, perhaps reflecting lower dependency of saplings on nutrient uptake from deeper mineral soils. While both species have comparable foliar levels of Mg and P, fir typically had higher levels of foliar Ca by approximately 50% (Figures 14 and 15). Foliar aluminum, on the other hand, were approximately 4 times higher for fir than for spruce (Figures 16 and 17). Thus Ca: Al levels in fir foliage were significantly lower in fir than in spruce saplings. This suggests the possibility that fir may be more sensitive to aluminum induced uptake of calcium than red spruce even though the total foliar levels of Ca were higher in fir.
Foliar potassium and nitrogen from red spruce and Fraser fir saplings and mature spruce and fir trees from 4 elevations (not inclusive for mature fir) on Clingman's Dome, GSMNP, in the 1995 survey.
Foliar Mn concentrations of spruce and fir at four elevations

Fig. II-11. Foliar manganese from red spruce and Fraser fir saplings and mature spruce and fir trees from 4 elevations (not inclusive for mature fir) on Clingman's Dome, GSMNP, in the 1995 survey.
Fig. II-12. Foliar zinc, iron, and aluminum from mature red spruce trees from 4 elevations on Clingman's Dome, GSMNP, in the 1995 survey.
Mature spruce foliar nutrient content at four elevations

Fig. II-13. Foliar calcium, phosphorous, and magnesium from mature red spruce trees from 4 elevations on Clingman’s Dome, GSMNP, in the 1995 survey.
Fig. II-14. Foliar calcium, phosphorous, and magnesium from Fraser fir saplings from 4 elevations on Clingman's Dome, GSMNP, in the 1995 survey.
Fig. II-15. Foliar calcium, phosphorous, and magnesium from red spruce saplings from 4 elevations on Clingman's Dome, GSMNP, in the 1995 survey.
Spruce sapling foliar nutrient content at four elevations

Fig. II-16. Foliar zinc, iron, and aluminum from red spruce saplings from 4 elevations on Clingman’s Dome, GSMNP, in the 1995 survey.
Fir sapling foliar nutrient content at four elevations

Fig. II-17. Foliar zinc, iron, and aluminum from Fraser fir saplings from 4 elevations on Clingman’s Dome, GSMNP, in the 1995 survey.
TASK III. PHYSIOLOGICAL INDICATORS OF PLANT FUNCTION

RESEARCH SUMMARY

Objectives: To evaluate effects of base cation depletion by acidic deposition on indicators of plant nutritional stress and impaired plant function of high elevation red spruce and Fraser fir.

Experiment 1. Influence of calcium nutrition on growth and physiology of red spruce and Fraser fir
This experiment included three tasks: A. Elevation survey of Fraser fir nutrition and gas exchange physiology; B. Fertilization studies with Fraser fir; and C. Fertilization studies with red spruce.

Findings: A. Survey. A survey of Fraser fir gas exchange physiology and foliar nutrition indicated that reduced calcium availability at high elevations in the GSMNP was associated with reduction in the photosynthesis:respiration ratio in a manner similar to that observed for red spruce; B. Fertilization of Fraser fir saplings with Ca. Results of liming/fertilization studies are still being analyzed as a part of a Ph. D. project, but suggest that Fraser fir is sensitive to increased inputs of nitrate and other strong anions to the system. C. Fertilization of red spruce saplings with Ca. A single application of CaCl₂ (200 kg/ha) to high elevation red spruce saplings led to stimulated photosynthesis, reduced respiration, and increased stem growth during the year after application.

Experiment 2. Exploratory Fluorescence analysis of cellular partitioning of calcium partitioning within plant cells.

Findings: Laser-induced fluorescence can be used to detect calcium with either pulsed or continuous-wave (cw) lasers. There may be advantages from using a pulsed (and possibly tunable) laser in this process, which might include the ability to detect two-photon fluorescence, study dynamic processes in cells, or use phase-sensitive detection techniques to enhance selectivity. From a practical aspect achieving penetration of Ca-specific fluorescent dyes into living tissues must still be resolved to accurately detect levels and the binding status of in situ calcium in tissues.

Experiment 3. Evaluation of relationships among mature red spruce growth rate, wood chemistry, and wood structural characteristics.

Findings: New techniques in materials testing were applied to the characterization of wood that documented important relationships among wood chemistry and wood structural properties. Energy dispersive X-ray spectroscopy was used in combination with a transmission electron microscope and a materials hardness tester to show that reduced levels of wood calcium in cell walls are associated with reduced lignin content and reduced levels of elasticity. Exploratory analyses of red spruce cores from high elevation sites demonstrated that reduced lignin, and reduced wall elasticity were associated with reduced levels of calcium in cell walls in more recently formed wood and in trees growing at higher elevations. Analysis of the annual growth patterns in relationship to historical and recent monthly climate data show that mature red spruce trees are responding more negatively to both temperature and precipitation in recent decades than previously. The negative response of red spruce to warmer temperatures and increasing precipitation is compatible with increased sensitivity of growth to acidic deposition through two mechanisms that are stimulated by acidic deposition, increased respiration and increased nutrient depletion through leaching.
TASK III. PHYSIOLOGICAL INDICATORS OF PLANT FUNCTION

Principal Task Scientists: S. B. McLaughlin and S. D. Wullschleger, Oak Ridge National Laboratory; R. Wimmer, Austrian Agricultural University, Vienna; R. F. Haglund, Vanderbilt University; and A. T. Stone, University of Tennessee Graduate Ecology Program

RATIONALE

Because of its roles in membrane stabilization and in the formation of crosslinks in cell walls and its limited mobility in plants, calcium has the potential to alter a wide variety of essential plant processes. Acidic deposition alters cation availability, particularly calcium, to growing plants through several mechanisms. These include increased leaching losses from foliage, increased mineralization and leaching from soils, and inhibition of cation uptake due to release of aluminum from soils.

Evidence from studies in the NAPAP sponsored Spruce-Fir Research Cooperative strongly implicates changing availability of calcium, in particular, and magnesium in some of the physiological disorders associated with adverse effects of acid deposition on red spruce in the Eastern U.S. (McLaughlin and Kohut, 1992). These include both increasing plant respiration and reduced cold tolerance, both thought to be due to a loss of plant membrane integrity.

