Chapter 5

VEGETATION AND ACIDIFICATION

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1. INTRODUCTION

In this chapter, the impact of watershed acidification treatments on WS3 at the Fernow Experimental Forest (FEF) and at WS9 on vegetation is presented and summarized in a comprehensive way for the first time. WS7 is used as a vegetative reference basin for WS3, while untreated plots within WS9 are used as a vegetative reference for WS9. Bioindicators of acidification impacts that will be considered include several measures of tree and stand growth rates, foliar chemistry, bolewood chemistry, and herbaceous species composition and diversity. These studies enhance our understanding of the inter-relationships of changes in soil conditions caused by the acidification treatment and the condition of forest vegetation. Treatments on these watersheds were described in Chapter 2. Scientific names of plant species mentioned by common name in the text are given in the Appendix.

Although the original objective of the Fernow acidification studies was to evaluate the effects of acidic deposition on solution chemistry and ecosystem processes, it is also important to understand the linkage between soil and solution processes and biotic effects. In particular, in this chapter we report the results of a number of studies designed to investigate the impact of soil acidification treatments on the health and growth of forest vegetation.

Productivity of vegetation is often used as a measure to detect effects of treatments on vegetation. Common measures of forest productivity include

aboveground biomass production and stand growth. Stand growth is measured in terms of basal area, biomass or cubic volume. Growth can also be measured as individual tree growth rates, leaf area production or net primary productivity. Plants growing in chronically acidified soils may exhibit decreased calcium (Ca) uptake over time due to competition with

almost all tree species. If treatments on WS3 caused significant Al mobilization which led to accelerated base cation leaching and decreased availability of important nutrients to critically low levels, then radial and basal area growth for all tree species on WS3 could be significantly reduced. Diameter growth expressed as basal area increment has been routinely used to show impacts of fertilization on tree growth (Wilmot et al. 1996, Long et al. 1997, Demchik and Sharpe 2000). Species composition and diversity are increasingly important measures which are believed to be linked to productivity. The effects of various treatments can also be considered by their effects on chemical constituents of the vegetation, or on physiological processes.

Foliar chemical analysis is a tool that is commonly used to assess changes in growth rates and health of forest vegetation. Our understanding of critical foliar nutrient values is most developed for commercial conifer species, but we can evaluate treatment effects in hardwood species as well. The utility of foliar analysis is based on the assumption that adding nutrients to a stand of trees increases availability of nutrients in the soil, which, in turn, is reflected in nutrient uptake and nutrient content of the vegetation. The application of ammonium sulfate fertilizer was expected to increase the availability of nitrogen (N), which could result in growth stimulation if this stand, or a particular species within the stand, were limited by N availability. Increased N availability could also lead to nutrient imbalances, which might be manifest as growth declines. Soil chemical differences that were hypothesized as a result of the acidification treatment were expected to result in differences in availability of exchangeable cations such as Ca, magnesium (Mg), manganese (Mn) and Al. Acidification-induced reductions in base cations such as Ca and Mg may lead to decreased uptake of these nutrients and increased stress for plants, while increased levels of Mn and Al can create toxic conditions for trees.

Study of the chemical element content of bolewood xylem has been used to assess the impact on forest health in cases where the soil has been acidified or limed (DeWalle et al. 1991, DeWalle et al. 2003). Changes in the availability of cations in soil solution cause changes in ion content of tree

ons in tree sap tend to become adsorbed within cell walls of xylem sap-

icting tissue. As trees grow and add a new growth ring of xylem tissue

each year, the sequence of chemical element concentrations in tree rings can potentially preserve a record of soil water chemical changes.

Bolewood concentrations of Ca, Mg, Mn and molar ratios such as Ca/Mn have been commonly used to indicate changes in soil acidity. Lower levels of bolewood Ca, Mg, and Ca/Mn and higher levels of bolewood Mn are thought

tors of increased stress in trees. Although soil acidification may

ised Al in soil solution, increased levels of Al in bolewood are generally not found due to exclusion at the soil-root interface (Taylor 1987) or retention by the root cortex (Tomlinson 1990).

Field studies investigating the effects of N on species diversity of plant communities, such as the Park Grass Experiment in Rothamsted, England (Silvertown 1980) and the Cedar Creek Natural Area (e.g., Tilman 1987), have found that excess N can profoundly alter species composition and decrease species diversity of plant communities. Unfortunately, most such studies have been carried out on herb-dominated ecosystems, whereas relatively few have been done in forested ecosystems (Gilliam et al. 2006). Because of the coincidence of forest ecosystems with regions of high N deposition and because most of the biodiversity of forests occurs in the herbaceous layer, it is imperative to understand the response of herb layer composition and diversity to excess N.

The response of herb-dominated communities to experimental additions of N can be rapid. Tilman (1987) reported significant changes in species composition of the Cedar Creek Natural Area to all levels of N additions within one year following treatment. Although growth and foliar nutrient concentrations of herb layer plants are generally sensitive to soil fertility (Muller 2003), it might be expected that the herb layer of forests may display a lag-response to experimental additions of N, given the structural complexity of forest ecosystems, wherein resource availability can be greatly altered by other vegetation strata.

2. VEGETATION DESCRIPTION

2.1 Fernow

2.1.1 Stand Conditions – WS3 and WS7

Interpretation of the impacts of acidification on vegetation on WS3 relative to WS7, depends in part upon understanding the status and dynamics of the species composition of the forest during the experiment. Forest vegetation on both watersheds, which was generally described in Chapter 2, was approximately the same age, having largely originated after cutting and

herbicide treatments ending in 1970. However, watersheds differed in the nature of earlier vegetation treatments and aspect, which may have influenced species composition and growth at the time of treatments. Specifically, prior to 1970 WS7 was maintained barren with herbicides for several years, while WS3 was allowed to regenerate immediately following

all trees grea

cm diameter at breast height (dbh) and dominant/co-dominant trees on WS3 and WS7 during the experiment based upon inventories in 1990/91, 1996, 1999, and 2003. Basal area of black cherry, expressed as % of dominant/ co-dominant trees on WS3 and WS7, gradually increased over the course of the study, in contrast to most other species. Yellow-poplar was also an increasingly important species on WS7, but a mix of other species still dominated. Red maple represented a small but relatively constant proportion of overstory trees during the experiment on both watersheds. Early successional species, such as sweet birch, fire cherry, and black locust, decreased gradually in the overstory, while more shade tolerant species, such as sugar maple and American beech, increased in relative abundance.

2.1.2 Herbaceous Layer Characteristics – WS3 and WS7

The herbaceous layer was studied initially on four watersheds of FEF – WS3, WS4, WS7, and WS13 – in 1991 and 1992, with further emphasis placed on WS3, WS4, and WS7 in conjunction with *in situ* soil incubations in 1994 (see Chapters 3 and 7). Despite large differences in stand age among these watersheds, with WS3 and WS7 trees being approximately 20 years old versus 80-100 years for WS4 and WS13 in 1991, there were minimal differences in herb layer composition, cover, biomass, and species diversity (Gilliam et al. 1995, Gilliam 2002). This was in sharp contrast to measures of tree communities, which varied more predictably with stand age along a successional gradient. Whereas WS3 and WS7 were higher in stem density, lower in basal area, and dominated by early successional species, such as yellow-poplar, black cherry, and sweet birch, WS4 and WS13 had higher basal area, lower stem density, and were dominated by late successional species, such as northern red oak and sugar maple (see Chapter 2 and Gilliam and Roberts 2003).

A complete floristic study of WS3, WS4, and WS7 was carried out by Aulick (1993). Using Sorensen's Coefficient of Similarity (Barbour et al. 1999), she found that WS3 was floristically more similar to WS4 (similarity coefficient of 0.58) than it was to WS7 (0.49). Rather than attribute this discrepancy in floristic similarity

concluded that it arose from the l

Table 5-1. Dominant species (% basal area) for WS3 and WS7 for all stems >2.54-cm dbh and dominant/codominant stems during acidification experiment. WS3 initial=1990, WS7 initial=1991. Data obtained from 25 0.04-ha permanent growth plots on each watershed. For scientific names of trees, see Appendix A.

	1990/91		1996		1999		2003	
		Dom/	All	Dom/ Codom	All	Dom/ Codom	All	Dom/ Codom
	%							
WS3								
Black cherry	36.9	55.8	45.4	57.5	47.9	61.4	51.0	60.9
Yellow-poplar	4.5	5.8	4.6	6.3	4.9	6.8	5.7	6.1
Red maple	14.4	7.7	13.1	6.3	12.0	5.7	11.5	7
Sweet birch	5.4	3.8	5.4	3.8	4.9	3.4	5.1	3.5
N. red oak	4.5	5.8	4.6	5.0	4.9	5.7	5.1	5.2
Amer. beech	2.7	0	2.3	0	2.1	0	2.5	0
Sugar maple	1.8	0	.5	0	.4	0	3	0
Fire cherry	9.0	11.5	8.5	11.3	7.7	10.2	6.4	7.0
Black locust	2.7	9	5	.3	.4		.3	0.9
Fraser magnolia	4.5	3.8	3.8	2.5	4.2	2.3	3.8	2.6
White ash	0.9	0	0.8	0	0.7	0	0.6	0.9
WS7								
Black cherry	15.1	18.6	17.3	20.6	17.9	22.4	20.5	23.6
Yellow-poplar	17.4	25.6	21.4	27.0	23.6	29.9	26.2	30.3
Red maple	8.1	2.3	8.2	3.2	8.5	3.0	8.2	3.4
Sweet birch	19.8	20.9	20.4	19.0	20.8	17.9	20.5	19.1
N. red oak	1.2	2.3	1.0	1.6	0.9	1.5	.6	
Amer. beech	0	0	0	0	0	0	0	0
Sugar maple	4.7	0	5.	0	4.7	0	4.9	1.1
Fire cherry	9.3	11.6	7.	9.5	5.7	7.5	3.3	4.5
Black locust	9.3	14.0	10.2	12.7	9.4	11.9	9.0	11.2
Fraser magnolia	0	0	0	0	0	0	0	0
ash	2.3	2.3	2.0	3.2	2.8	5	2.5	2.2

fern, Christmas fern – see Table 5-2) on WS7 that were either absent from or of low cover on the other watersheds. The prominent fern component on WS7 was ascribed to its more north-northeast slope aspects, wherein ambient temperatures are generally lower and soil moisture generally higher than the southeast-south-southwest slope aspects of WS3 and WS4. These conditions are generally conducive to greater success of ferns (George and Bazzaz 2003). Carvell and Perkey (1997) found that wood and Christmas ferns could be used as diagnostic plants to identify mesic sites. Gilliam et al. (1994) summarized herbaceous layer cover and foliar chemistry for the 1991 sampling of WS3, WS4, and WS7, and they concluded that there were few differences among watersheds in any measured variable that could be attributed to the acidification treatment on WS3.

Table 5-2. Herbaceous layer species names and codes.

Species name	Common name	Code
Acer pensylvanicum L.	striped maple	ACPE
A. saccharum Marsh.	sugar maple	ACSA
Dryopteris marginalis (L.) A. Gray.	shield fern	DRMA
Laportea canadensis (L.) Webb	stinging nettle	LACA
Panicum spp.	panic grass	PANI
Polystichum acrostichoides (Michx.) Schott.	Christmas fern	POAC
Prunus serotina Ehrh.	black cherry	PRSE
Rubus spp.	blackberry	RUBU
Sassafras albidum (Nutt.) Nees	sassafras	SAAL
Smilax glauca Walter.	catbrier	SMGL
S. rotundifolia L.	greenbrier	SMRO
Stellaria pubera Michx.	star chickweed	STPU
Viola spp.	violet	VIOLA

2.2 Stand Conditions – WS9

The Clover Run watershed, hereafter known as WS9, was also subjected to acidification treatments following a history of mountain farming, removal of regrowing hardwood forest following farm abandonment, and reforestation with planted Japanese larch, as described in Chapter 2. Larch were planted in 1984 and acidification began in April 1987 after about three years of growth. A buffer zone of regrowing hardwoods, which received treatments was left along the perennial stream channel on the watershed. Effects of treatment on WS9 were evaluated by comparing response on six 30-m x 30-m control plots randomly located on the watershed that did not receive surface applications of chemicals with the response on the adjacent treated areas. See Chapters 3 and 4 for a map of WS9 with plot layouts and treatment assignments.

3. METHODS

3.1 Tree and Stand Growth

Two methods were employed to evaluate potential impacts of the acidification treatment on tree growth on the experimental watersheds. One method employed repeated measurements of tree diameter on growth plots located on WS3 and WS7, and measurements of height and diameter of larch trees on treatment and control plots on WS9. The second method involved annual radial growth analysis of increment cores and wood disks from trees sampled for dendrochemical analysis.

3.1.1 Growth Plot Analyses

Twenty-five randomly located 405-m^2 plots were established in 1990 (WS3) and 1991 (WS7), 1 and 2 years after treatments began, respectively. The diameter of all trees greater than or equal to 2.54 cm was recorded and the trees were permanently tagged. These plots were re-measured in the dormant season of 1996, 1999 and 2003. The data set included more than 5,600 trees. Changes in diameter over time on these plots were converted into basal area growth, volume growth using local volume tables for the FEF, and biomass growth from equations developed by Brenneman et al. (1978). Basal area and biomass included all stems $\geq$2.54 cm dbh and cubic volume included all stem $\geq$12.7 cm dbh. Analyses presented here are for net volume growth on plots including the effects of growth of surviving trees, ingrowth of trees reaching measurable size, and mortality. For comparative purposes the statistical analyses were conducted for the 1990-1996 six-year period for WS3, the 1991-1996 five-year period for WS7, and the 1996-2004 eight-year period for both watersheds. Since the total analyses included net volume growth of all species and trees on each plot, further analyses were also repeated for two clusters of plots based on the relative abundance of black cherry and yellow-poplar (n = 15 for WS3 and n = 14 for WS7) or sweet birch and red maple (n = 10 for WS3 and n = 11 for WS7). A multiple-factor repeated measures analysis of variance with watershed and species group as class variables, plot as the subject, initial plot relative density as a covariate and annual growth components as the repeated measure was used to compare annual net growth (Nowak 1996).

For WS9, the effects of the acidification treatments on the growth and development of the Japanese larch plantation were evaluated after 9 growing seasons using six 12.2 m x 15.2 m unfertilized control plots with buffers and 43 0.004-ha circular fertilized plots (Kochenderfer et al. 1995). Three condition classes were used to describe the larch trees: 1. good vigor – dominant or

co-dominant trees. 2. medium vigor – intermediate trees. 3. poor vigor – low intermediate or overtopped trees. Diameter and height were measured on all trees within the measurement plots.

3.1.2 Tree Core Analysis

The objective of radial growth analysis was to determine if annual radial and basal area growth differed significantly between WS7 and WS3 over time during the experiment. No comparable radial growth studies were conducted on WS9. Radial and basal area growth for yellow-poplar, black cherry, red maple and black locust trees on WS3 and WS7 were compared after 12 years of treatment. The methods used to conduct this research are condensed here, but are discussed in depth elsewhere (Odenwald 2002). To analyze growth rates, a wood core was extracted at breast height with increment borers from ten yellow-poplar, black cherry, and red maple trees on WS3 and WS7; thus the data set included a total of 60 trees. Trees on permanent growth plots were not cored. In preparation for tree-ring growth analysis, these cores were dried, mounted on wood blocks, sanded, and examined using a high resolution scanner and WinDENDRO 6.5d software manufactured by Regent Instruments Inc. (Quebec, Canada). Alternatively, black locust trees were sampled by collecting wood disks at breast height from ten felled trees on each watershed. Black locust disk surfaces were prepared for growth analysis similar to the wood cores described above. Black locust growth data have not been previously published.