Foliar nutrition in relation to gas exchange processes was of interest in these experiments because previous studies of red spruce physiology along elevational gradients in the GSMNP showed that reduced foliar calcium occurring at high elevation sites was associated with a decreased efficiency of carbon metabolism (McLaughlin et al., 1990, 1991). Subsequent laboratory studies confirmed the capacity of acidic mist and rain to reproduce those symptoms and reduce tree growth and depth of rooting (McLaughlin et al., 1993). These results suggested that Fraser fir growing in this same environment might also be experiencing nutrient deficiency and physiological dysfunction on these same sites.

Weakening of Fraser fir may be an important factor in both the susceptibility of these trees to attack by the balsam wooly aphid as well as in their capacity to recover following attack. Thus exploring the extent to which physiological weakening of fir may have occurred may provide very important insights related to host - pathogen relationships which are currently a dominant influence on the diversity and stability of the high elevation spruce - fir ecosystem.

The importance of calcium in cell wall formation suggested to us that wood formed during periods of reduced calcium availability might have a lower structural integrity and hence be more susceptible to the normal wind and icing stresses of the high elevation environment. Since calcium is essential for cell wall growth and is currently physiologically limiting at high elevation sites in the Southern Appalachians, we suspected that wood quality may have been affected by reduced calcium supply. We further anticipated that any weakening in wood properties would lead to enhanced canopy and stem damage by winds and ice at high elevation sites that receive the highest levels of acidic deposition.

Such changes might explain the canopy deterioration associated with losses of branches and twigs currently observable in some declining canopy dominant trees at high elevation sites across the Appalachians.
This set of experiments was designed to examine the role of calcium nutrition on physiological processes of red spruce and Fraser fir. Processes examined included growth, foliar gas exchange, changes in distribution of calcium in leaf cells and the effects of reduced wood calcium levels on wood chemistry and wood structural properties.

DESCRIPTION OF RESEARCH ACTIVITIES

Three types of experiments were being conducted under this task to provide additional documentation of the importance of calcium to plant nutrition and growth of Fraser fir.

Experiment 1. Influence of soil chemistry and foliar nutrition on carbon assimilation of Fraser fir and red spruce saplings

This experiment consisted of two principal components: an initial survey of foliar and soil nutrition and foliar gas exchange physiology of Fraser fir along elevational gradients and a study of the effects of fertilizing with calcium on sapling growth and physiology. The survey was conducted on two mountains, Mt. Rogers in southwest Virginia and Clingman's Dome in the GSMNP. These two locations have had important historical differences in susceptibility of indigenous Fraser populations to adelgid attack, with high elevation sites on Mt. Rogers having had an apparent resistance to adelgid induced mortality.

Based on this exploratory survey we subsequently initiated a Ph.D. research project (April Stone, Ecology and Evolutionary Biology, University of Tennessee) to examine the effects of variations in atmospheric and soil chemistry on growth and gas exchange physiology of Fraser fir.

The experimental design for the survey study included obtaining concurrent measurements on foliar nutrient status, soil chemical status, and foliar rates of photosynthesis and dark respiration from 10 sapling (2-3 m tall) Fraser fir trees at each of two elevations on Mt. Rogers (1300 m and 1700 m (summit) and three elevations (1750 m, 1850 m, and 1950 m (summit)) of Clingman's Dome in the GSMNP. Data on sapling height, diameter, apparent age (node count), and needle characteristics (length and thickness) were also collected.

For the more intensive study with Fraser fir which followed on Clingman’s Dome, Plots were set up at 1750 m and 1950 m to examine the effects of soil nutrient status and acidic deposition on Fraser fir physiology and growth. Three soil treatments (complete fertilizer addition, addition of calcium only, and control) were applied to eight 2-3 m tall fir saplings at each of the two study sites on Clingman’s Dome during the spring of 1993. These were the same sites on which red spruce was previously examined by McLaughlin et al.(1990 and 1991).

Fertilization rates begun in the spring of 1993. They were designed to add 200 kg/ha of Ca in the Ca only treatment, a complete fertilizer with 200 kg/ha of Ca and 100 kg/ha of Mg, and 100 kg/ha of nitrogen with micronutrients. Soil solution chemistry was measured with 16 lysimeters at each elevation and throughfall volume and chemistry were tracked with 8 collectors at each elevation. The method of calcium addition was changed from CaCl₂ to CaO prior to the third year of this experiment due to high levels of chloride, a strongly acidifying ion detected in high quantities in the soil solutions. A detailed outline of the design of this experiment and the types of data collected can be found in Appendix A.
RESULTS

A. Fraser fir elevational transect - Tables 1-3 summarize the physical data on the 8-10 sapling trees evaluated at two elevations on Mt. Rogers (MR) and three elevations on Clingman’s Dome (CD). Height, diameter, age (estimated by whorl counts), and cover data in Table 1 indicate that, in general, comparable sized trees were measured at all locations. However trees at the highest elevation sites on both mountains were growing more slowly as judged by calculated height/age ratios. Estimated mean height and diameter growth per year for the highest elevation sites were 20.4 cm/y and .36 cm/y for MR and 19.2 cm/y and 0.43 cm/y for CD. At the lowest elevation sites height and diameter growth were 31.5 cm/y and 0.68 cm/y on MR and 27.6 cm/y and 0.54 cm/y on CD. The most significant tree level difference was the somewhat more open condition of trees measured in the forest/shrub transition zone of the lower elevation MR site.

Node length and diameter data in Table 2 for the last three years terminal growth reflect similar trends observed for the whole trees as discussed above. Needle size and thickness data in Table 3 reflect generally similar morphological characteristics across the sites with the exception being the low elevation MR site where the thicker, wider needles reflect the expected morphological response to more open canopy conditions.

Data on foliar gas exchange (Table 4) indicated that Fraser fir shows a decrease in photosynthesis to respiration ratio with increasing elevation and decreasing calcium and aluminum on Clingman’s Dome (elevation range = 5700 ft to 6400 ft). The Ps:Rs ratio on CD showed an almost linear decline with increasing elevation with the Ps:Rs value at 1950 m being approximately half that at the 1750 m elevation. This was a very similar pattern to that shown previously with red spruce at the same sites.

Interestingly, on the summit of Mt Rogers where adelgid damage has been slowest to develop, an inverted Ps: Rs gradient was observed with comparable Ps: Rs values to CD at the high site but Ps:Rs values reduced by approximately 33% at the lowest elevation. MR-Low Ps:Rs values were only about a third of those of the CD-Low site.

Analyses of differences in soil (Table 5) and foliar (Table 6) nutrient levels with elevation on the two mountains identified significantly lower calcium and magnesium, and significantly higher aluminum and iron in the upper rooting zone at CD. On MR by contrast, this gradient was reversed with the low elevation site having significantly lower Ca and pH, and significantly higher aluminum levels. As expected from these data the elevational trends in Al:Ca ratios were also reversed between the MR and CD.

Trends in foliar analyses (Table 6) reflected the previously discussed soil analyses except that calcium in foliage was lowest on MR - high despite that the somewhat higher soil Ca levels. These data suggest that calcium uptake and/or retention at the upper elevation sites may be reduced relative to soil supply by foliar leaching increasing the relative importance of the frequent exposure of higher elevation forests to acidic mists.

Statistical analyses of the gas exchange data will be included in the Ph. D dissertation based on this work, however means and standard deviations reported in Table 5 suggest that elevational differences in Ps, Rs, and Ps/Rs will be statistically significant. We have determined that dark respiration on MR
Table III-1. Physical dimensions and approximate age of Fraser fir saplings sampled in gradient studies on Mt. Rogers and Clingman's Dome, GSMNP, during the summer of 1992. Data are averages of 8-10 trees per site.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>Height (cm)</th>
<th>Diameter (cm)</th>
<th>Age</th>
<th>MNFT(^a)</th>
<th>CC(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. Rogers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>297 ± 46</td>
<td>6.45 ± 1.71</td>
<td>9.4 ± 1.6</td>
<td>4.6 ± 1.4</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>High</td>
<td>243 ± 31</td>
<td>4.33 ± 0.81</td>
<td>11.9 ± 1.3</td>
<td>5.0 ± 0.9</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Clingman's Dome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>265 ± 64</td>
<td>5.21 ± 1.33</td>
<td>9.5 ± 1.7</td>
<td>4.3 ± 1.1</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Medium</td>
<td>231 ± 41</td>
<td>4.50 ± 1.15</td>
<td>8.8 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>3.1 ± 0.9</td>
</tr>
<tr>
<td>High</td>
<td>286 ± 37</td>
<td>6.46 ± 1.31</td>
<td>14.9 ± 2.4</td>
<td>8.8 ± 1.6</td>
<td>2.0 ± 0.5</td>
</tr>
</tbody>
</table>

\(^a\) Number of nodes from measured branch to top of tree.

\(^b\) Indicator of canopy closure, 1 open, 4 closed.
Table III-2. Comparative recent terminal node growth patterns of Fraser fir saplings sampled in gradient studies on Mt.Rogers and Clingman's Dome, GSMNP, during the summer of 1992.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>Node length (cm)</th>
<th>Node diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mt. Rogers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>22.2 ± 4.7</td>
<td>20.3 ± 4.2</td>
</tr>
<tr>
<td>High</td>
<td>14.2 ± 3.7</td>
<td>16.6 ± 2.7</td>
</tr>
<tr>
<td>Clingman's Dome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>16.2 ± 1.3</td>
<td>16.3 ± 1.8</td>
</tr>
<tr>
<td>Medium</td>
<td>12.7 ± 2.0</td>
<td>14.0 ± 2.1</td>
</tr>
<tr>
<td>High</td>
<td>11.6 ± 2.5</td>
<td>12.4 ± 1.9</td>
</tr>
</tbody>
</table>
Table III-3. Comparative needle morphology of Fraser fir saplings sampled in gradient studies on Mt. Rogers and Clingman's Dome, GSMNP, during the summer of 1992.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>Needle length (mm)</th>
<th>Needle width (mm)</th>
<th>Needle thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mt. Rogers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19.5 ± 2.4</td>
<td>1.87 ± 0.10</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>High</td>
<td>21.4 ± 2.5</td>
<td>1.61 ± 0.12</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td><strong>Clingman's Dome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>21.5 ± 1.6</td>
<td>1.58 ± 0.12</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Medium</td>
<td>22.3 ± 3.2</td>
<td>1.55 ± 0.10</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>High</td>
<td>20.0 ± 1.5</td>
<td>1.60 ± 0.08</td>
<td>0.35 ± 0.02</td>
</tr>
</tbody>
</table>
Table III-4. Comparative rates of photosynthesis and respiration of Fraser fir saplings sampled in gradient studies on Mt. Rogers and Clingman's Dome, GSMNP, during the summer of 1992.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>Photosynthesis</th>
<th>Respiration</th>
<th>( \frac{\text{Ps}}{\text{Rs}} )</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg CO₂ g⁻¹ h⁻¹</td>
<td></td>
<td>°C</td>
<td></td>
</tr>
<tr>
<td>Mt. Rogers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>6.30 ± 0.77</td>
<td>1.46 ± 0.17</td>
<td>4.4 ± 0.9</td>
<td>26.8 ± 1.0</td>
</tr>
<tr>
<td>High</td>
<td>8.54 ± 1.76</td>
<td>1.30 ± 0.18</td>
<td>6.7 ± 1.7</td>
<td>20.8 ± 1.1</td>
</tr>
<tr>
<td>Clingman's Dome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>9.23 ± 1.10</td>
<td>0.76 ± 0.16</td>
<td>12.7 ± 3.3</td>
<td>19.5 ± 1.3</td>
</tr>
<tr>
<td>Medium</td>
<td>7.88 ± 1.18</td>
<td>0.82 ± 0.12</td>
<td>9.8 ± 1.5</td>
<td>19.8 ± 1.0</td>
</tr>
<tr>
<td>High</td>
<td>6.38 ± 0.68</td>
<td>0.93 ± 0.11</td>
<td>6.9 ± 1.3</td>
<td>18.9 ± 1.4</td>
</tr>
</tbody>
</table>