Use of radial growth increment comparisons requires correction for differences in tree age or diameter and site specific conditions. In this section use is made of relative radial growth rates (RRG) (Odenwald 2002) defined as:

$$\text{RRG} = \frac{\text{annual radial increment (mm) for a year after treatment}}{\text{mean annual radial increment (mm) in that tree over 14 years before treatment.}}$$

The RRG thus indexes growth in each tree to average radial growth in that same tree for years prior to treatment to account for site differences. The RRG is expected to gradually decline during the treatment period as radial increment declines and diameter increases. Mean RRG for all trees of a species was compared between treatment and reference watersheds year by year.

Annual basal area increments in mm^2 (BAI) were also computed for each tree in each year before and after treatment (Odenwald 2002). Using BAI data from Odenwald (2002), regression relationships for mean annual BAI between WS3 and WS7 for years prior to treatment (Fig. 5-1) were used to

predict expected BAI for years after treatment for black cherry, yellow-poplar and red maple trees. Relationships in Figure 5-1 adjust for the growth effects of differences in site and tree size between WS3 and WS7 prior to treatment. The differences between predicted BAI and actual BAI measured for years after treatment began were tested for significance and used as a measure of treatment effect.

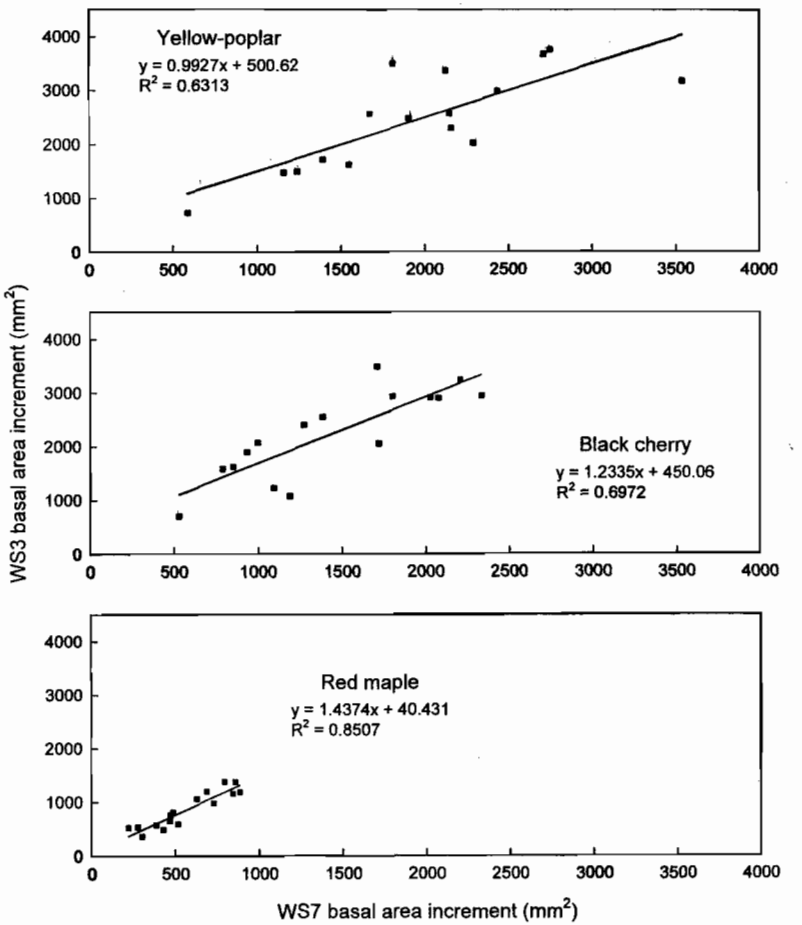

Figure 5-1. Regression relationships between mean annual basal area increment (mm^2/tree) of yellow-poplar, black cherry, and red maple trees on WS3 vs. WS7 for pretreatment years (1973-1988, 10 trees per species each year, based upon data from Odenwald 2002).

3.2 Foliar Chemistry

Foliage was sampled in 1992 from WS3 and WS7 (sweet birch, yellow-poplar, black cherry and red maple) as part of a total biomass sampling for nutrients (Adams et al. 1995). In 1997 and 2002, foliage was collected from the upper crown of five trees from each of six species (the four listed above plus northern red oak and sugar maple) from WS3 and WS7, and in 2002 from WS4. Because the data are most complete for WS3 and WS7, for the original four tree species, statistical comparisons were made between watersheds using these data. No foliar data were available for these watersheds prior to the beginning of this study. All of these heretofore unpublished data were collected by personnel with the USDA Forest Service, Northeastern Research Station at Parsons, West Virginia. All tissue nutrient analyses were conducted at the University of Maine Soil and Plant Testing Laboratory, using protocols described by Adams et al. (1995). Samples were collected from the upper crown of two trees per species per treatment plot, where present. Samples were dried and ground (1-mm mesh) and analyzed for N, Ca, potassium (K), Mg, phosphorus (P), Al, boron (B), copper (Cu), iron (Fe), Mn, and zinc (Zn).

Pickens (1995) and Pickens et al. (1995) reported on foliar chemistry of planted Japanese larch trees on WS9 sampled in 1992 and 1993, or six and seven growing seasons after treatments had begun. Thirty larch trees were randomly selected and sampled from a single 25 m x 25 m control and adjacent treated plot, each with a 5-m buffer zone, in the upper northwest corner of the catchment. All needle whorls were sampled on a lateral shoot at breast height just below the terminal shoot from selected trees in each year. Foliar samples were analyzed by inductively coupled plasma emission spectroscopy for Al, Ca, K, Mg, Mn, sulfur (S), and P, and for N using the Kjehldahl method at the Agricultural Analytical Services Laboratory, The Pennsylvania State University. Total height and dbh were also measured.

3.3 Dendrochemistry

Dendrochemistry of three tree species (yellow-poplar, black cherry, and red maple; see Appendix A for scientific names) on WS3 and WS7 was evaluated after four, eight and 12 years of treatment to help assess effects on nutritional status. Black locust was sampled after only 12 years of treatment. Results given here update those previously published (DeWalle et al. 1995, Tepp 1995, DeWalle et al. 1999, Odenwald 2002). Methods employed in the dendrochemical study are detailed elsewhere (DeWalle et al. 1999) and are only summarized briefly here. On WS3 and WS7, wood for most species was extracted as cores at breast height with increment borers from five trees of

each species (four cores per tree). Wood from cores from each tree was later separated and composited into 4-yr segments. Wood tissue was destructively analyzed for chemical element content using inductively-coupled plasma emission methods. Simple t-tests were used to determine significance of differences in mean concentration or ratios between treated and control trees. Wood from black locust was only collected after 12 years of treatment. For the locust trees, wood from each annual increment was taken from wood discs collected at breast height from boles of ten felled trees on WS3 and WS7. Wood from each annual growth ring in black locust was combined among all treated and control trees for chemical analysis to give detail for each year. Due to the necessity of such compositing, differences between treated and control trees could not be evaluated statistically for black locust; however, having annual growth ring data can help to show how tree-ring chemistry changes between heartwood and sapwood regions. Some black locust dendrochemistry data have been previously published (DeWalle et al. 2002).

Dendrochemistry of young planted Japanese larch trees on untreated and treated plots on WS9 was evaluated in 1994, seven growing seasons after initiation of treatment in spring 1987. Four increment cores were taken from each of five treated and control trees. Procedures were similar to those described for the Fernow dendrochemistry comparisons (DeWalle et al. 1999). Planted Japanese larch were considered desirable for these comparisons since larch generally has narrow bands of xylem for sapflow that may preserve a record of past soil chemistry changes in heartwood. Additional dendrochemistry studies of larger sawtimber red oak and black cherry in the WS9 streamside buffer zone and nearby control areas were conducted five years after treatments began (DeWalle et al. 1995, 2003).

3.4 Herb Layer Dynamics

The methods employed have been described in detail in other publications (e.g., Gilliam et al. 2001, Gilliam 2002), so we summarize them briefly here. The herbaceous layer was sampled by identifying and visually estimating cover (%) of all vascular plants ≤1 m in height within ten 1 m^2 circular sub-plots in each of seven 0.04-ha circular sample plots in WS3, WS4, and WS7, for a total of 2100 1 m^2 sub-plots. Sampling was carried out monthly from May to October of 1994. Mineral soil was collected by hand trowel at five points within each of the seven plots per watershed to a depth of 5 cm. These five samples were composited, thoroughly mixed, and then placed in two polyethylene bags – one brought back to the laboratory for immediate extraction and analysis (see below) and the other incubated *in situ* by burying it 5 cm beneath the mineral soil surface for ~30 days during the same period described for the herb layer.

Data were analyzed in three ways, the first two to assess temporal variability at different scales and the third to assess spatial variability. First, year-to-year variability was examined by comparing mean cover for the more dominant herb layer species. This was done by combining the 1991/1992 observations with the 1994 study of Hockenberry (1996). To ensure uniformity in these comparisons, data from 1991/1992 were taken as a sub-set of the original 15 plots sampled in each watershed to match the seven plots sampled by Hockenberry (1996). Similarly, only the July samples from Hockenberry (1996) were used to match the sample time used in 1991/1992. Next, seasonal variability was analyzed specifically to address the relationship of herb layer cover to N availability, and hence test for possible responses to the N additions on WS3. Accordingly, monthly mean herb layer cover was compared to monthly rates of mean net nitrification for each watershed separately for the 1994 data.

Spatial variability was assessed with canonical correspondence analysis (Gilliam and Saunders 2003) using Canoco for Windows 4.5. This analysis was performed on each watershed separately and used available N pools (i.e., extractable ammonium (NH_4) and nitrate (NO_3)), rates of net nitrification, and foliar N and N:P ratios in *V. rotundifolia* as environmental variables. This was performed on the July sample only (see above).

4. RESULTS

4.1 WS3 vs. WS7 (Fernow) Results

4.1.1 Tree Growth

4.1.1.1 Growth Plots

In general, annual net growth on WS3 growth plots exceeded that observed on WS7 for the 14 year period. This result was consistent for basal area, biomass and cubic volume growth using repeated measurements on permanent growth plots. Stratifying the analysis by plot species composition and time period indicated that most of the differences in growth between the watersheds occurred during the period from 1990/91 to 1996 and on plots dominated by black cherry and yellow-poplar (BC/YP in Fig. 5-2). There was no evidence that productivity differed in either direction between the watersheds for plots dominated by red maple and sweet birch (RM/SB in Fig. 5-2). For the BC/YP plots, growth on WS3 was greater than WS7 for all measures and time periods ($p < 0.03$), except basal area growth during the period from 1996-2004 ($p = 0.12$). For the RM/SB plots, growth on WS3 and

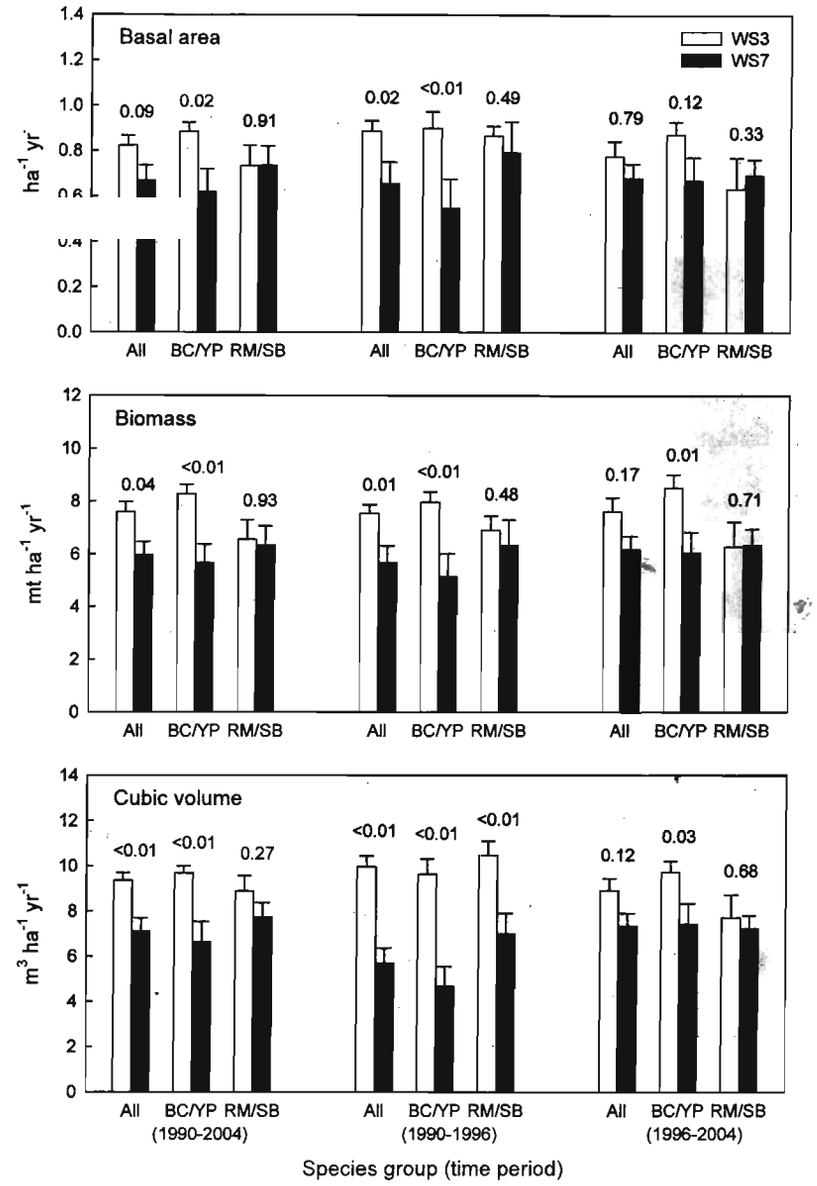

Figure 5-2. Comparison of net annual growth of trees on growth plots, by species composition and time period. Plots were stratified by a higher occurrence of black cherry/yellow-poplar (BC/YP, n = 14 or 15) or red maple/sweet birch (RM/SB, n = 10 or 11) for WS3 and WS7, respectively. Initial measurements were recorded in 1990 for WS3 and 1991 for WS7. Numbers above means and standard errors are significance levels, indicating probability >F statistic for each comparison.

WS7 did not differ by any measure or time period ($p > 0.33$), except cubic volume growth during the period from 1990-1996, where growth on WS3 was greater ($p < 0.01$).

There was no evidence that the acidification treatment reduced productivity from one time period to the next within WS3 on the BC/YP plots (Fig. 5-3). Annual basal area, biomass and cubic volume growth were virtually unchanged for all plots combined ($p > 0.07$) and for the BC/YP plots ($p > 0.59$). On the RM/SB plots, annual basal area growth ($p = 0.02$) decreased on WS3 during the period from 1996 to 2004, but biomass growth ($p = 0.26$) and cubic volume growth ($p = 0.06$) were unchanged. On WS7, both basal area and biomass growth were unchanged during the two time periods for both species groups, while cubic volume growth increased for the BC/YP plots in the second time period ($p < 0.01$).