\( ^a \) The ratio of photosynthesis divided by respiration.
Table III-5. Nutrient analyses of current year foliage samples collected from Fraser fir saplings sampled on Mt. Rogers and Clingman’s Dome, GSMNP, during the summer of 1992.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>CA (mg/L)</th>
<th>P (mg/L)</th>
<th>Mg (mg/L)</th>
<th>N (%)</th>
<th>Al (ppm)</th>
<th>Fe (ppm)</th>
<th>Bo (ppm)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Al/CA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mt. Rogers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.32</td>
<td>0.17</td>
<td>0.07</td>
<td>1.48</td>
<td>365</td>
<td>59.5</td>
<td>13.1</td>
<td>3.74</td>
<td>30.1</td>
<td>1165</td>
</tr>
<tr>
<td>High</td>
<td>0.25</td>
<td>0.17</td>
<td>0.07</td>
<td>1.59</td>
<td>115</td>
<td>63.3</td>
<td>12.7</td>
<td>3.63</td>
<td>23.8</td>
<td>473</td>
</tr>
<tr>
<td>P &gt; F</td>
<td>0.0115</td>
<td>0.7946</td>
<td>0.9561</td>
<td>0.0802</td>
<td>0.0001</td>
<td>0.8617</td>
<td>0.7883</td>
<td>0.7411</td>
<td>0.0154</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Clingman’s Dome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.20</td>
<td>0.13</td>
<td>0.07</td>
<td>1.41</td>
<td>110</td>
<td>45.6</td>
<td>9.7</td>
<td>3.06</td>
<td>17.3</td>
<td>594</td>
</tr>
<tr>
<td>Med</td>
<td>0.22</td>
<td>0.15</td>
<td>0.08</td>
<td>1.41</td>
<td>152</td>
<td>80.0</td>
<td>8.4</td>
<td>3.46</td>
<td>20.3</td>
<td>704</td>
</tr>
<tr>
<td>High</td>
<td>0.17</td>
<td>0.14</td>
<td>0.05</td>
<td>1.23</td>
<td>182</td>
<td>247.2</td>
<td>7.0</td>
<td>2.83</td>
<td>17.2</td>
<td>1115</td>
</tr>
<tr>
<td>P &gt; F</td>
<td>0.0188</td>
<td>0.0985</td>
<td>0.0001</td>
<td>0.0028</td>
<td>0.0023</td>
<td>0.0331</td>
<td>0.1699</td>
<td>0.3656</td>
<td>0.1406</td>
<td>0.0017</td>
</tr>
</tbody>
</table>
Table III-6. Nutrient analyses of composite soil samples collected from the upper rooting zone (typically 6-12 cm deep) of 8-10 Fraser fir saplings sampled on Mt. Rogers and Clingman’s Dome, GSMNP, during the summer of 1992.

<table>
<thead>
<tr>
<th>Site/elevation</th>
<th>Ca</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Al</th>
<th>Fe</th>
<th>Zn</th>
<th>pH</th>
<th>OM</th>
<th>Al/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. Rogers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>190</td>
<td>28.6</td>
<td>137</td>
<td>48.6</td>
<td>1926</td>
<td>162</td>
<td>6.6</td>
<td>4.2</td>
<td>34</td>
<td>13.5</td>
</tr>
<tr>
<td>High</td>
<td>290</td>
<td>18.4</td>
<td>99</td>
<td>33.7</td>
<td>423</td>
<td>117</td>
<td>5.8</td>
<td>3.4</td>
<td>72</td>
<td>1.8</td>
</tr>
<tr>
<td>P &gt; F</td>
<td>0.03</td>
<td>0.10</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.25</td>
<td>0.55</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Clingman’s Dome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>345</td>
<td>12.3</td>
<td>69</td>
<td>44.6</td>
<td>394</td>
<td>180</td>
<td>6.2</td>
<td>3.5</td>
<td>63</td>
<td>2.6</td>
</tr>
<tr>
<td>Med</td>
<td>307</td>
<td>12.6</td>
<td>88</td>
<td>52.0</td>
<td>802</td>
<td>367</td>
<td>3.2</td>
<td>3.8</td>
<td>26</td>
<td>3.8</td>
</tr>
<tr>
<td>High</td>
<td>130</td>
<td>13.3</td>
<td>102</td>
<td>37.7</td>
<td>2338</td>
<td>375</td>
<td>2.1</td>
<td>3.9</td>
<td>46</td>
<td>21.0</td>
</tr>
<tr>
<td>P &gt; F</td>
<td>0.06</td>
<td>0.88</td>
<td>&lt; 0.01</td>
<td>0.16</td>
<td>&lt; 0.01</td>
<td>0.05</td>
<td>&lt; 0.01</td>
<td>0.12</td>
<td>&lt; 0.08</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
was negatively related to foliar Ca levels ($P \leq 0.04$) while Ps:Rs was positively influenced by foliar Ca ($P \leq 0.06$) across both mountains and all elevations combined.

B. Fraser fir liming experiments - Three years of data have been collected in this experiment and will be finally analyzed as a part of the Ph.D. dissertation of April Stone. The data set contains growth data on 48 sapling trees, gas exchange physiology on current year foliage produced during each treatment year by these trees, and water chemistry from rainfall, throughfall, and soil lysimeter data. The water chemistry data represent collections on 12 dates in 1993, a dry year; 14 collections during 1994, a wet year; and 9 collections during 1995 a relatively dry year. These data offer excellent opportunities to examine the relationship between rainfall amount, distribution, and chemistry on soil solution chemistry, and in turn the relationship between soil solution chemistry and its chemical and physical drivers and growth of 20 large spruce trees for which seasonal growth dynamics were monitored over the same time intervals as solution chemistry dynamics will be evaluated.

Results from these experiments to date have reinforced the earlier findings of a nutrient related physiological difference between Fraser fir at upper and lower elevations. Our ability to alter the nutrient or gas exchange physiology of fir by additions of Ca (as CaCl$_2$) has been limited by the apparent negative effects of the Cl ion on soil solution chemistry. Similarly the effects of addition of nitrogen-containing fertilizer have been negative for both sapling growth and physiology, further affirming the negative effects of strong anion additions on this poorly buffered soil system. Because of the potential of Cl to mobilize Al, further exacerbating nutrient stress, Ca additions during the third year (1995) were delivered as CaO leading to and overall increase in growth of treated trees. Soil solution chemistry data and the absence of foliar effects on herbaceous vegetation on treated plots in 1995 support the rationale for this decision. Final remeasurements of these trees will be made in October of 1996.

C. Red spruce liming experiment - Previous liming studies with red spruce saplings in the GSMNP had provided evidence of foliar Ca deficiency based on classical nutrient content: needle mass relationships (Van Miegroet et al., 1993). Observed nutrient responses in that study were not evaluated with respect to tree growth or foliar physiology. The present experiment was designed to couple foliar nutrient response data to data on stem growth and foliar gas exchange physiology at the most base cation deficient site, CD -High. Ca was added as CaCl$_2$ at 200 kg/ha to 8 trees at CD - in May of 1993 with foliar gas exchange measurements being made in August of 1993 and sapling growth data being collected in May of 1994 as difference from initial measurements in June of 1993.

Gas exchange, growth, and foliar nutrient data from this experiment are shown in Tables 7, 8, and 9, respectively. These data reflect an increase in Ps (7%, NS) and a significant decrease in dark respiration (16%, $P \leq 0.05$) for the trees fertilized with Ca. The resultant Ps: Rs ratio was increased by approximately 28% by liming ($P \leq 0.05$).