A comparison of growth between species groups within each watershed and time period indicated that growth in the BC/YP plots was enhanced by the acidification treatment. On WS7, where no treatment was applied, the RM/SB plots grew slightly faster than the BC/YP plots during the period from 1991-1996, and grew at an equal rate during the period from 1996 to 2004 (Fig. 5-4). The growth comparison between species groups was different on WS3, where the species groups had equal growth from 1990 to 1996 and the BC/YP plots grew faster than the RM/SB plots during the period from 1996-2004. This pattern of response was consistent for annual basal area, biomass and cubic volume growth.

Net annual biomass production in 2004, when the stands were 34 years old, averaged 6.0 mt ha^{-1} on WS3 and 4.6 mt ha^{-1} on WS7. These rates are comparable to data summarized by Tritton and Hornbeck (1982), in which annual biomass production of 30-year-old beech-birch-maple stands averaged 4.7 mt ha^{-1}. Periodic net annual biomass growth since the acidification treatments began was 7.6 mt ha^{-1} on WS3 and 6.0 mt ha^{-1} on WS7 (Fig. 5-2).

4.1.1.2 Tree Cores

Results of analyses of RRG ratios indicated that growth of black cherry, red maple and yellow-poplar trees on WS3 had significantly decreased compared to those from WS7 by 1998, after nine years of treatment (Figs. 5-5 through 5-8). RRG ratios declined to levels below 1.0, as is expected with increasing tree diameter on both watersheds, but RRG ratios for each species were significantly lower on WS3 than on WS7 after nine years of treatment. The RRG ratio for black cherry in 2000 did not differ significantly between watersheds.

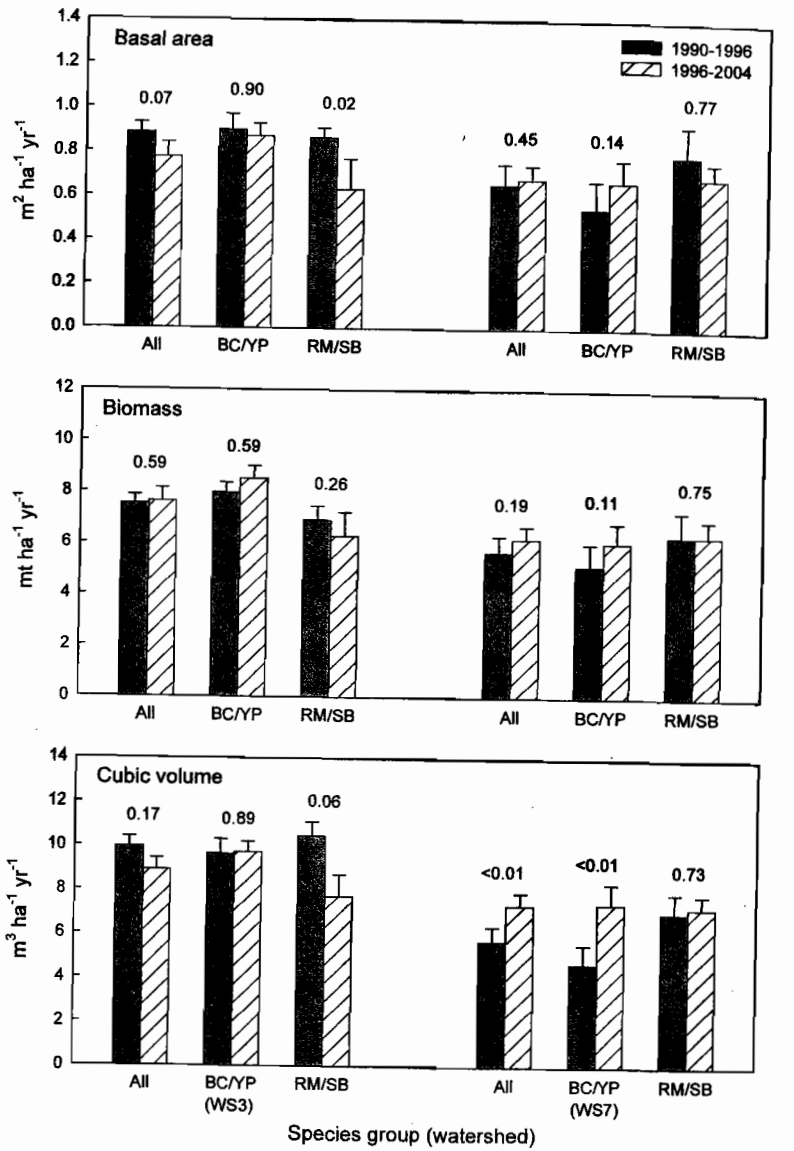

Figure 5-3. Comparisons of net annual growth between time periods for each watershed. Initial measurements were recorded in 1990 for WS3 and 1991 for WS7. Numbers above means and standard errors are significance levels, indicating probability >F statistic for each comparison.

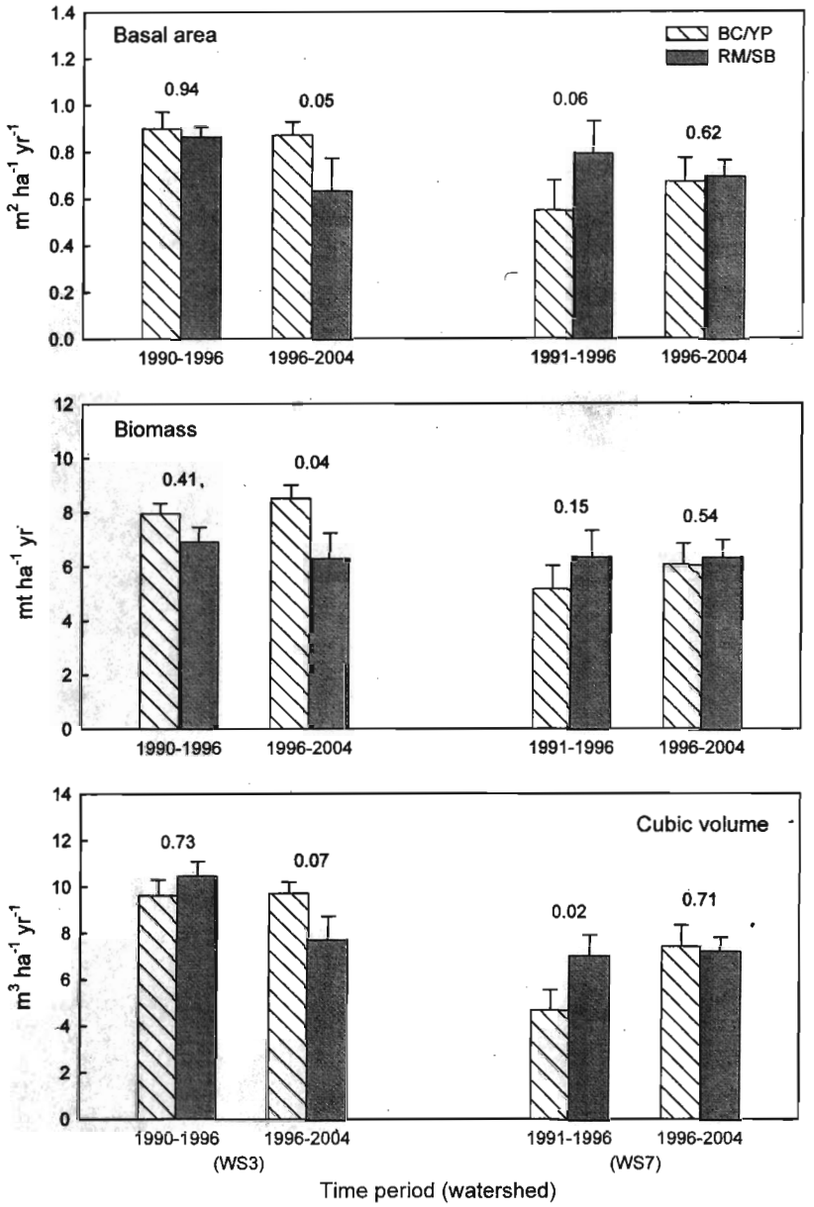

Figure 5-4. Comparisons of net annual growth by plot species group and time period for each watershed. Species groups include black cherry/yellow-poplar (BC/YP) and red maple/sweet birch (RM/SB). Numbers above means and standard errors are significance levels, indicating probability >F statistic for each comparison.

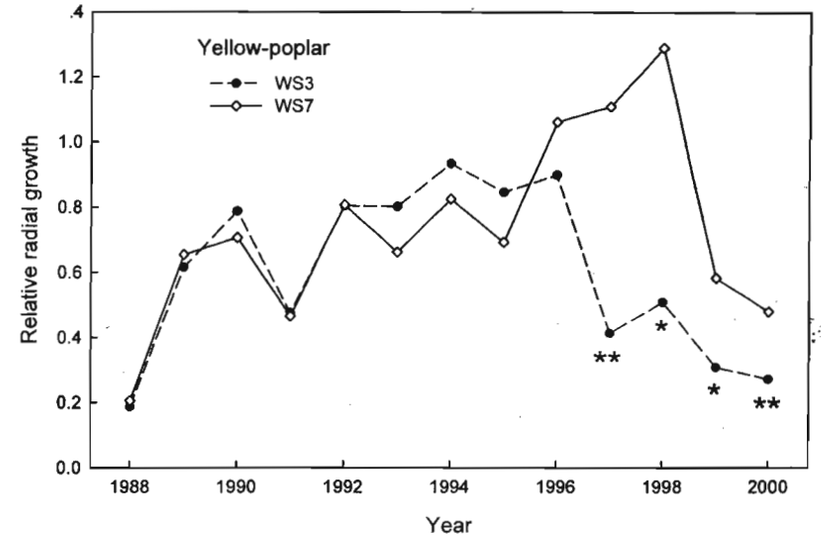

Figure 5-5. Relative radial growth patterns for 10 yellow-poplar trees on WS3 and WS7 during acidification treatments which began in 1989. Asterisks by year indicate significant differences; * $\alpha = 0.1$ and ** $\alpha = 0.05$.

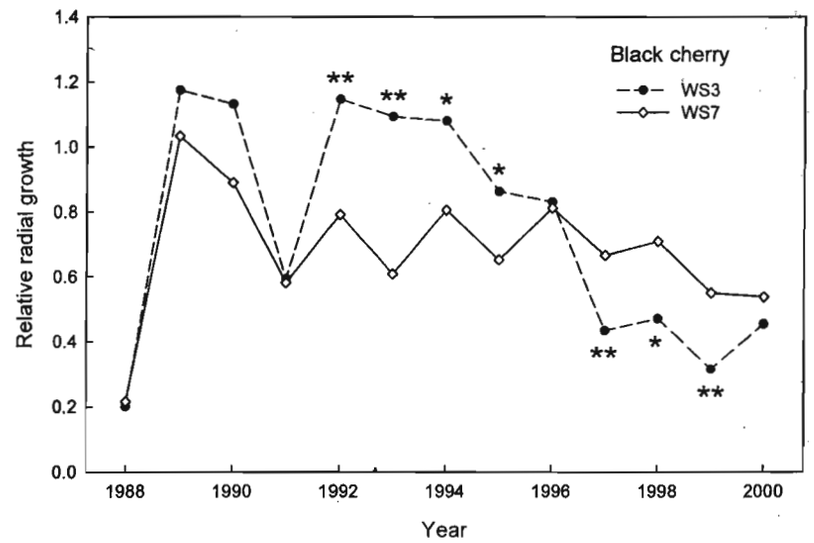

Figure 5-6. Relative radial growth patterns for 10 black cherry trees on WS3 and WS7 during acidification treatments which began in 1989. Asterisks by year indicate significant differences; * $\alpha = 0.1$ and ** $\alpha = 0.05$.

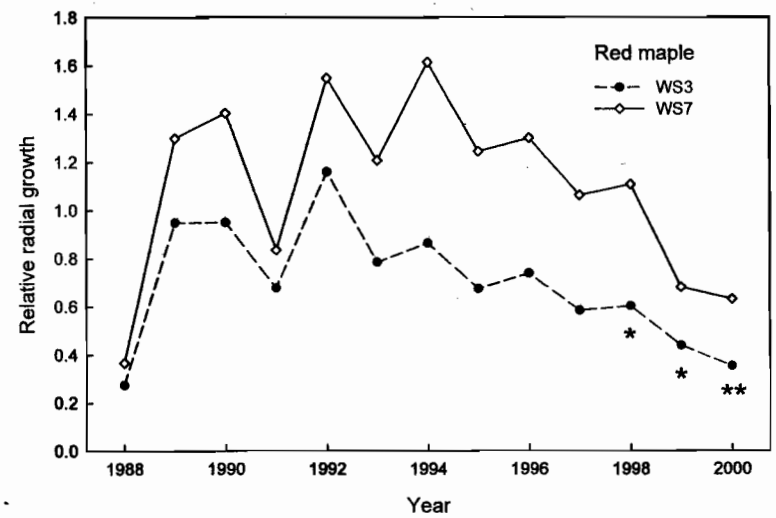

Figure 5-7. Relative radial growth patterns for 10 red maple trees on WS3 and WS7 during acidification treatments which began in 1989. Asterisks by year indicate significant differences; * $\alpha = 0.1$ and ** $\alpha = 0.05$.

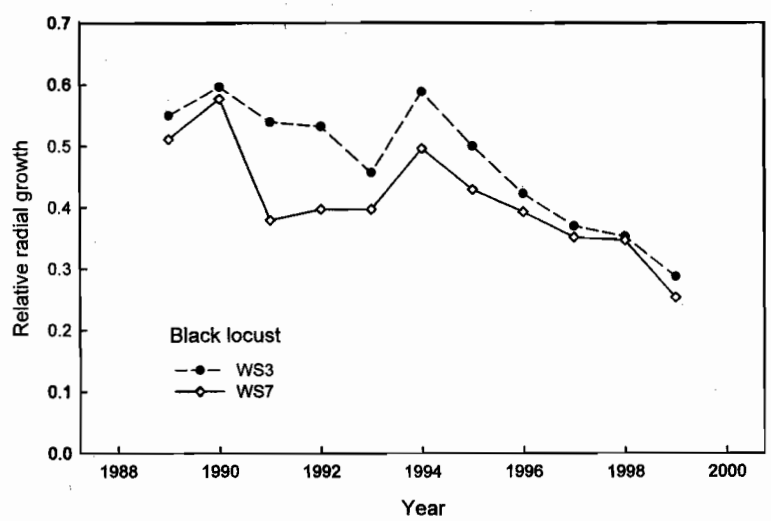

Figure 5-8. Relative radial growth patterns for 10 black locust trees on WS3 and WS7 during acidification treatments which began in 1989. Asterisks by year indicate significant differences; * $\alpha = 0.1$ and ** $\alpha = 0.05$.

All species, except red maple, also showed a pattern of increased growth on WS3 compared to WS7 from four to seven years after treatment began, but this early growth acceleration was only significant in black cherry trees. Black locust RRG ratios showed a trend similar to that of black cherry and yellow-poplar trees, with accelerated early growth and slowed later growth, but there were no statistically significant differences between watersheds. Black cherry RRG ratio analysis produced the greatest number of statistically significant differences between WS3 and WS7 (Fig. 5-6). Red maple trees did not show any evidence of an increase in RRG from 1989 to 2000, but did exhibit significantly lower RRG ratios during the last 3 years (Fig. 5-7).

Basal area growth patterns using tree core measurements, were similar to those obtained with the RRG. For yellow-poplar and black cherry (Fig. 5-9), actual mean basal area increments per tree were greater than predictions for about the first eight years of treatment and then became smaller than predicted from 1997 to 2000. Red maple trees exhibited lower BAI than predicted throughout the study period. After eight years of treatment, or about 1996, actual basal area was only about half the predicted values for all species. All species showed significantly lower BAI on WS3 vs. WS7 during 1997-2000 (Fig. 5-9). Actual BAI relative to predicted BAI in early

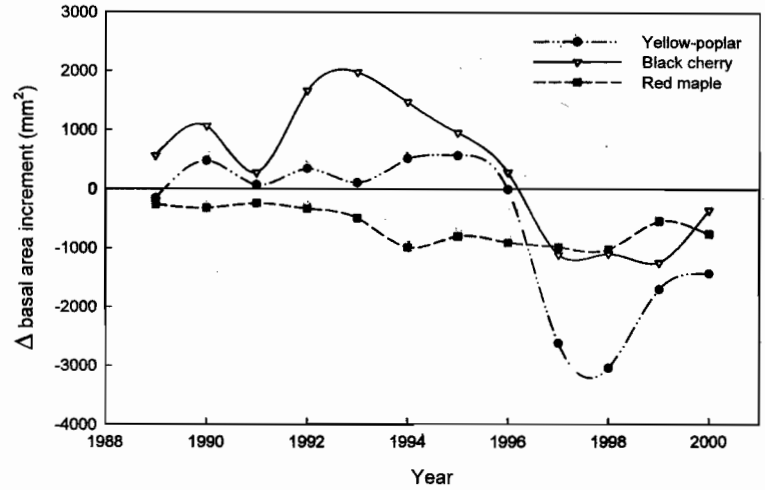

Figure 5-9. Actual minus predicted annual mean basal area increment per tree (mm^2) for yellow-poplar, black cherry, and red maple trees on WS3 for years after treatments began (based upon data from Odenwald 2002). Yellow-poplar differences were significant at the 0.01 level in 1990 and 1997-2000 and significant at the 0.05 level in 1994-95. Black cherry differences were significant at the 0.05 level in 1991 and at the 0.01 level in all other years except 1989 and 1996. Red maple differences were significant at the 0.01 level in all years except 1992.

years of treatment was equivalent to a maximum of about 2000 mm^2 or 80% increase for black cherry. Smaller positive BAI changes of up to 500 mm^2 or 20% of expected BAI were indicated for yellow-poplar in early years. As ndicated in Figure 5-9 increases were significant in yellow-poplar during 3 'ears and in black cherry during 7 years prior to 1996.

May et al. (2005) found diameter growth on WS3 reduced by 37% ompared to WS7 from 1999-2001 in three each of red maple, yellow-poplar, nd black cherry trees. These data were collected late in the treatment period nalyzed here and support the tree core analysis showing reduced radial growth had occurred.

4.1.2 Overstory Foliar Chemistry

Significant differences in mean foliar chemistry concentrations existed among species, as expected, and between WS3 and WS7 (Table 5-3). Trends in foliar nutrients varied over time within watershed and species suggesting that foliar condition at the time of sampling was important even though foliage was sampled in late summer each year. In 1992 and 1997, average foliar N concentrations for black cherry and red maple were greater

Table 5-3. Significant differences in foliar chemistry for four tree species between WS3 and WS7 in 1992, 1997 and 2002 which represents conditions 4, 9 and 14 growing seasons after treatments began. Species were SB = sweet birch, YP = yellow-poplar, BC = black cherry, and RM = red maple. All differences were significant at the $\alpha = 0.05$ level except that for iron (Fe) which was significant at $\alpha = 0.1$ (box).

	Analyte										
Species	N	Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Zn
						1992					
SB		X					X			X	
YP		X		X				X			X
BC	X										
RM	X	X			X		X				
						1997					
SB		X									
YP		X							$\boxed{X}$		
BC		X				X		X		X	
RM	X			X	X	X	X	X		X	
						2002					
SB								X			
YP											
BC			X			X	X	X			X
RM						X				X	

on WS3 than WS7. Nitrogen concentrations decreased over time, with the exception of yellow-poplar, for which concentrations increased slightly (Fig. 5-10). Calcium was significantly greater in foliage on WS7 for all species in 1992 and/or 1997, but no significant differences occurred in 2002 (Fig. 5-11).

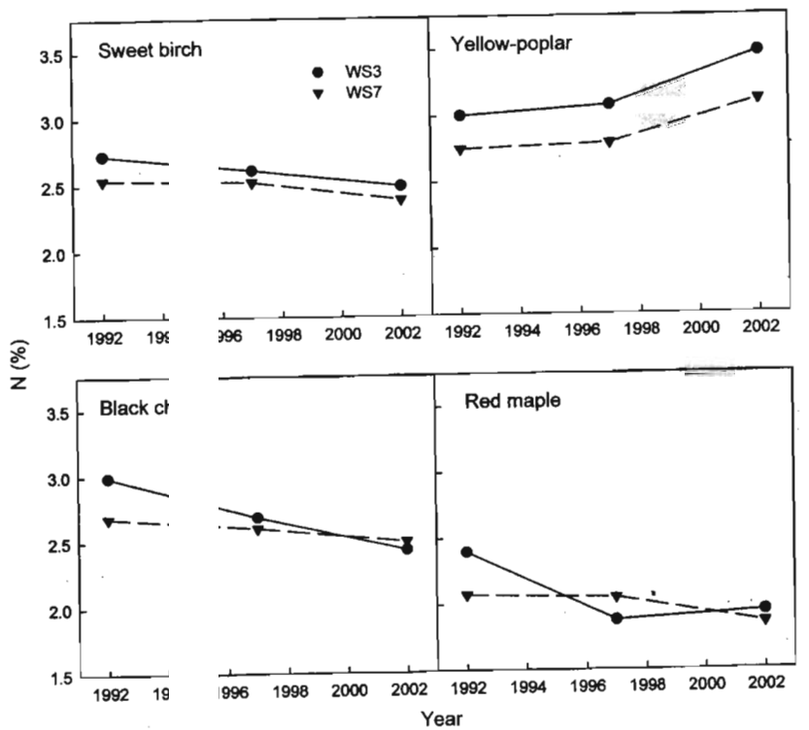

Figure 5-10. Foliar ntent for sweet birch, yellow-poplar, black cherry, and red maple trees on WS3 and WS7 i 2, 1997 and 2002 (4, 9 and 14 growing seasons after treatments were initiated in 1989).

Generally, f Ca increased over time, with a peak in 1997 (Fig. 5-11). WS7 yellow-po consistently exhibited the highest Ca concentrations – nearly twice tha yellow-poplar concentrations on WS3. For Mn there was no consistent tr n average concentrations over time, but levels in foliage were generally greater on WS3 than WS7 (Fig. 5-12) in all four species. Differences in foliar Mn concentrations between WS3 and WS7 were significant for sweet birch in 1992, yellow-poplar and red maple in 1996 and red maple in 2002 (Table 5-3). Foliar Mn concentrations in black cherry did not differ between the two watersheds.

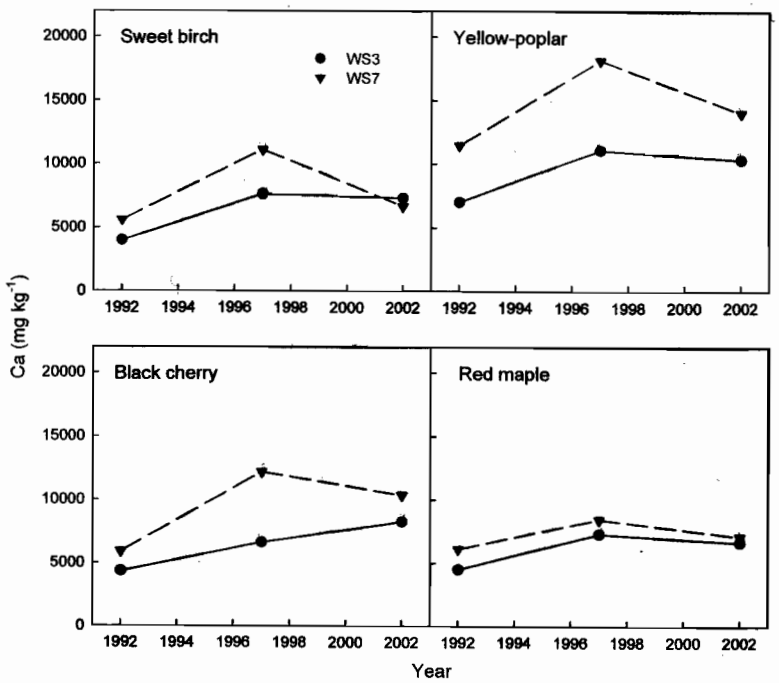

Figure 5-11. Variation in mean foliar Ca (mg kg^{-1}) in four tree species after 4, 9 and 14 growing seasons of treatment on WS3 and WS7.

For yellow-poplar, foliar Al concentrations decreased significantly in 1997, a trend opposite that displayed for foliar Ca, while all others showed at least a slight increase (data not shown). Generally foliar Al concentrations were greater on WS3 than WS7, except in 2002 for sugar maple, yellow-poplar and oak, 1992 for sweet birch, and 1997 for northern red oak.

Mean foliar nutrient concentrations were compared with regional means and extremes from the Northeastern Ecosystem Research Cooperative foliar chemistry database for those species for which there was an acceptable number of samples. Mean values for N, Ca, Mg and Al concentrations in sweet birch, black cherry and red maple foliage all fell within the range of values observed across the northeastern United States for these species (Northeastern Ecosystem Research Cooperative 2004). Foliar P concentrations in black cherry, however, were at the lower end of the range reported for black cherry in the northeastern United States. The P concentrations reported here are similar to those found elsewhere on the Fernow (Adams et al. 1995, Adams

et al. 2005), and may suggest the potential for P limitation on this site. Additional data from this and other nearby sites (W. Peterjohn, unpublished data) lend support to this hypothesis.

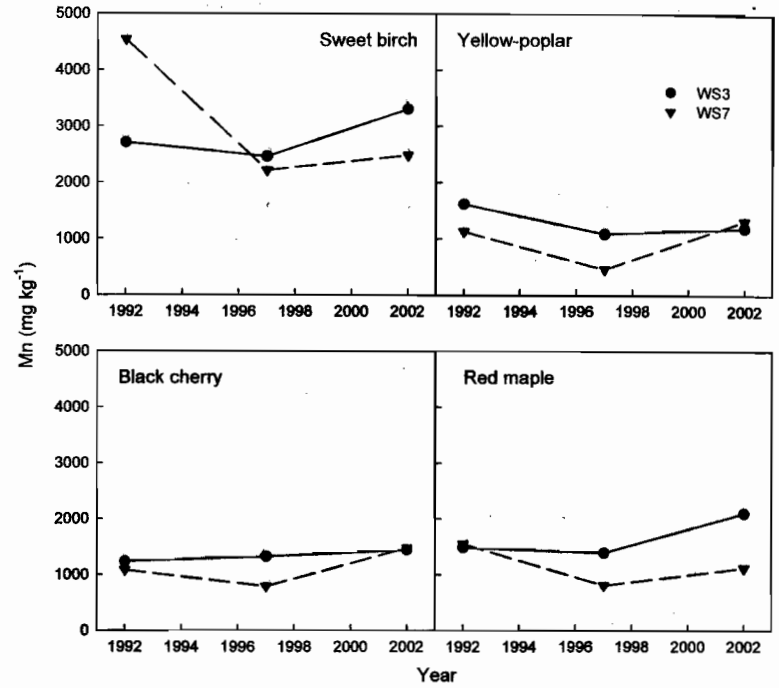

Figure 5-12. Variation in mean foliar Mn (mg kg^{-1}) in four tree species after 4, 9 and 14 years of treatment on WS3 compared to WS7.

4.1.3 Dendrochemistry

Analyses of dendrochemistry results showed shifting patterns between treated and control trees after four years of treatment as opposed to eight and 12 years of treatment. Figures 5-13 through 5-16 show patterns of Ca, Mg, Mn and Ca/Mn found in bolewood at the times of sampling on WS3 and control areas in the four tree species. Many, but not all, of these differences between treated and control tree mean concentrations and ratios were significant in t-tests by species and age segment (DeWalle et al. 1999). WS7 control tree chemical concentrations were similar over time. Only average values for control trees are shown to simplify presentation; different control

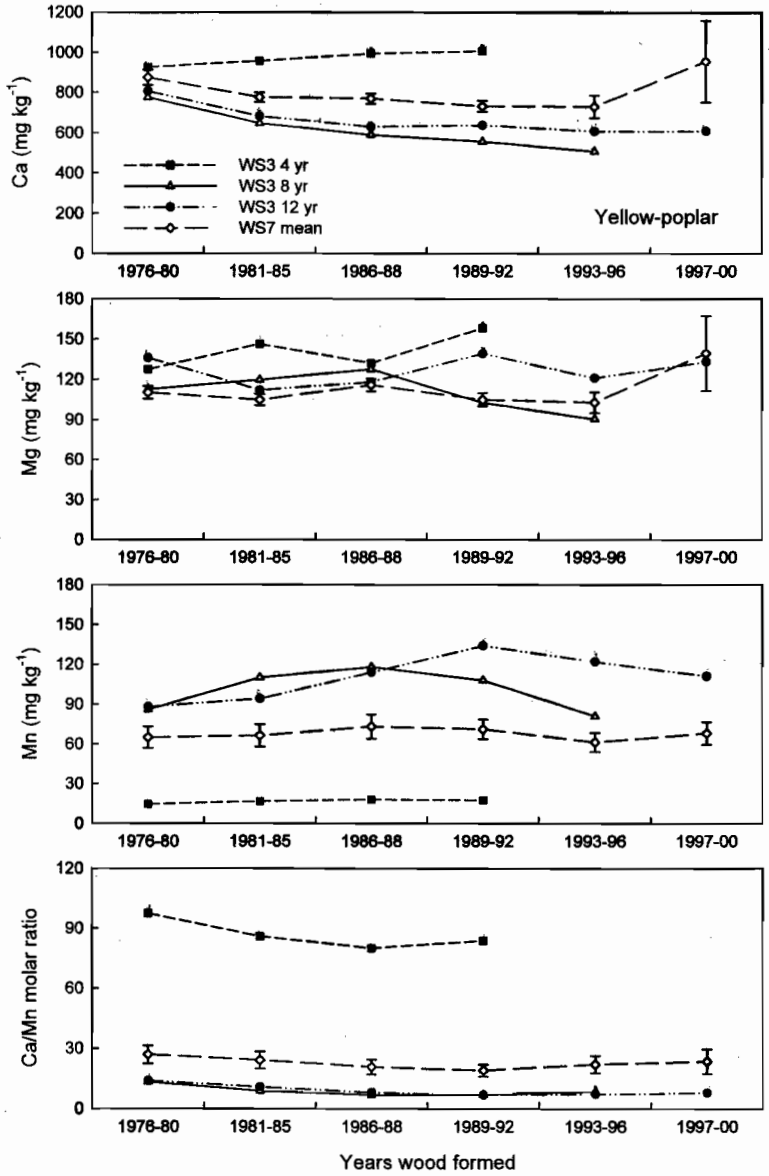

Figure 5-13. Yellow-poplar bolewood Ca, Mg, and Mn concentrations and Ca/Mn molar ratios on WS3 and WS7 measured after 4, 8 and 12 years of treatment. WS7 untreated tree data were similar over time and only means ± standard errors are shown. Treatments began in 1989.