Sapling growth data (Table 8) showed an approximate 30% increase in the length of new branch growth ($P \leq 0.05$) for limed saplings while diameter increases (+5-15%) were not significant.

Foliar nutrient data in Table 9 reflect rather small increases (typically 10%) in most foliar nutrients including Ca, Mg, N as well as for Al and Mn, however differences were not statistically significant for Ca and most other nutrients. It is important to note however that the physiologically active component of cellular Ca may be quite small (see McLaughlin et al, 1993). The one nutrient for which uptake was statistically significant was N. The improvement of nutrient uptake by Ca addition
Table III-7. Comparative rates of photosynthesis and dark respiration of foliage from limed and unlimed red spruce saplings at a high elevation (1950 m) site on Clingman's Dome as measured in September of 1993 following addition of 200 kg/ha of calcium (CaCl₂) on June 11 of 1993. N = 8 per treatment and means ± 1 sd are shown.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Foliar gas exchange (μmol g⁻¹ s⁻¹)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Photosynthesis</td>
<td>Respiration</td>
<td>Ps:Rs</td>
</tr>
<tr>
<td>Control</td>
<td>40.9 ± 10.3 a</td>
<td>7.6 ± 1.5 a</td>
<td>5.5 ± 1.6 a</td>
</tr>
<tr>
<td>Plus Ca</td>
<td>43.8 ± 5.7 a</td>
<td>6.4 ± 1.0 a</td>
<td>7.0 ± 1.2 a</td>
</tr>
<tr>
<td>P &gt;</td>
<td>T</td>
<td></td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table III-8. Comparative annual new growth of mid-canopy branches of red spruce saplings at a high elevation (1950 m) site on Clingman's Dome as measured in May of 1993 following addition of 200 kg/ha of calcium (CaCl₂) on June 11 of 1993. N = 8 per treatment and means ± 1 sd are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 meter Length (mm)</th>
<th>Diameter (mm)</th>
<th>2 meters Length (mm)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.1 ± 17.4</td>
<td>2.4 ± 0.5</td>
<td>46.8 ± 9.4</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Calcium fertilized</td>
<td>64.0 ± 17.7</td>
<td>2.5 ± 0.6</td>
<td>61.5 ± 18.1</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>P &gt;</td>
<td>T</td>
<td></td>
<td>0.055</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table III-9. Comparative weight and nutrient content of needles from mid-canopy branches of red spruce saplings at a high elevation (1950 m) site on Clingman’s Dome as measured in September of 1993 following addition of 200 kg/ha of calcium (CaCl₂) on June 11 of 1993. N = 8 per treatment and means ± 1 sd are shown.

<table>
<thead>
<tr>
<th>Needle size and nutrient content</th>
<th>Soil treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Calcium fertilized</td>
<td>P &gt;</td>
<td>T</td>
</tr>
<tr>
<td><strong>Needle weight (mg/100 needles)</strong></td>
<td>137 ± 27</td>
<td>138 ± 21</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>N (%)</strong></td>
<td>1.09 ± 0.10</td>
<td>1.19 ± 0.12</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td><strong>P (µg/g)</strong></td>
<td>1113 ± 99</td>
<td>1138 ± 130</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>K (µg/g)</strong></td>
<td>5425 ± 590</td>
<td>5175 ± 684</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Ca (µg/g)</strong></td>
<td>1125 ± 158</td>
<td>1250 ± 267</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mg (µg/g)</strong></td>
<td>600 ± 119</td>
<td>638 ± 92</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mn (µg/g)</strong></td>
<td>294 ± 81</td>
<td>364 ± 181</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Fe (µg/g)</strong></td>
<td>35 ± 8</td>
<td>39 ± 16</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Zn (µg/g)</strong></td>
<td>14.8 ± 1.6</td>
<td>14.3 ± 2.4</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Al (µg/g)</strong></td>
<td>55 ± 13</td>
<td>63 ± 13</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td><strong>Cu (µg/g)</strong></td>
<td>3.6 ± 0.5</td>
<td>3.7 ± 0.4</td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>
indicates that ambient Ca levels in soil solutions are low enough that root growth and/or root function is impaired. The fact that N uptake is improved in the face of a nitrogen saturated soil system supports our earlier hypothesis that reduced N uptake by tree roots is a likely contributor to nitrogen accumulation in these high elevation soils.

**Experiment 2. Leaching losses and physiological alteration of cation compartmentalization**

While measurements of changes in whole tissue calcium have been adequate to describe the effects of acid deposition on processes such as growth, most recent studies in the laboratory suggest that it is the distribution more than the amount of calcium that is responsible for observed responses. Thus, localized changes in the Ca content of specific membranes may be the key to macroscopic responses that we detect at grosser levels of analysis. This task was initiated to explore fluorescence spectroscopy as a tool for detecting changes in subcellular levels of calcium that may play an important role in process level responses of tissues or whole plants.

Results indicate that laser-induced fluorescence can be used to detect calcium with either pulsed or continuous-wave (cw) lasers. There may be advantages from using a pulsed (and possibly tunable) laser in this process, which include the ability to detect two-photon fluorescence, study dynamic processes in cells, or use phase-sensitive detection techniques to enhance selectivity. Studies with fluorescent calcium indicators were performed to evaluate dye detection capabilities for calcium on three categories of samples: Ca ions in dye buffer solutions; Ca ions in a solution of "spruce juice" expressed from spruce needles; and Ca ions detected in dye-soaked spruce needles. Samples were excited with light from a 532-nm frequency double output of a Nd:YAG laser using a wavelength setting range of 5500 Å to 8500 Å. Similarities of fluorescence peaks (679 nm) between samples from ground material and intact needles allowed to absorb the dye suggested that similar levels of binding and cellular integrity were represented in both samples. From a practical aspect achieving penetration of Ca specific fluorescent dyes into living tissues must still be resolved to accurately detect levels and the binding status of in situ calcium in tissues. Results of this exploratory project suggest that high resolution (1-5 um) scanning could be possible using fiber optic probes and a diode array spectrometer to reduce sample time and enhance selectivity of Ca detection in biological samples.