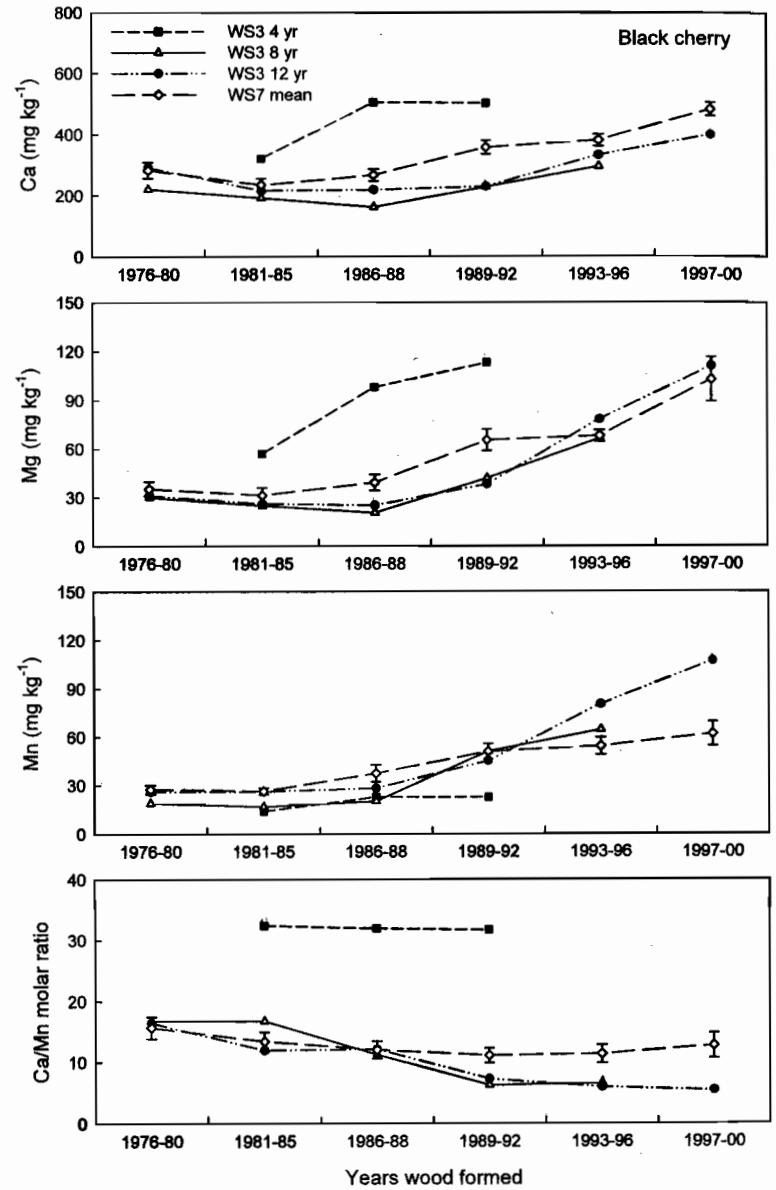

Figure 5-14. Black cherry bolewood Ca, Mg, and Mn concentrations and Ca/Mn molar ratios on WS3 and WS7 measured after 4, 8 and 12 years of treatment. WS7 untreated tree data were similar over time and only means ± standard errors are shown. Treatments began in 1989.

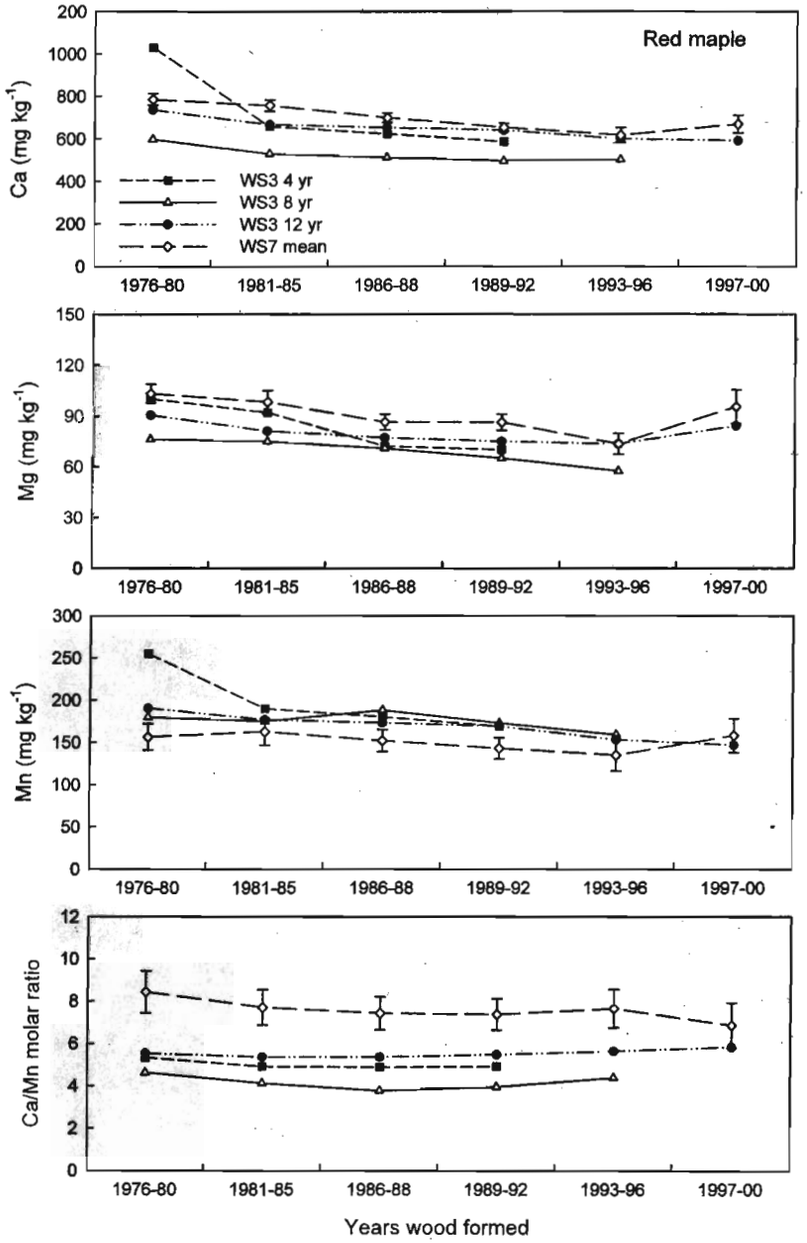

Figure 5-15. Red maple bolewood Ca, Mg, and Mn concentrations and Ca/Mn molar ratios on WS3 and WS7 measured after 4, 8 and 12 years of treatment. WS7 untreated tree data were similar over time and only means ± standard errors are shown. Treatments began in 1989.

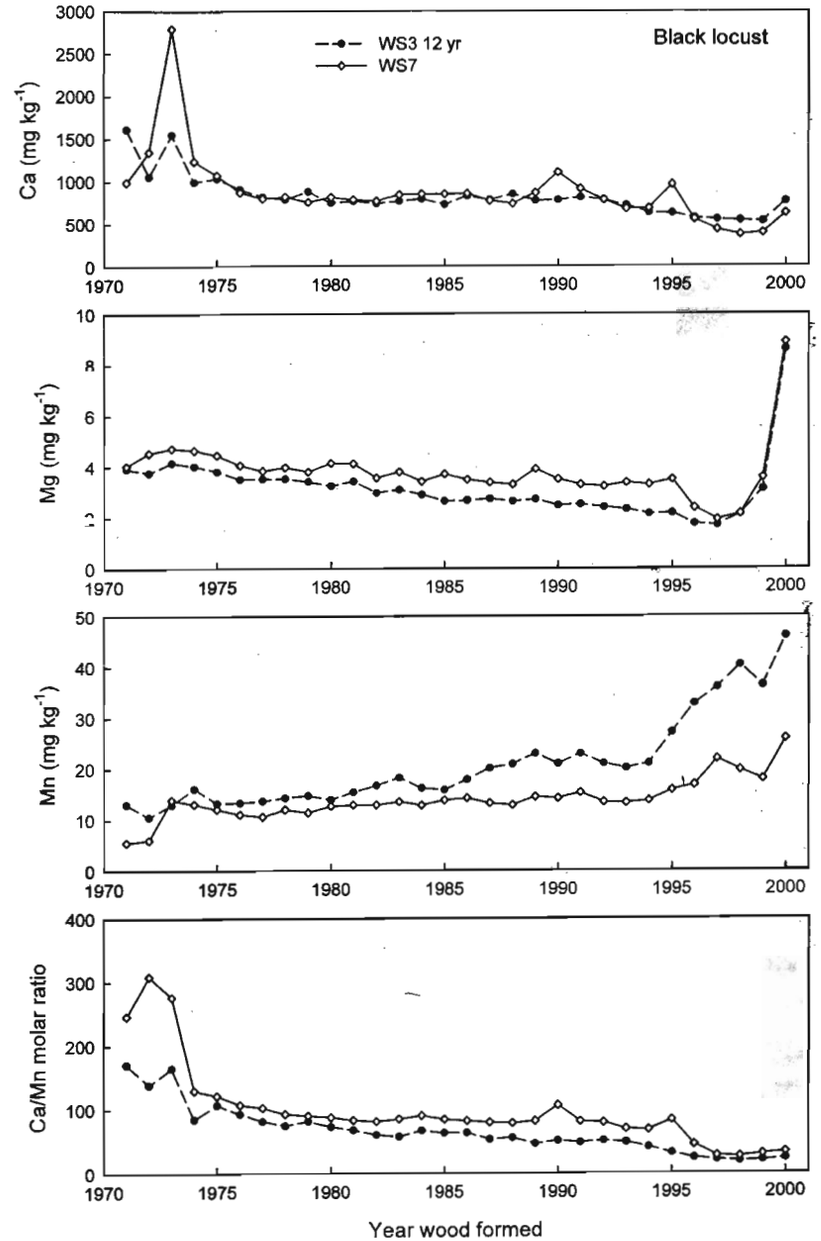

Figure 5-16. Black locust bolewood Ca, Mg, and Mn concentrations and Ca/Mn molar ratios on WS3 and WS7 measured after 12 years of treatment. Treatments began in 1989.

trees were sampled after four, eight, and 12 years of treatment. For the three species sampled repeatedly (black cherry, yellow-poplar, and red maple) mean Ca and to a lesser extent Mg concentrations were generally greater in ood of treated trees after four years of treatment. After eight and 12 of treatment, treated trees generally showed lower levels of Ca and Mg nt compared to control trees. Although there were some deviations from pattern due to sample variations and species characteristics, the data ared to suggest an initial period of base cation mobilization occurred wed by a period of base cation depletion.

Manganese trends in species sampled repeatedly were essentially the opposite with treated trees showing lower levels than controls after four years and higher levels of Mn after eight and 12 years of treatment. Ratios of Ca/Mn magnify these trends and show higher ratios in treated compared to control trees after four years of treatment and lower ratios after eight and 12 years of treatment. This suggests that base cation mobilization of Ca relative to Mn occurred after four years of treatment and cation depletion of Ca relative to Mn occurred after eight and 12 years of treatment.

Relatively small, inconsistent differences existed in dendrochemistry between eight and 12 years of treatment. However, greater levels of Mn were observed for all species, except red maple, from eight to 12 years, which suggests a continuation of soil acidification.

Dendrochemistry response to treatment varied somewhat among the various tree species. Red maple trees did not show the elevated Ca and Mg levels after four years of treatment (Fig. 5-15) observed in yellow-poplar and black cherry trees (Figs. 5-13 and 5-14). Red maple trees sampled were largely understory and intermediate canopy position trees, while all yellow-poplar and black cherry trees sampled were dominants or co-dominants. Slower growth of understory red maple trees may have limited their response to treatment due to intense competition for nutrients by the overstory trees. Red maple was the only species that did not show increased radial growth in the early stages of treatment. This suggests a linkage between bolewood chemistry and radial growth (see discussion section). Black cherry trees showed increases in Mn levels in wood formed only after four to eight years of treatment, due to the preservation of prior trends of Mn depletion in heartwood that existed from the base cation mobilization phases (Fig. 5-14).

The response of black locust trees to the fertilization treatment deserves special mention (Fig. 5-16). Sapwood in this species was only 3-4 growth ies was expected to better preserve a record of soil ings than other species. However, sapwood rings did er base cation levels on WS3. In addition, the was formed well before treatments began appeared nd reduced Ca/Mn ratios due to treatment. This k locust heartwood may not be chemically stable. Black locust

trees appeared to be unsuited to recording chemical changes in the soil, possibly due to its nitrogen fixing capability.

4.1.4 Herb Layer Effects

Gilliam et al. (2001) suggested that one of the main effects of the acidification treatment on WS3 was to decrease spatial heterogeneity of N processing, using several measures that included net N mineralization and nitrification, available N pools, and tissue concentrations of nutrients of an herb layer species – *Viola rotundifolia* – that occurred on all sample plots in the study. It was concluded that foliar nutrient concentrations in *V. rotundifolia* represented a useful and adequate indicator of soil nutrient availability, including N, P, Ca, Mg, and K. Spatial variability in virtually all measures of soil N dynamics (particularly foliar N in *V. rotundifolia*) was substantially less in WS3 than in WS4 and WS7 (Gilliam et al. 2001). Mechanisms potentially controlling spatial variability on these watersheds, including soil chemistry and degree of soil weathering, were investigated further by Gilliam et al. (2005).

Hockenberry (1996) repeated assessment of the herbaceous layer of WS3, WS4, and WS7 in 1994, but used a different sampling approach than those used in the first two years of the study. The 1991 and 1992 samplings were carried out on 15 plots per watershed, but only done once (July) in the year. By contrast, Hockenberry (1996) did monthly measurements of herb layer cover and composition (from May to October) in a seven-plot sub-set of the original 15 used in 1991 and 1992. Yurish (1996) carried out monthly *in situ* soil N incubations on the same seven plots as Hockenberry (1996). To date, no study has synthesized these two studies to better assess the potential effects of the acidification treatment on the herbaceous layer of WS3. Accordingly, we report the results of further analysis of data collected in 1994, five years after treatments began. In doing so we assess (1) temporal patterns of herbaceous layer cover and net nitrification on a plot-by-plot basis, and (2) spatial patterns of herb layer composition, soil N processing, and foliar nutrients of *V. rotundifolia*.

Comparisons of mean cover for individual species of the herbaceous layer among watersheds and years of sampling, despite being based on more extensive sampling comprising a four-year period, support the findings of earlier studies (e.g., Gilliam and Turrill 1993, Gilliam et al. 1994), which were based on one-time sampling (Table 5-4). In particular, there were (1) notable similarities in the herb layer between these watersheds that differ greatly in forest stand age and history, and (2) negligible, if any, responses of yer to the N additions to WS3 during the first five growing seasons ients began. Dominance of the herbaceous layer by fern species on ot change in the four-year period of the study, with Christmas fern

and shield fern combining for 20-25% cover (Table 5-4). This is substantial, considering that it is approximately the same as the total herb layer cover for each of WS3 and WS4. There were no significance differences among years for WS3, except for black cherry seedlings in 1994, strongly indicating that there has been little measurable response of herb layer composition to the acidification treatment on WS3 (Table 5-4).

Table 5-4. Percent cover for dominant species/species groups in July of 1991, 1992, and 1994 (>1% cover on any watershed in any year) on WS3, WS4, and WS7. Values given are mean cover ± 1 SE of mean. Yearly means for a given watershed followed by the same letter are not significantly different at $p < 0.05$. See Table 5-2 for species codes. Data from Hockenberry (1996).

Species code	1991	1992	1994
		%	
		WS3	
LACA	–	–	–
STPU	–	–	–
POAC	–	–	–
DRMA	0.5 ± 0.2a	0.5 ± 0.1a	0.6 ± 0.3a
VIOL	3.8 ± 1.1a	2.9 ± 0.9a	3.9 ± 1.0a
SMRO	3.0 ± 0.9a	2.3 ± 0.7a	3.4 ± 1.0a
RUBU	0.7 ± 0.4a	1.3 ± 0.6a	2.3 ± 0.7a
ACPE	1.7 ± 0.8a	2.7 ± 0.5a	1.8 ± 0.6a
PRSE	0.4 ± 0.2a	0.3 ± 0.1a	0.9 ± 0.2b
		WS4	
LACA	3.5 ± 2.5a	1.8 ± 1.4a	2.4 ± 2.4a
STPU	–	–	–
POAC	1.7 ± 1.0a	1.1 ± 0.6a	0.8 ± 0.3a
DRMA	1.1 ± 0.5a	1.7 ± 0.7a	0.4 ± 0.1a
VIOL	3.0 ± 1.1a	1.3 ± 0.6a	4.6 ± 1.4a
SMRO	1.0 ± 0.2a	0.9 ± 0.3a	1.0 ± 1.2a
RUBU	1.9 ± 1.0a	0.8 ± 0.7a	1.7 ± 1.0a
ACPE	2.2 ± 0.7a	0.9 ± 0.5a	1.0 ± 0.4a
PRSE	1.2 ± 0.9a	1.0 ± 0.5a	0.9 ± 0.3a
		WS7	
LACA	2.7 ± 1.8a	0.4 ± 0.4a	0.8 ± 0.6a
STPU	1.0 ± 0.9a	2.9 ± 1.0a	2.6 ± 0.2a
POAC	5.6 ± 2.5a	6.7 ± 2.7a	3.9 ± 1.4a
DRMA	15.2 ± 5.3a	20.5 ± 6.8a	15.2 ± 5.1a
VIOL	3.8 ± 1.1a	5.2 ± 0.9a	5.9 ± 1.5a
SMRO	1.1 ± 0.6a	3.5 ± 1.0a	1.0 ± 0.5a
RUBU	0.5 ± 0.3a	1.0 ± 0.4a	1.3 ± 0.6a
ACPE	0.2 ± 0.1a	0.2 ± 0.1a	0.2 ± 0.1a
PRSE	0.2 ± 0.1a	0.3 ± 0.1a	0.5 ± 0.1a

Although species composition and cover of the herb layer do not appear to have responded to five years of the acidification treatment, other results

suggest that it may have altered plant and soil relationships, especially the relationship between soil N availability and herb cover. This can be seen in differences in patterns of correlation among watersheds for net nitrification versus mean herb layer cover. Monthly rates of net nitrification were significantly correlated among watersheds. For WS4 versus WS7 this was significant at $p < 0.01$ ($r^2 = 0.88$), whereas for WS4 versus WS3 it was significant at $p < 0.001$ ($r^2 = 0.98$), indicating the seasonal patterns in soil net nitrification were essentially identical among watersheds (Fig. 5-17). By contrast, mean cover for the herb layer was significantly related to net nitrification only for WS4 and WS7 (despite higher overall cover and large fern component on WS7) but not for WS3 (Fig. 5-18). Thus, the seasonal pattern of change in herb cover that is consistent with seasonal patterns of change in net nitrification on WS4 and WS7 appears to be absent on WS3, likely related to decoupling of N cycle from biotic controls due to acidification treatments, as noted by Gilliam et al. (2001).

Multivariate analysis also revealed alterations in spatial patterns of plant – soil relationships potentially caused by the acidification treatment on WS3. We used canonical correspondence analysis (CCA), a multivariate technique that performs a least-squares regression of plot scores (species' weighted averages) as dependent variables onto environmental variables as independent variables (ter Braak 1988). Thus, CCA is a form of direct gradient analysis (Palmer 1993). In addition to generating ordination diagrams with plot and species locations, CCA also generates environmental vectors originating from the center of the ordination space. The lengths of these vectors represent the gradient lengths of each measured environmental variable, such that vector length is proportional to the importance of an environmental gradient in explaining species' patterns. Thus, shorter lines represent gradients of lesser importance; longer lines represent gradients of more importance (ter Braak 1988, Palmer 1993, Gilliam and Saunders 2003).

The environmental factors included in our analyses were chosen to represent aspects of soil N availability and cycling at treated and untreated watersheds at FEF: extractable N pools (NH_4 and NO_3), rates of net nitrification, and foliar N and N:P ratios in *V. rotundifolia*. The latter (N:P ratios) was included to assess the likelihood of P-limitation on herb layer vegetation. Gilliam et al. (2001) found higher N:P ratios, indicative of P-limitation (Koerselman and Meuleman 1996), in *V. rotundifolia* on WS3 compared to that on WS4 and WS7.

Although the individual species appearing in the ordinations (Figs. 5-19 through 5-21) varied among watersheds, N:P ratio was the shortest (i.e. least important) vector on the two untreated watersheds (Figs. 5-20 and 5-21).

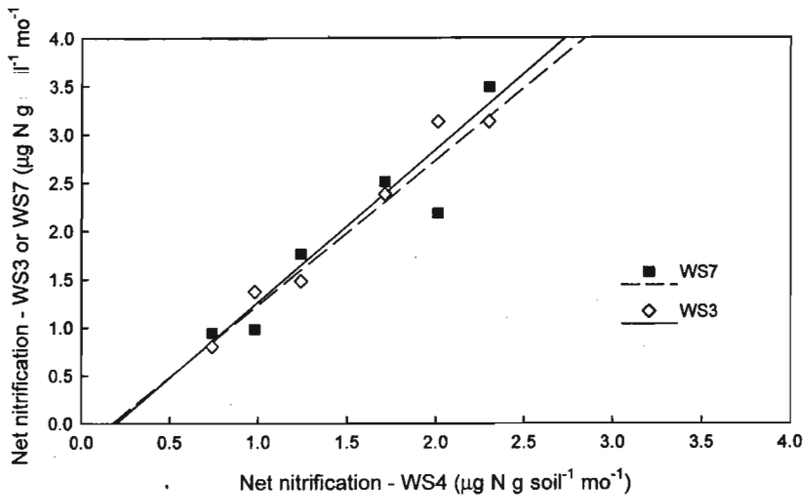

Figure 5-17. The relationship between net nitrification on WS3 and WS7 versus WS4. Each point represents mean monthly values, May to October 1994. Solid line represents significant linear relationship between net nitrification on WS3 and WS4: $y = -0.31+1.57x$, $r^2 = 0.98$, $p < 0.001$; dashed line represents significant linear relationship between net nitrification on WS7 and WS4: $y = -0.27+1.50x$, $r^2 = 0.88$, $p < 0.01$.

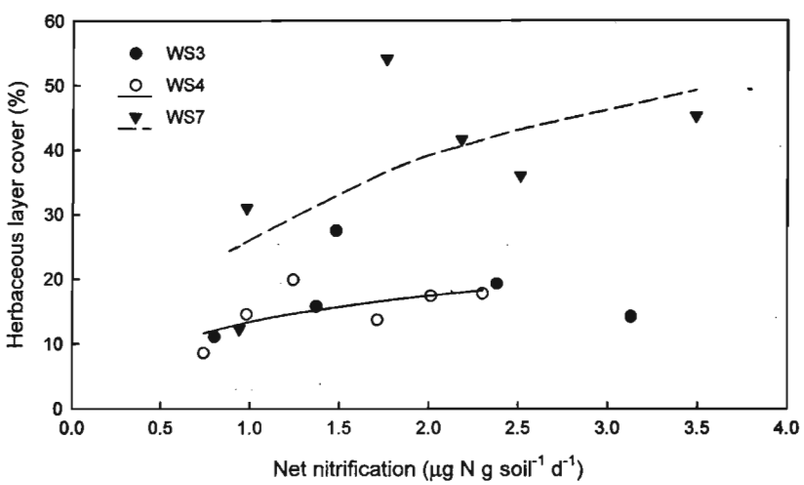

Figure 5-18. The relationship between herbaceous layer cover and net nitrification in mineral soil for WS3, WS7 and WS4. Each point represents mean monthly values, May to October 1994. Lines represent significant logarithmic relationships for WS7 ($Y = 18.4(\ln X) + 26.2$, $r^2 = 0.45$, $p < 0.05$), and WS4 ($Y = 5.8(\ln X) + 13.4$, $r^2 = 0.41$, $p < 0.05$). Relationship was not significant ($p > 0.10$) for WS3.

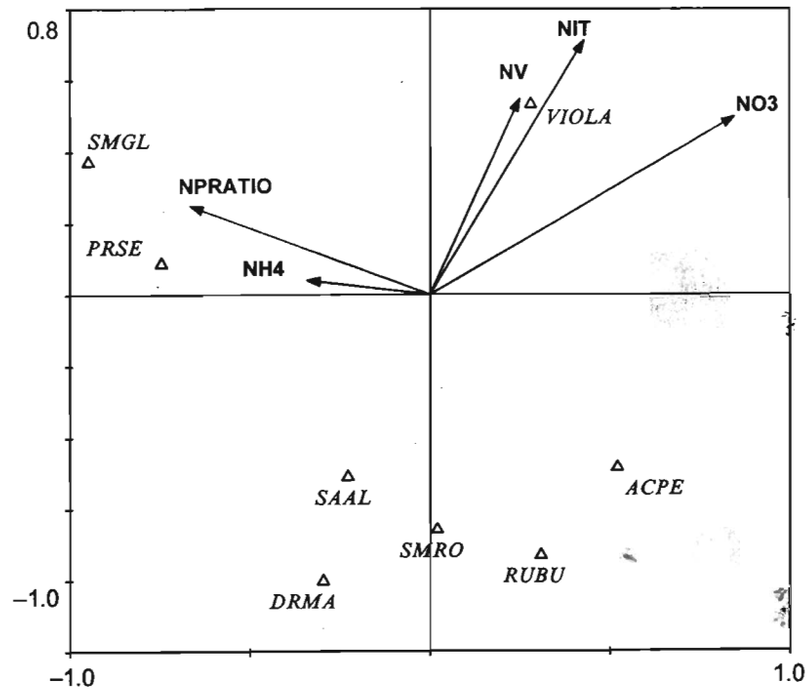

Figure 5-19. Canonical correspondence analysis of herbaceous layer species and indices of N availability/dynamics on WS3. NH4 and NO3 are extractable pools of soil NH_4 and NO_3, respectively; NIT is net nitrification; NV is foliar N in *Viola rotundifolia*; NPRATIO is foliar N:P ratio in *V. rotundifolia*. See Table 5-2 for species codes.

However, N:P ratio was among the longer (i.e. more important) vectors for treatment WS3 (Fig. 5-19). This suggests that the acidification treatment, particularly the N content of the treatment, has created P limitations by saturating the plant demand for soil N. As a result, P availability (measured in the case by foliar N:P ratios) exerts a significant influence on the spatial patterns of herbaceous layer species on WS3, but not on WS4 or WS7, likely the result of the excess N being added to WS3.

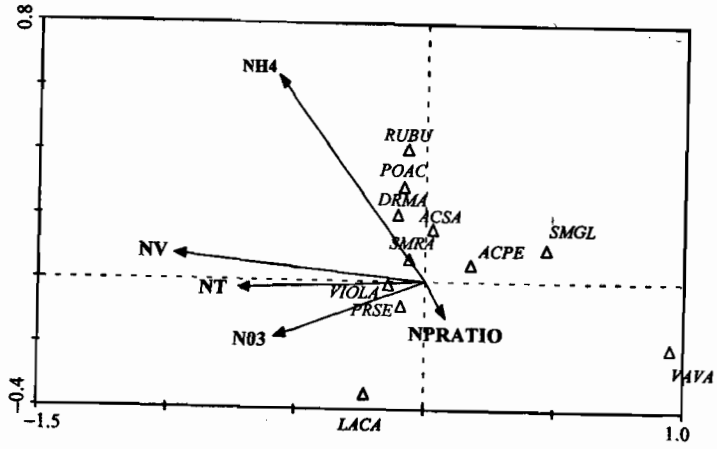

Figure 5-20. Canonical correspondence analysis of herbaceous layer species and indices of N availability/dynamics on WS4. See Figure 5-19 for meaning of environmental vectors and Table 5-2 for species codes.

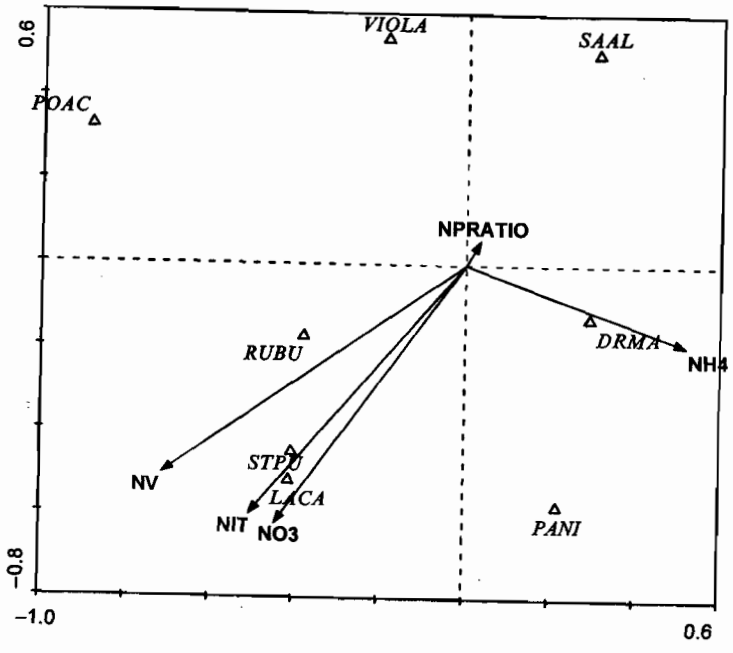

Figure 5-21. Canonical correspondence analysis of herbaceous layer species and indices of N availability/dynamics on WS7. See Figure 5-19 for meaning of environmental vectors and Table 5-2 for species codes.

4.2 WS9 (Clover Run) Results

4.2.1 Tree Growth

In 1993, after nine growing seasons of treatment, Kochenderfer et al. (1995) reported that average height of the treated larch trees was significantly lower ($\alpha = 0.05$) than that for the control trees in high, medium and low condition classes (Table 5-5). There was no significant difference in height of larch trees growing on the treated area and control plots prior to initiation of treatments (1986) for any of the condition classes (Table 5-5). The mean height of all larch trees after nine growing seasons was 5.7 m on the control plots and 4.8 m on the treated area. Average diameter of treated trees in condition class 1 and 2 was significantly smaller than that of control trees in 1993 (Table 5-5). Growth of the WS9 larch after nine growing seasons compared favorably with that reported at other locations, but acidification treatments appeared to cause a significant reduction in both height and diameter growth.

Table 5-5. Comparison of height (m) and diameter (cm) growth of treated and control larch trees for three condition classes on WS9. Values are means with standard errors in parentheses. For a given year and condition class, means followed by different letters are significantly different α=0.05 (analysis of variance followed by Tukey's HSD test).