**Experiment 3. Effects of reduced cation availability on structural integrity of wood**

Results: This exploratory experiment has examined a variety of state of the art techniques for testing both the chemistry and mechanical properties of red spruce wood from high elevation sites in the GSMNP. In initial studies, energy dispersive X-ray spectroscopy was used in combination with a transmission electron microscope to examine Ca and Lignin distribution at various locations within early- and late-wood tracheids. Wood formed at various times over the past 100 years in trees from both higher (>1900 m) and lower (<1700 m) elevations was examined. The wood examined in these studies was sampled from thin sections of tree cores removed by a microtome and subsequently embedded in resin. For the first time we have used a computer operated microprobe to examine the mechanical properties of wood at a cellular level. This approach reported in Wimmer and McLaughlin (1997) has allowed us to evaluate for the first time relationships among wood structure and wood organic chemistry across gradients in wood inorganic chemistry. Measurements of the changes in wood hardness, the modulus of elasticity (a measure of deformation potential), wood calcium, and wood lignin levels are shown in Table 10 for analyses of wood formed at different times (1890s vs 1990s).
Table III-10. Comparison of wood chemistry and mechanical properties for tree rings formed in recent years (1991-1993) versus previous years (1889-1893) for one red spruce tree at a high elevation (1950 m) site; Cell regions: SW = secondary wall; CML, compound middle lamella; CC, cell corner.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Region</th>
<th>previous (1889-1893)</th>
<th>recent (1991-1993)</th>
<th>Diff %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (ppm)</td>
<td>SW</td>
<td>660</td>
<td>370</td>
<td>-44</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1100</td>
<td>2650</td>
<td>+140</td>
</tr>
<tr>
<td></td>
<td>Pit Torus</td>
<td>1100</td>
<td>2650</td>
<td>+140</td>
</tr>
<tr>
<td>Lignin (counts)</td>
<td>SW</td>
<td>1827</td>
<td>9230</td>
<td>-52</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>17656</td>
<td>15942</td>
<td>-10</td>
</tr>
<tr>
<td>Hardness (GPa)</td>
<td>SW</td>
<td>0.299</td>
<td>0.265</td>
<td>-11</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0.299</td>
<td>0.265</td>
<td>-11</td>
</tr>
<tr>
<td>Modulus of elasticity (GPa)</td>
<td>SW</td>
<td>8.98</td>
<td>8.53</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>8.98</td>
<td>8.53</td>
<td>-5</td>
</tr>
</tbody>
</table>
for a single red spruce tree. In Table 11 contrasts in these same parameters are made between red spruce wood formed in 1889 at two elevations (1736 m and 1950 m).

These results provide evidence that wood structural integrity and wood chemistry, notably calcium content of wood, are related. Lower levels of calcium whether they reflected decreasing Ca availability in more recently formed wood or decreasing Ca availability with increasing elevation were associated with reduced lignin levels, reduced wood hardness, and reduced resilience properties of wood. A second manuscript from this work (Wimmer, 1995 - see Appendix B) has advanced our understanding of the relationship between latewood formation and wood density in pine.

These exploratory analyses have been followed additional more systematic sampling of red spruce and Fraser fir from an elevational gradient on Mt. LeConte in 1994 and from selected forest health inventory plots on Clingman’s Dome during the 1995 survey. The latter samples have the advantage of being tied to the soil and foliar nutrient analyses for the same plots. Structural and chemical analyses from these cores are continuing. Preliminary signals indicate that Fraser fir responds to adelgid attack with increased lignin formation and that a period of increased lignin formation may be followed by return to normal lignin values in resistant trees that survive attack.

In addition, we have examined the growth patterns of mature red spruce trees sampled during the 1995 survey and evaluated the most recent growth trends and response to climate of mature red spruce relative to historical patterns. These analyses are included in McLaughlin et al., (1997). The growth trends in Figure 1 highlight the shift to slower growth that occurred at widespread high elevation sites in the GSMNP around 1960. The possibility of a trend toward recovery during the past 10 years is suggested by these data.

Data in Figure 2 and 3, which evaluate recent growth responses to temperature and precipitation in relationship to historical growth responses suggest that red spruce growth in recent decades has responded differently to climate than in the earlier decades of this century. Currently growth appears to be more negatively influenced by warm temperatures and by precipitation than previously. Both increased precipitation and increased temperature were previously recognized as positive signals by red spruce, whereas more recently red spruce has been negatively influenced by these variables. We consider these responses to be compatible with an increasing importance of acidic deposition in enhancing carbon loss through increased calcium depletion and enhanced dark respiration (McLaughlin et al., 1993) of red spruce.

The initiation of an upward trend in growth in the last decade shown in Figure 1 is likely the result of both climatic and anthropogenic factors, including years of low rainfall in 1988, 1993, and 1995. In addition the reduction in SO₂ emissions by TVA from a peak of around 2.3 M tons in 1978 to around 1 M ton in 1985 (TVA, personal communication) has reduced annual input of SO₄ to the east Tennessee region. It will be important to follow these growth and chemical trends in the future to substantiate the relative roles of chemical and physical climate on observed growth responses.
Table III-11. Comparison of wood chemistry and wood mechanical properties for tree rings formed in the year 1889 at two different elevations; Cell regions: SW = secondary wall; CML, compound middle lamella; CC, cell corner.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Region</th>
<th>1736 m</th>
<th>1950 m</th>
<th>Diff %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (ppm)</td>
<td>SW</td>
<td>940</td>
<td>950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CML</td>
<td>970</td>
<td>410</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>640</td>
<td>170</td>
<td>27</td>
</tr>
<tr>
<td>Lignin (counts)</td>
<td>SW</td>
<td>9685</td>
<td>9723</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CML</td>
<td>13369</td>
<td>6803</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>18028</td>
<td>6956</td>
<td>39</td>
</tr>
<tr>
<td>Hardness (GPa)</td>
<td>SW</td>
<td>0.393</td>
<td>0.158</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0.388</td>
<td>0.282</td>
<td>73</td>
</tr>
<tr>
<td>Modulus of elasticity (GPa)</td>
<td>SW</td>
<td>12.6</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A 170-year chronology of annual ring widths from 42 red spruce trees (72 cores) extracted from 13-15 trees from each of three elevations in the Great Smoky Mountains National Park, NC and TN.
Correlation analyses relating red spruce growth and monthly precipitation data for the period 1900-1995, including the previous (p) June to current October (16 months) revealed a shift to more negative influences of growing season precipitation during the current growing season.
Correlation analyses relating red spruce growth and monthly temperature data for the period 1900-1995, including the previous (p) June to current October (16 months) revealed a shift to more negative influences of growing season temperature during the current growing season.
SYNTHESIS

This research was designed to address five issues that we considered basic to measuring and better understanding the current and future health of the Southern Appalachian Spruce-Fir ecosystem. We have organized the synthesis of these results around those same issues as discussed below:

ISSUE I. What is the significance of atmospheric sources of nitrogen to foliar nutrition and relative rates of nitrogen uptake from the soil environment?