Condition Class	Pretreatment (1986)[a]		Post-treatment (1993)	
	Treated	Control	Treated	Control
	Height			
	m			
1	1.2 (0.03)a	1.2 (0.03)a	5.8 (0.09)b	6.7 (0.09)c
2	0.8 (0.03)a	0.8 (0.03)a	4.4 (0.12)b	5.1 (0.12)c
3	0.5 (0.03)a	0.5 (0.03)a	2.9 (0.15)b	3.4 (0.18)c
	Diameter			
	cm			
1	–	–	5.3 (0.3)a	6.1 (0.3)b
2	–	–	3.0 (0.3)a	3.8 (0.3)b
3	–	–	2.3 (0.3)a	2.0 (0.3)a

[a]Treatments began in April 1987.

Pickens (1995) also compared the height and diameter of larch trees within one of the untreated control plots on WS9 that did not receive treatment and an adjacent treated plot in 1992 and 1993 after six and seven years of treatment, respectively. Mean larch tree height and diameter in the Pickens (1995) study were significantly greater ($\alpha \leq 0.05$) on the control plot

than the treated plot in 1992 and 1993 (Fig. 5-22). On the treated area, mean larch tree height was about one meter less and mean diameter at breast height was about 1-2 cm less than on the control area. These differences agree with those found after nine years of treatment shown in Table 5-5 and indicate that acidification treatments with ammonium sulfate reduced larch height and diameter gro

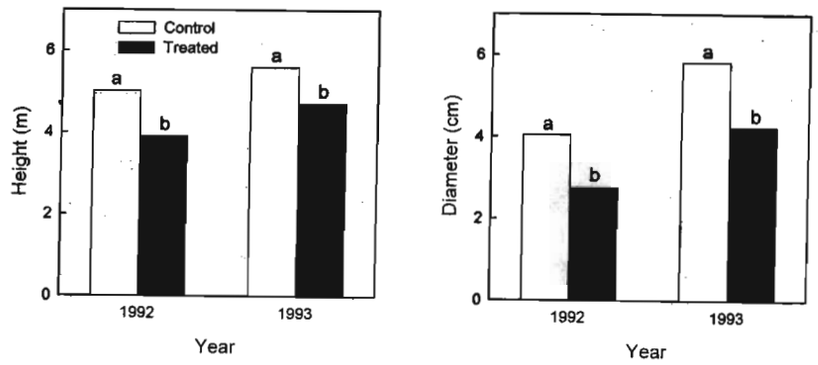

Figure 5-22. Mean heights (m) and diameters at breast height (cm) of Japanese larch trees on a control and treated plot measured in 1992 and 1993, six and seven years, respectively, after initiation of treatment on WS9 (Pickens 1995). Different letters above bars indicates significant difference at the $\alpha = 0.05$ level between control and treatment plots in that year.

Although it has been more than 60 years since farming ceased, soil analysis indicated that the soils on WS9 are very nutrient-deficient and highly acidic, with an average pH of 4.5 in the top 30.5 cm of soil. The average concentration of Mn extracted from soil samples collected in 1994 at the same locations where foliar samples were collected, indicated no significant difference ($\alpha = 0.05$) between treated and control soils (Kochenderfer et al. 1995). However, average concentrations of Mn were significantly different in both soils between the 0-15.2 cm soil layer (56.5 mg kg^{-1}) and the deeper 15.2-30.5 cm layer (21.2 mg kg^{-1}). In contrast, soils analyzed using a strontium chloride ($SrCl_2$) extraction method (Chapter 3, Table 3-4 and Fig. 3-14) showed significantly reduced exchangeable Ca and Mg and significantly increased Al and Mn in treated soils. Acidification of soil water by ammonium sulfate treatments on WS9 was also indicated, based upon soil lysimeter data (Chapter 4, Fig. 4-12 and Table 4-2) and
r sampling at baseflow and peakflow (Chapter 4,
4-24 and 4-29). Thus, it appears the reduced growth
ed on WS9 could be related to low levels of soil
ated by the acidification treatment.

Unfortunately, no detailed analysis of tree cores was conducted on Clover Run to determine if patterns of increasing growth followed by decreasing growth occurred in Japanese larch. Since growth was already reduced after six years of treatment (Pickens, 1995), the period of accelerated growth, if any, would have to have been relatively short compared to Fernow WS3.

4.2.2 Foliar Chemistry

An analysis of foliar samples collected from dominant and co-dominant larch trees (Kochenderfer et al. 1995) indicated that concentrations of macro-elements and most metals were within the medium range of concentrations reported for coniferous tree species. Of the foliar elements analyzed, only the concentration of Mn was significantly greater on the fertilized plots. Pickens (1995) also found significantly reduced Mg and P and elevated Mn and Al in larch foliage on the treated portions of WS9 and DeWalle et al. (1999) showed both depleted Ca and increased Mn in bolewood of treated trees (see Section 4.2.3 of this chapter).

Results from Pickens et al. (1995) showed that trees on the treated plots had significantly lower foliar Mg and P and significantly higher foliar Mn and Al content in 1992 and 1993 than those from the control plots (Fig. 5-23). No significant differences in foliar K and Ca between treated and control larch trees were found. However, Ca in treated red maple seedling, blackberry and mountain laurel foliage on WS9 was significantly lower than in control leaves during the same years. Foliar N was significantly greater in treated than control trees in 1993.

4.2.3 Dendrochemistry

For WS9, dendrochemistry analyses suggest that Japanese larch trees appeared to preserve a record of dendrochemical changes due to treatment over time (Fig. 5-24, DeWalle et al. 1999). Wood formed in treated trees after 4-5 years of treatment showed higher concentrations of Ca and Mg relative to control trees, while wood formed more than five years after treatments began, showed relative depletion. Differences in mean Ca and Mg concentrations were significant only in 1996. Manganese concentrations were elevated relative to control trees in all years (significant in 1992-1994) and Ca/Mn showed significant reductions in all years. Depletion in bolewood base cations may have occurred a few years sooner on WS9 than on WS3, due to differences in soil buffering capacity, but precise timing of these changes is difficult to determine.

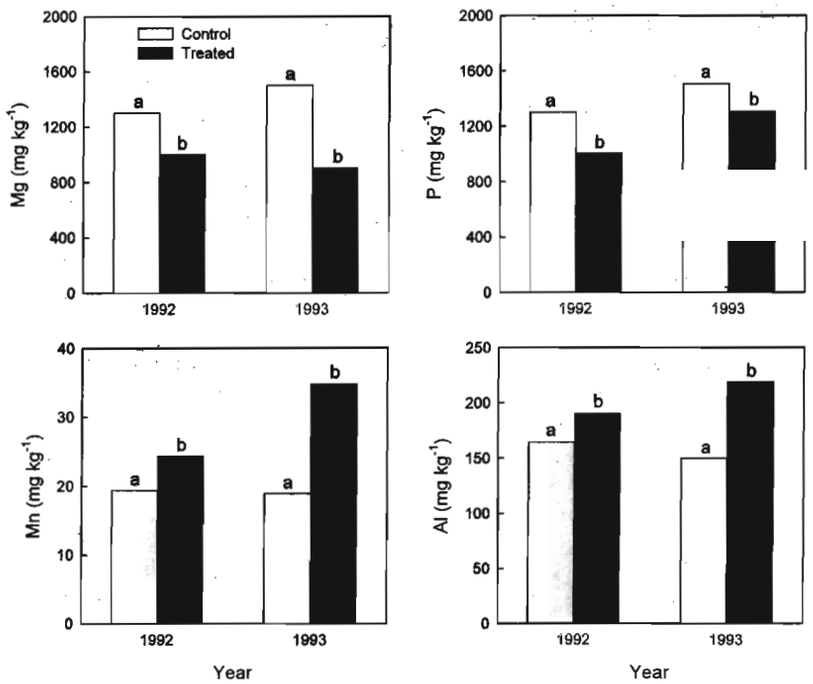

Figure 5-23. Comparison of Japanese larch foliar chemistry on WS9 treated and control plots in 1992 and 1993 (Pickens 1995). Significant differences between treated and control trees ($\alpha = 0.05$) are shown by different letters above the bars.

5. DISCUSSION

The results of analyses of treatment effects on forest growth, based upon inventory growth plot and tree core data analyses at Fernow WS3 and WS7 did not completely agree. Tree core analysis indicated that radial and BAI growth was species specific and either increased or remained unchanged in the early stages of treatment, and in later stages of treatment tree cores showed that growth was reduced in all species. Growth plot data generally only showed increased growth or no reductions in stand productivity due to treatment. All measurements of treatment effects on growth of Japanese larch on WS9 Clover Run showed reduced growth. Possible reasons for these differences in research results on treatment effects on forest growth and comparisons with results from other research are discussed below.

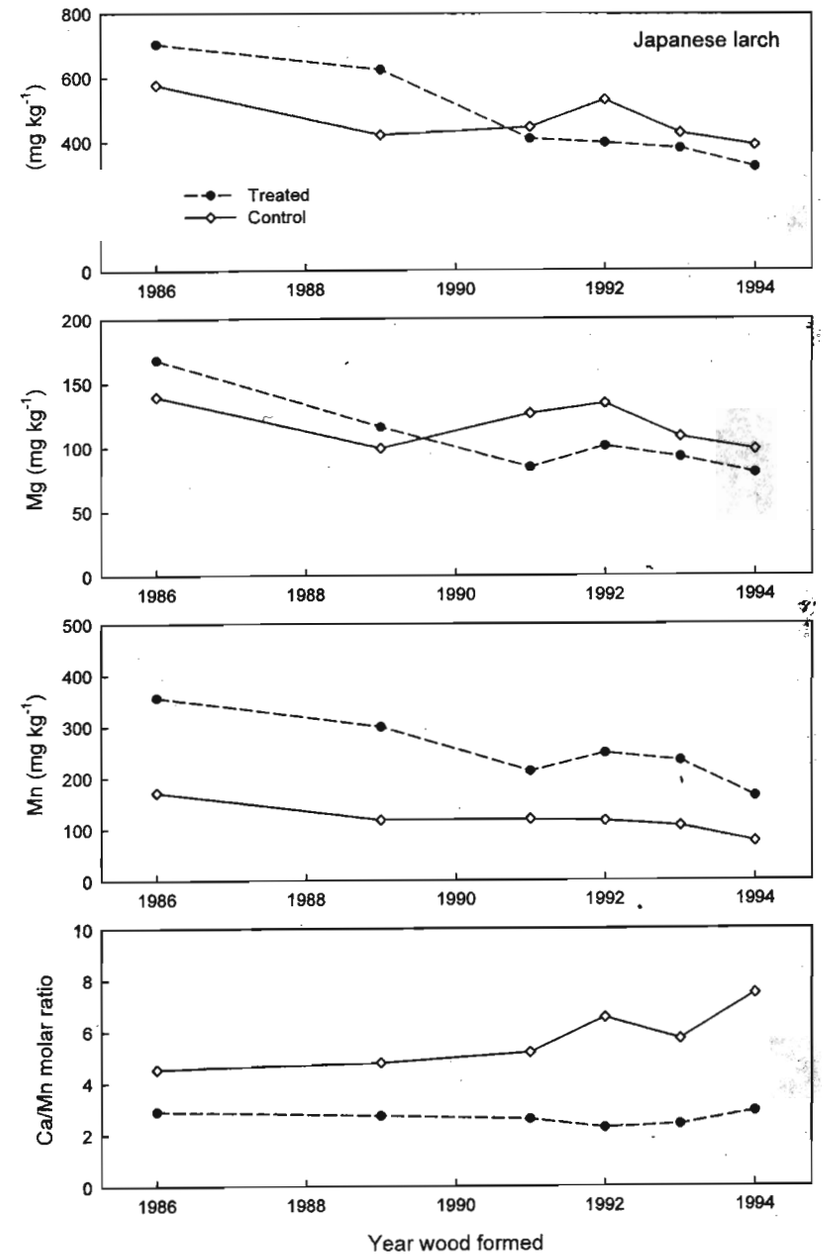

Figure 5-24. Japanese larch bolewood Ca, Mg, Mn and Ca/Mn molar ratios on WS9 treated and untreated plots after seven years of treatment. Treatments began in spring 1987.

Comparisons of annual radial growth data from tree cores with stand level growth plot results is problematic due to differences in measurement approach, as well as differing time periods available for comparisons. Since the micro-site conditions, competitive status, and genetic makeup of each tree determines its growth rate, direct comparisons between net volume growth of a mix of trees of varying size and species on plots over a multi-

and annual radial growth or basal area increment based upon tree-core analysis of individual tree specie should be made with caution.

Forest growth/productivity is not constant in forest stands and follows consistent trends represented by a sigmoid curve described by Assman (1970). Growth in the young stand increases at an increasing rate to an inflection point where growth essentially increases at a constant rate for a number of years. Beyond the inflection point, growth increases at a decreasing rate until it reaches a peak and begins to decline. Although the trend is robust, the actual timing can be affected by site quality, species composition, stand density, and disturbance regimes. Since these two watersheds differed in initial stand density, volume, species composition and biomass, interpreting differences between watersheds to determine growth effects is complicated. For example, Auchmoody (1985) obtained the greatest response to a N+P fertilizer treatment in Allegheny hardwood stands when the fertilizer treatment was combined with a thinning. Some of the growth differences between WS3 and WS7, could have resulted from the stands being in slightly different stages of development. Stand development on WS7 may have lagged behind WS3 due to the herbicide treatments that preceded the acidification study. However, with no conclusive evidence of significant changes in growth over time, it is clear that stand development in both watersheds is characteristic of the middle stages of stand development described by Assman (1970).

Regardless, some similarities in growth results between plots and tree cores at Fernow were found. Greater annual net volume growth on WS3 growth plots with more black cherry and yellow-poplar (BC/YP) from 1990/91-2004 and greater cubic volume growth on plots with more red maple and sweet birch (RM/SB) during 1990/91-96 agreed in general with the greater relative radial growth and BAI in tree cores found for black cherry, yellow-poplar and black locust during this period. Black cherry typically responds positively to N inputs in the study region (Auchmoody and Filip 1973), thus was more likely to be affected by improved soil fertility conditions due to treatment in the base cation mobilization period. Increased foliar N was found on WS3 in 1992 early in the treatment period which suggests N fertilization could have been influencing growth. It appears that ammonium sulfate treatments had a positive impact on growth of some species in this mixed hardwood stand.

Similar positive growth response to N additions have been found in other studies. BAI growth responses to ammonium sulfate treatments have been reported in sugar maple in Maine (BBWM) during the first eight years of treatment (Elvir et al. 2003). Magill et al. (2000) also reported significant increases in wood production due to high experimental levels of N deposition (150 kg N ha^{-1} yr^{-1}) in Massachusetts hardwood stands dominated by oak l Thomsen (2001) likewise found significant increases in radial increment in Norway spruce trees after 20 years of atmospheric N deposition in southern Norway. Solberg et al. (2004), also working in southern Norway, similarly concluded that atmospheric deposition may have increased conifer forest growth by up to 25%. All of these results show that initial stages of enhanced atmospheric deposition can increase growth of forests due to the fertilizer effect of the added nitrogen and other related changes in ecosystem biogeochemistry.

Not all species studied showed an initial positive growth response to treatment in the West Virginia experiments, however. Red maple RRG and BAI results from tree cores at WS3 and Japanese larch height and diameter measurements at WS showed generally reduced growth due to treatment throughout the treatment period. RM/SB growth plot data analysis for Fernow WS3 (Figs. 5- through 5-4) also showed no significant differences in growth due to treatment for the 1996-2004 later growth period, unlike the BC/YP plots. Thus, Fernow growth data did suggest that some differences in the response to treatment exist among tree species over time.