Experiments in Task I, Nitrogen Deposition and Cycling, demonstrated that foliar uptake of nitrogen by the forest canopy is significant and that at below cloud base canopy openings may experience increased N fluxes to soil surfaces due to the absence of overstory canopy retention of deposited N. Atmospheric deposition, particularly cloud deposition represents an important source of nitrogen input to this system, particularly above cloud base. Our direct measurements of nitrogen deposition and accumulation using N\textsuperscript{15} isotope addition demonstrated that both wet and dry deposited nitrogen are taken up by the canopy and incorporated into foliage and stems. Thus canopy uptake will supplant soil uptake and may serve to reduce uptake of N from the soil. Soil retention of nitrogen deposited as NH\textsubscript{4} is relatively greater than when N is deposited as NO\textsubscript{3}, and, in general, N is very mobile in this high elevation system. The fact that approximately \( \geq 75\% \) of the deposited NO\textsubscript{3} and \( \geq 55\% \) of deposited NH\textsubscript{4} move through the soils indicates that atmospheric deposition contributes significantly to mobilization and leaching of Ca and Al.

ISSUE II. How is soil chemistry, including mineralization of cations and mobilization of aluminum of the upper organic soil horizons where fine roots are most prolific, influenced by short term inputs of high anionic and acidic content?

The high mobility of nitrate indicates that atmospheric inputs of both nitrate and sulfate in this poorly buffered soil system will exert a strong influence on soil solution chemistry and cation leaching. Our fertilization studies with nitrogen demonstrate the adverse effects of additional strong anion additions to the soil resulting in mobilization of both base cations and aluminum in soil solutions. Stream chemistry studies conducted by Nodvin et al., 1995 support this conclusion and indicate that this system is nitrogen saturated with strong losses of calcium and aluminum accompanying losses of nitrate to streams. Lysimeter data to be analyzed in an ongoing Ph.D. study should provide additional quantification of the mobilization and loss patterns relative to atmospheric inputs and intraannual climatic variation.

ISSUE III. How is fine root uptake of calcium and magnesium influenced by the high levels of aluminum mobilized in upper organic and underlying mineral soil by acidic deposition?

Fertilization studies at Whitetop Mountain have demonstrated that reducing the Al:Ca ratio in soil solution increases uptake of calcium and other nutrients into fine roots of mature red spruce. This apparently occurs by increasing the efficiency of transport of base cations across the root cortex and into the inner conducting root stele. The effects of improved uptake helped overcome foliar deficiencies, including both calcium and zinc, leading to improved growth of needle tissue. Aluminum levels in soil solutions of poorly buffered high elevation soils are in the range where atmospheric inputs of strong anions interfere with nutrient uptake and exacerbate growth-limiting nutrient deficiency. We suspect that reduced efficiency of nitrogen uptake associated with enhancement of
aluminum mobilization by acidic deposition is contributing to nitrogen saturation of these soils and increased inputs of nitrate to streams.

ISSUE IV. How does reduced calcium uptake and incorporation into woody tissues influence the structural integrity and strength of wood formed in high elevation spruce and fir.

Our exploratory analyses and development of new techniques for relating wood calcium levels, wood lignin levels, and wood structural properties provide initial evidence to indicate that reduced availability of calcium to mature red spruce trees will lead to wood which is chemically altered, with lower lignin content, and structurally altered with reduced elasticity. Such changes, which must be validated with additional analyses, may explain the increased thinning of crowns noted at high elevation sites in the past few years. Our preliminary analyses and an earlier report indicate that increased lignin formation is a part of the reaction of Fraser fir to attack by the wooly adelgid. If as we suspect, increased lignin formation is a part of the process by which Fraser fir resists fatal attack by the balsam wooly adelgid, then reduced calcium uptake at higher elevations by Fraser fir as detected in our field survey may reduce the capacity of Fraser fir to respond successfully to such attack.

ISSUE V. What are the physiological limitations placed upon Fraser fir growing in an environment in which red spruce has experienced physiological dysfunction associated with acid deposition-induced limitations on cation availability?

Our data from three experiments indicate that Fraser fir growing at high elevation sites experiences reduced availability of calcium. Ongoing studies on Clingman’s Dome indicate that Fraser fir is responding very similarly to red spruce under these conditions with reduced photosynthetic rates, but more significantly, increased rates of dark respiration, which would lead to reduced efficiency of carbon metabolism. Fraser fir saplings may be more sensitive to soil solution aluminum than red spruce based on foliar nutrient data. Fir foliage contains approximately twice the Ca levels of red spruce, but three times the foliar aluminum levels. Thus foliar Ca:Al levels are approximately one third lower for fir than for spruce. Collectively our experiments to date indicate that Fraser fir is also being physiologically impaired by reduced cation availability and can be expected to be at least as sensitive to strong anion limitations of cation supply by acidic deposition as is red spruce.

In conclusion, the results of these studies strongly support, the earlier synthesis of Johnson and Lindberg (1992) regarding the importance of acidic deposition to nutrient cycling, and the synthesis of McLaughlin and Kohut (1992) regarding the importance of calcium deficiency to physiological function of red spruce in the Southern Appalachian Spruce Fir Ecosystem. Our studies have extended the implications of that earlier work by examining the relative importance and differential behavior of nitrogen, as both NH$_4$ and NO$_3$, as it interacts with both the forest canopy and the soil; providing contrasting physiological data for Fraser to evaluate in relationship to the larger body of physiological data from earlier experiments with red spruce; by providing survey data on soil and nutrient status of both Fraser fir and red spruce saplings and mature trees along an elevational gradient that includes periodic evaluation of historical changes in stand composition and canopy condition; and finally by developing exploratory methods that for the first time have examined the relationship between wood chemistry and wood structural characteristics.

The information provided by these studies indicates that acidic deposition is affecting many facets of ecosystem function that can substantially alter future patterns of development of this sensitive high
elevation ecosystem. The responses we have observed include processes related to nutrient deposition, nutrient cycling, nutrient uptake and root function, carbon metabolism, and wood formation and are anticipated to make this system increasingly sensitive to interacting environmental stresses including changes in temperature and precipitation patterns, susceptibility to physical stress from wind and ice, and decreasing resistance to insect pests, such as the balsam wooly adelgid.