Species differences in response to acidification or N treatments have also been found elsewhere. For example, Magill et al. (2000) reported that wood production in a Massachusetts red pine forest was reduced experimentally by high N and N+S additions, while hardwoods responded positively to the same treatment. No significant increases in BAI of red spruce trees were found at BBWM due to ammonium sulfate treatments (Elvir et al. 2003) even though a positive response was found for sugar maple. Other research has suggested that conifer species are more susceptible to effects of soil acidification than hardwoods (Fenn et al. 1998, Fernandez et al. 2003). Differing response to treatment between species groups, especially conifers vs. hardwoods, is not uncommon in the literature.

What is most surprising in the Fernow study of vegetative growth is the pattern of the initial radial growth increases followed by radial growth decreases in hardwood species based upon tree core analysis (Figs. 5-5 through 5-9). This pattern of growth changes was not supported by the growth plot data at Fernow. However, a similar pattern of radial growth increases and decreases attributed to effects of acidification, N deposition and climate stress was found by Nelleman and Thomsen (2001) based upon radial growth analysis on >31,000 Norway spruce growth plots in southern Norway. McNulty et al. (1996) also reported a shift from positive to negative

net growth response due to experimental N additions in a high elevation spruce-fir stand including some birch and maple trees. Boxman et al. (1998) also found that reducing N deposition in throughfall on roofed plots at Ysselsteyn, Netherlands resulted in significantly improved radial growth of Scots pine, suggesting that growth was being suppressed by ambient throughfall. Finally, May et al. (2005) also noted reduced diameter growth in black cherry, yellow-poplar, and red maple trees at Fernow from 1999 to 2001, during the period of decline in growth noted by the tree cores. Theories of forest ecosystem acidification discussed in Chapter 1 suggest that a pattern of increased followed by decreased growth can result from prolonged exposure to acidic deposition. The cause of this response pattern may be either development of N saturation or base cation depletion with Al mobilization and can not be determined from the available data. The Fernow tree core results are the first to show a pattern of increasing followed by decreasing tree growth in mixed hardwood species.

The pattern of increasing then decreasing tree growth matches the pattern exhibited by soil solution chemistry and stream chemistry (Chapter 4) and the patterns of treatment effects on bolewood chemistry reported in this chapter. In Chapter 4, a base cation mobilization phase during the first four years of treatment was hypothesized based on solution chemistry. Such a mobilization of base cations could have altered bolewood chemistry and improved the nutritional status of trees relative to control conditions. Mobilization of base cations in soil solution was followed by a base cation depletion phase; we hypothesize that after eight years of treatment this depletion of cations had impaired the relative nutrient status of these same trees. The early positive effect on net annual volume growth of WS3 could be attributed to this mobilization of base cations or to improved N status. However, the growth plot data do not indicate a decrease in growth with the apparent cation depletion, as growth has remained significantly higher on WS3 in the 1996-2004 period, especially on the BC/YP plots. Treatment of Fernow WS3 beyond eight to 12 years only produced marginally greater reductions in nutrient status of trees, suggesting that an equilibrium was approached after eight years.

Larch on WS9 exhibited decreases in growth in response to treatment. One might have expected increased growth in response to treatment unless other nutrients are limiting such that the trees could not utilize the increased available N. The overall poor fertility of the eroded soils on WS9 may be limiting in other nutrients, including P or Mg. Visible symptoms of Mg deficiency were observed on WS9 (Fig. 5-25).

Foliar chemistry analyses on WS3 and WS7 provide some support for the idea that early growth response on WS3 was due to N response (Fig. 5-10) but also provide support for the mobilization of base cations (Fig. 5-11), depending on the particular tree species. The foliar data do not provide

clear indications however, of a base cation depletion stage. There is a suggestion that P may be limiting to black cherry on both watersheds. Other supporting data (Gilliam et al. 2001, W.T. Peterjohn, unpublished data) also provide evidence of a P limitation.

Figure 5-25. Yellow needle tips on Japanese larch indicate Mg deficiency on WS9 (Photo courtesy of W. E. Sharpe).

WS9 foliar chemistry results showed the differences that were hypothesized due to treatment. We hypothesized that additions of NH_4, which was converted to mobile NO_3 anions by nitrification, along with applied SO_4 anions in excess of soil SO_4 adsorption capacity, would leach base cations such as Ca and Mg and ultimately reduce their availability to plants. A decrease in soil base cation availability would lead to soil acidification, making Mn and Al more available. Increased tissue N due to the added NH_4, may have led to reduced P availability due to formation of Al-PO_4 precipitates (Pickens 1995, VanGoor 1953). Recommended N:P ratios for larch needles ranged from 4 to 5, while treatment and control plots had foliage with ratios of 17 and 15, respectively, indicating reduced P availability.

Altered nutrient availability due to mobilization of Al and depletion of Ca in soils on WS3 caused by accelerated soil acidification processes may explain growth differences between trees on WS3 and WS7 based upon tree core analysis. The observed increases in relative radial growth of yellow-poplar and black cherry trees on WS3 a few years after treatment was

initiated, may be due to a temporary increase in base cations in soil solution (see Chapter 4) that resulted in concurrent increases in bolewood Ca and Mg levels. Seven to eight years after treatments began, trees of both species on WS3 declined in radial growth significantly, concurrent with a change in Ca status from a mobilized phase into a depletion phase (DeWalle et al. 1999). The lack of mobilization response by red maple trees to the treatment may

sensitive to base cation availability than red maple.

In order to examine concurrence of trends in growth and bolewood chemistry, relative radial growth rates (RRG) from the above analysis can be combined with changes in bolewood chemistry (Ca/Mn ratios) for black cherry, yellow-poplar and red maple after four, eight, and 12 years of treatment. Although it is natural for RRG to decline with time, bolewood Ca/Mn decreases were accompanied by larger decreases in RRG on WS3 than on WS7 from treatment year 4 to treatment year 8 (Fig. 5-26). Patterns of change for black cherry and yellow-poplar on WS3 suggested a delay or threshold of Ca/Mn decline beyond which RRG rapidly decreased. Because each species has a characteristic range of bolewood Ca/Mn it is difficult to specify a threshold, but RRG declines occurred approximately when ratios fell below the minimum Ca/Mn found on WS7 for that species. Again, red maple did not show a base cation mobilization response and experienced RRG declines that were not closely linked to Ca/Mn. Lack of relationship for red maple may be due to the intermediate to suppressed canopy position of this species in this stand or to the relative lack of response of this species to changes in Ca availability.

A different pattern of changes in relative radial growth rates vs. bolewood Ca/Mn ratios from treatment years 4 to 12 was found on the WS7 reference watershed. Ca/Mn ratios actually increased with time of treatment for both black cherry and yellow-poplar even though RRG declined as expected. Thus it appeared that bolewood chemistry was linked differently to RRG on WS3 compared to WS7.

The Japanese larch dendrochemistry results were similar to those for WS3 vs. WS7 comparisons with the added benefit that the larch trees seemed to preserve a record of soil changes over time. The only exception was that Mn levels appeared to be elevated even during the base cation mobilization phase, unlike results on WS3 where bolewood Mn appeared to be reduced during base cation mobilization. Soils on WS9 had lower levels of exchangeable bases and higher exchangeable Al than those at Fernow WS3 and WS7 and exhibited higher levels of Mn in soil solution even before treatments began. No soil Mn data are available for comparison, but Table 3-1 in Chapter 3 does show lower exchangeable Ca and Mg and higher exchangeable Al on WS9 than either WS3 or WS7 in the 0-10 cm layer.

Thus, Mn ions already present were likely mobilized along with base cations in the early stages of treatment. Otherwise, dendrochemical responses for larch on WS9 were quite similar to those of other species on WS3 at Fernow.

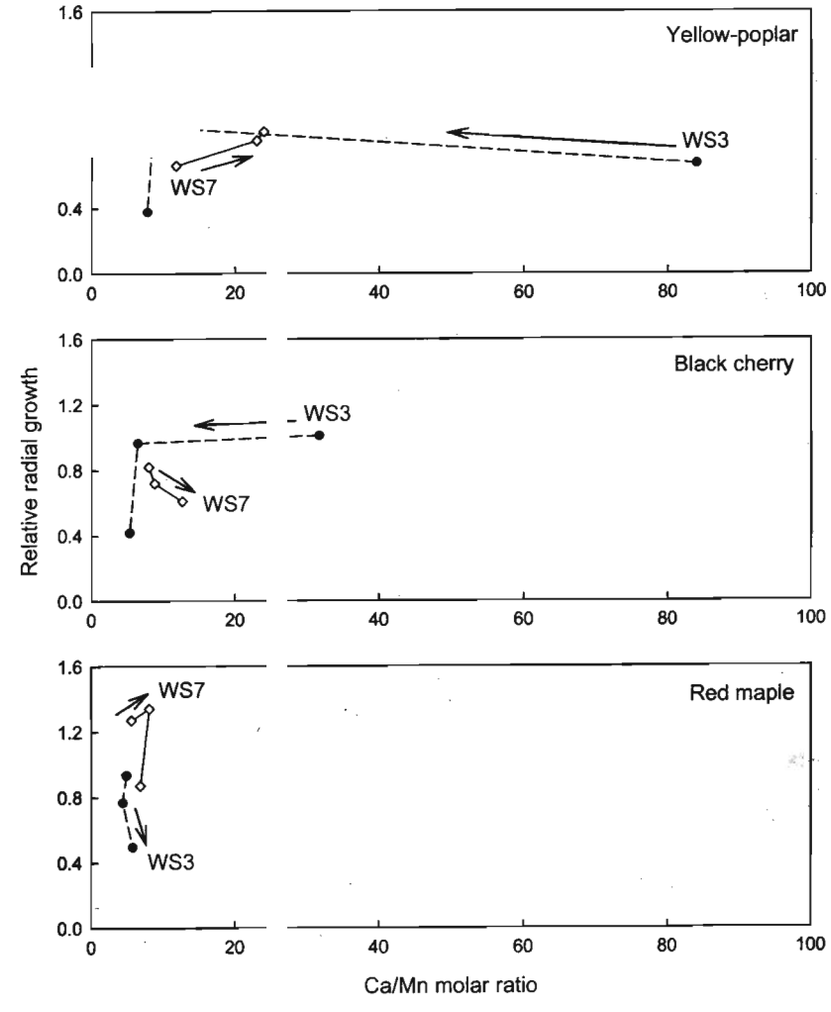

Figure 5-26. Patterns of mea elative radial growth rate and bolewood Ca/Mn ratio changes after 4, 8, and 12 years of tre nent for three tree species on WS3 and WS7. Arrows indicate the direction of progression fr n treatment year 4 to treatment year 12.

The similarities between WS3 and WS9 results also extended to the time required for base cation mobilization and depletion to occur. Larch tree rings began to show base cation depletion in about 1991 or about five years after

treatments began. Since tree ring wood was only analyzed for individual years as the planted larch trees grew large enough for rings to be easily sampled, the exact timing of mobilization and depletion can only be detected within about ± 2 years. Regardless, despite the differences in soils and past land use history between WS3 and WS9, the time required for trees to show base cation mobilization and depletion effects were about the same. A resampling of larch trees on WS9 to determine later trends in dendrochemistry could be fruitful.

Dendrochemistry response time of the forest ecosystem to a change in deposition can be roughly scaled based upon total loading from this experiment. The estimation was developed based upon assumptions that ecosystem change occurs over a time interval that is proportional to the total loading of acid computed as the product of the magnitude of the deposition change and the number of years over which it acts. Thus, a small deposition change (say 10% increase in annual deposition rate D from 1.0D to 1.1D) would take a longer time to reach an equilibrium condition (X years) than the eight years of essentially triple the atmospheric deposition load (D + 2D = 3D) that was imposed by the experimental Fernow treatment, e.g.

$$1.1D \bullet X \text{ years} = 3D \bullet 8 \text{ years}$$
$$X = 21.8 \text{ years.}$$

The dendrochemical response time (X) indicated for a 10% deposition increase was thus 21.8 years. If 12 years were needed to produce the same change, rather than eight years, then the response time to a 10% increase in deposition would be scaled up to about 33 years. Regardless, data suggest that the impacts of a relatively small 10% increase in atmospheric deposition would require a time scale of two to three decades for a dendrochemical response to occur. Thus, long-term monitoring would be required to detect impacts of such a fairly small deposition change and results could vary from Fernow response time depending on site fertility.

6. CONCLUSIONS

Forest basal area, biomass, and cubic volume growth was increased by experimental additions of ammonium sulfate at FEF (WS3 vs. WS7) on growth plots with more black cherry and yellow-poplar trees during 1990-2004 and cubic volume growth was increased on growth plots with more red maple and sweet birch during 1990-1996. Growth plot analyses included the effects of growth of all trees on the plots, including the effects of ingrowth and mortality, and represent an expression of changes in site productivity over multi-year time periods.

Tree core analysis suggested BAI growth was increased for black cherry and yellow-poplar overstory trees on WS3 during the first six to seven years of treatment (1989-1996), followed by decreased BAI in these species plus red maple during the last four years of treatment (1996-2000). Similar declines in growth were not found on permanent growth plots. Tree core analysis showed BAI treatment response for individual tree species for each year.

Red maple growth on WS3 did not positively respond to treatment, based upon tree core analyses. Our results support previous work that generally show positive response of hardwood species to N fertilization, but shows that individual hardwood species, like red maple, may respond differently.

Japanese larch diameter and height growth on WS9 (Clover Run) was significantly reduced by ammonium sulfate treatments. The WS9 results are consistent with several past studies showing reduced conifer growth in response to N fertilization and soil acidification. Reductions in growth on WS9 may have been exacerbated by past abusive farming that left the soil depleted of nutrients.

Patterns of bolewood chemistry over time on both WS3 and WS9 showed a period of relative base cation mobilization during the early years of treatment followed by a period of base cation depletion for overstory black cherry, yellow-poplar, and Japanese larch. Periods of bolewood base cation depletion were accompanied by increased concentrations of Mn. Only red maple showed bolewood base cation depletion throughout the experiment. Since red maple also did not show increased radial growth during the early stages of the experiment, a similarity in patterns of bolewood chemistry change and radial growth change is suggested. Black locust sapwood did not show effects of treatment on base cations and this species did not appear suitable for dendrochemical detection of acidification.

Acidification treatments to both WS3 and WS9 resulted in changes in foliar chemistry of trees, although larch on WS9 seemed to be more responsive than the hardwood trees on WS3. Where statistically significant differences were detected, concentrations of Ca and Mg were generally decreased and levels of Mn and/or Al were elevated in foliage. Foliar chemistry response was inconsistent and did not show initial periods of base cation mobilization followed by base cation depletion, that were found with bolewood chemistry.

The acidification treatments, through the effects of elevated N, also may have created P limitations for the herbaceous and overstory plant species on WS3. The seasonal changes in herbaceous cover that are normally linked to net nitrification were absent on WS3 due to a decoupling of N cycle from biotic controls due to treatments.

The pattern of increasing growth followed by decreasing growth found in tree cores was synchronous with changes in bolewood chemistry and other

temporal patterns of soil solution and stream chemistry described in Chapter 4. The pattern of changes in growth (tree cores only), soil solution and stream chemistry fits the expected response of forest ecosystem to acidification described in Chapter 1. Ecosystem response time for bolewood chemistry changes in these experiments suggests that two to three decades would be required for detection of a 10% decrease in deposition in ecosystems similar to the FEF.

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Environmental Pollution

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