The geographical and ecological uniqueness of the Southern Appalachian Spruce Fir Ecosystem and specifically the rich history of data collection in the Great Smoky Mountains National Park make this system a unique indicator system. It has the potential to provide important additional insights into the interactions between physical and chemical climate and anthropogenic and natural stresses that must underlie future environmental regulation designed to sustain forest health. We believe our research has made an important beginning in that direction.
REFERENCES


ACKNOWLEDGEMENTS

This research was sponsored by the U.S. Environmental Protection Agency under Interagency Agreement 1824-F036-A1 under Martin Marietta Energy Systems, Inc., contract DE-AC05-84OR21400 with the U.S. Department of Energy. We are indebted to Dr. Ralph Baumgardner, of the U.S. EPA, Atmospheric Research Exposure Laboratory, Ecological Exposure Research Division, for providing us funding to continue the excellent previous research that was conducted in the Southern Appalachian Spruce Fir Ecosystem during the NAPAP era, and to Dr. Deborah Mangus for subsequent support. We also gratefully acknowledge the support of the U.S. Forest Service and of Susan Fox, Program Director of the Southern Global Change Program for providing complementary support for portions of this work. In addition, we thank Dr. Niki Nicholas of the Tennessee Valley Authority for her very helpful collaboration in the sampling of forest health monitoring plots on Clingman’s Dome, GSMNP.
APPENDIX A

Outline of experiments being conducted by April Stone as a Ph.D. Dissertation at the University of Tennessee, Graduate Program in Ecology and Evolutionary Biology.

Great Smoky Mountains National Park
Fraser Fir Study
1993 - 1996

I. Treatments
   A. Control - no treatment
   B. Calcium
      1. CaCl during 1993 to 1994 (added spring and mid summer)
      2. Dolomitic limestone 1995 (added in spring)
   C. Complete fertilizer (including micronutrients)
      1. Low nitrogen fertilizer, CaCl, and MgCl from 1993 to 1994 (added spring and mid summer)
      2. Slow-release fertilizer and dolomitic limestone in 1995 (added in spring)

II. Two site
   A. Clingman's Dome - Upper site
      1. Twenty-four trees (2-3 meter saplings)
         a. Eight trees per treatment
      2. Eight throughfall water collectors (two per treatment)
      3. Eight soil plots (two meters square)
         a. Two per treatment
         b. Two lysimeters per plot
      4. Two rain gauges (with no overhead canopy)
   B. Noland Divide - Lower site
      1. Twenty-four trees (2-3 meter saplings)
         a. Eight trees per treatment
      2. Eight throughfall water collectors (two per treatment)
      3. Eight soil plots (two meters square)
         a. Two per treatment
         b. Two lysimeters per plot
      4. Two rain gauges (with no overhead canopy)

III. Tree measurements
   A. Overall height
   B. Bole circumference (midway)
   C. Apical growth
      1. Length
      2. Diameter
   D. Lateral branch growth
      1. Length
      2. Diameter
   E. Needle growth (eight shoots collected per tree)
      1. Average number of needles per shoot
      2. Average length of needles per shoot
3. Average width of needles per shoot
4. Average shoot length
5. Average shoot diameter
6. Average shoot compression and rebound
F. Root growth
   1. Nylon mesh bag placed at canopy edge (from soil surface to 10 cm deep)
   2. Dry weight of root growth per year
G. Licor measurements
   1. Photosynthesis
   2. Respiration

IV. Throughfall water collection
   A. Dry deposition and needle nutrient leaching

V. Soil plot measurements
   A. Soil solution nutrient content

VI. Rain gauges
   A. Total rainfall per week
   B. Rain water nutrient analysis

VII. Results - 1993
   A. Slight increase (not significant) in overall growth of treated trees at upper site
   B. Rapid die-back of herbaceous growth in soil plots after treatment
   C. Strong pulse of chloride noted in soil solution for several weeks after treatment
   D. Four to five fold increase in soil solution Ca
   E. Slight increase (not significant) in needle and shoot Ca
   F. No clear pattern noted in net photosynthesis between treatments
   G. Significant increase in overall tree growth at lower site versus upper site
   H. Overall low rainfall during summer

VII. Results - 1994
   A. Slight decrease in overall growth of treated trees at upper site
   B. Rapid die-back of herbaceous growth in soil plots after treatment
   C. Strong pulse of chloride noted in soil solution for several weeks after treatment
   D. Four to five fold increase in soil solution Ca
   E. Slight decrease in net photosynthesis of Ca treated trees
   F. Greater decrease in net photosynthesis of trees treated with complete fertilizer
   G. Significant increase in overall tree growth at lower site versus upper site
   H. Overall high rainfall during summer

VIII. Results - 1995
   A. Increase in overall growth of treated trees at upper site
   B. No die-back of herbaceous growth in soil plots after treatment
   C. No chloride pulse in soil solution
   D. Significant increase in overall tree growth at lower site versus upper site
   E. Overall low rainfall during summer

IX. Results - 1996 (not completed)
APPENDIX B

List of Publications Supported by This Project:


APPENDIX C

List of Databases from Which Additional Publications Will Follow:

I. Effects of Nutrient Supply on Soil Chemistry and Physiology of Fraser Fir

This data set is the centerpiece of a Ph.D. dissertation by April Stone of the Graduate Ecology Program at the University of Tennessee. It includes growth and development data on 48 sapling Fraser fir trees, physiology data on foliar gas exchange rates, and soil solution and throughfall chemistry data as outlined in Appendix A. We anticipate completion of this project, which was delayed by a funding shortfall during the past year, during the next calendar year.

II. Effects of Calcium Fertilization on Physiology and Growth of Red Spruce

These results, which are summarized in this report, are suitable for open literature publication and we will proceed in that direction. We delayed publication initially because of what appeared to be marginal statistical significance; however reexamination of these results in the process of preparing this report indicates that this dataset should make a strong publication.

III. Survey of Soil and Foliar Nutrient Variations Associated with the GSMNP Forest Health Survey

We have just begun to examine this data set from a substantive field research effort in the fall of 1995. Preliminary analyses based on data included in this report indicate that this will be a strong data set suitable for one or more open literature publications.

IV. Wood Chemistry - Wood Structure Relationships

We have a collection of cores from approximately 40 red spruce and Fraser fir trees on which we are examining structure -chemistry relationships. Preliminary analyses have proven interesting and the studies being completed at the laboratory of Dr. Wimmer in Austria should provide us an adequate basis for evaluating this concept. If preliminary data are substantiated by these more extensive analyses, we anticipate one or more significant open literature publications to follow from this work.

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