Tree-Ring Cation Response to Experimental Watershed Acidification in West Virginia and Maine

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ABSTRACT

The impact of experimental watershed acidification on xylem cation chemistry was evaluated in eight tree species at two sites in West Virginia (Clover Run and Fernow) and one site in Maine (Bear Brook). All sites had received regular additions of $(NH_4)_2SO_4$ equivalent to twice the ambient annual wet plus dry atmospheric deposition of N and S. Multiple wood cores were extracted from tree boles in five trees of each species on treatment and control areas at each site with increment borers. Cores were divided into several age segments and composited for each tree. Ground wood samples were destructively analyzed for Ca, Mg, Mn, and Al concentrations using inductively coupled plasma emission (ICP) methods. All tree species sampled at the two West Virginia sites exhibited significant Ca and/or Mg concentration decreases and Mn concentration increases in sapwood on the treated relative to control areas after 8 yr of treatment. At Bear Brook, tree-ring concentrations in three species showed similar trends after 5 yr of treatment, but differences were generally not significant. Sapwood molar ratios of Ca/Mn and Mg/Mn were better indices to soil acidification than Ca/Al, due to low Al concentrations and insensitivity of sapwood Al concentrations to treatments. Overall, sapwood chemistry appeared to be a reliable indicator of the current nutrient status of trees; but, except for Japanese larch (*Larix leptolepis* Sieb. and Zucc.), sapwood chemistry did not preserve a record of the chronology of past changes due to treatments.

Long-term impacts of atmospheric deposition on soils and the growth and development of forests in the northeast USA remain a major unresolved environmental issue. Increases in Al concentrations and depletion of base cations in the soil can occur due to atmospheric deposition (Robarge and Johnson, 1992; Ulrich, 1986). Federer et al. (1989) showed for the northeastern USA that leaching caused by atmospheric deposition and removals by forest harvesting could lead to depletion of Ca and other nutrients over a 120-yr period. Impacts of such soil changes on the growth and development of trees remain elusive, but several studies have shown Ca and/or Mg enrichment in xylem of red spruce (*Picea rubens* Sarg.) that was believed due to cation mobilization in soil associated with acidic atmospheric deposition during the 1950 to 1960s (Bondietti et al., 1990; Shortle et al., 1997; Shortle and Bondietti, 1992). In addition, Shortle et al. (1997) also found higher levels of foliar putrescine, a biochemical indicator of stress, in apparently healthy red spruce in the Northeast where lower Ca/Al ratios occurred in the soil organic layer. In watershed acidification/N saturation experiments at Fernow WS3 in West Virginia (Adams et al., 1993, 1997) and Bear Brook in Maine (Kahl et al., 1993; Norton et al., 1994) $(NH_4)_2SO_4$ applications equal to twice the normal wet plus dry atmospheric deposition quickly caused increased losses of base cations and, at least in Maine, Al from the watersheds in streamwater. At both sites increased loss of base cations in stream water was accompanied by increased nitrate concentrations suggesting rapid nitrification of added NH_4 was occurring. In a similar watershed experiment at Clover Run watershed in West Virginia $(NH_4)_2SO_4$ treatments appear to have caused increased soil water concentrations of sulfate, nitrate, and aluminum (Pickens 1995) and reduced height and diameter growth of Japanese larch (Kochenderfer et al., 1995). Such watershed experiments offer an excellent opportunity to study the effects of soil acidification on tree-ring chemistry.

Tree-ring element concentrations offer promise for detecting and establishing the chronology of long-term soil chemical changes due to atmospheric deposition. Cations are bound to sap-conducting tissue in tree boles (Ferguson and Bollard, 1976) and studies have shown that varying soil acidity conditions can influence the concentrations of divalent cations such as Mn, Ca, and Mg in xylem tissue (DeWalle et al., 1991; McClenahen et al., 1989; Guyette et al., 1992; McClenahen and Vimmerstedt, 1993; Kashuba-Hockenberry and DeWalle, 1994). Inferences concerning timing of environmental change based upon tree-ring chemical chronologies may be complicated in many species by the potential for radial translocation of elements in sapwood and/or longitudinal sapflow in a wide area of older sapwood that may blur the signal over time (Smith and Shortle, 1996). In some species, radial variations in the cation-binding capacity of wood may also affect the radial distribution of element concentrations (Momoshima and Bondietti, 1990). Cutter and Guyette (1993) ranked tree species for suitability for dendrochemical studies based on sapwood/heartwood properties and other factors, but in many settings ideal species are not available for study. Ideally, efficacy of dendrochemistry in detecting the timing of environmental change should be evaluated in an experimental setting where the exact timing and intensity of the causative agent can be documented and controlled.

Bondietti et al. (1989) proposed use of molar ratios of Al to either Ca or Mg in xylem as indicators of the effects of atmospheric deposition, since xylem base cations, Ca or Mg, are likely to be depleted and xylem Al increased by accelerated soil leaching. Cronan and

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Abbreviations: ICP, inductively coupled plasma emission spectroscopy; T/C, treated/control.

Grigal (1995) found little direct experimental evidence in support of use of Ca/Al ratios in xylem to detect effects of atmospheric deposition and argued further that extremely low Al relative to high Ca or Mg concentrations found in xylem caused computational problems with such ratios. DeWalle et al. (1991) reported that sapwood concentrations of Ca and Mg were higher, Mn concentrations were lower, and Al concentrations did not differ in less-acidic compared to acidic soils for several tree species, suggesting that Ca/Mn and Mg/Mn molar ratios in xylem may be more useful for detection of soil acidification effects than Ca/Al.

The purpose of this study was to evaluate the usefulness of dendrochemistry to determine environmental changes caused by $(NH_4)_2SO_4$ used to simulate accelerated rates of atmospheric deposition at three experimental sites in the eastern USA (Clover Run, Fernow, and Bear Brook). Specific objectives of the study were to: (i) evaluate the general direction and magnitude of tree-ring Ca, Mg, Mn, and Al concentration changes caused by treatments, (ii) evaluate the usefulness of Ca/Mn and Mg/Mn relative to Ca/Al molar ratios in xylem as indicators of soil acidification, (iii) determine the ability of annual growth rings to preserve a chronological record of soil changes caused by treatments, and (iv) compare the xylem chemistry response of the various tree species sampled at each site.

METHODS

At each of three experimental watershed acidfication sites, trees species commonly found at the site were cored with increment borers to obtain wood samples for chemical analysis from both treated and control areas. No tree cores were available from trees prior to treatment at any of the sites, but control areas were adjacent or in close proximity to treatment areas and exhibited similar tree species, stand age, topography, soil series, and geology as treatment areas.

Experimental Watersheds Sampled

The experimental watersheds sampled were Clover Run in northcentral West Virginia near St. George, WV; Fernow WS3 in north central West Virginia on the Fernow Experimental Forest near Parsons, WV; and Bear Brook in east-central Maine near Aurora, ME (Table 1). Clover Run soils are Calvin channery silt loam (loamy-skeletal, mixed, mesic Typic Dystrochrepts) and are derived from Hampshire formation sandstone and shale. Slope steepness on the 55 ha Clover Run treatment basin averages about 25%. Mean annual precipitation is about 163 cm and mean annual air temperature is about 10°C. The Clover Run watershed had been farmed until 1930, was allowed to regrow to poor-quality hardwoods until 1983 when it was cut, root-raked and planted in April 1984 with 2-yr-old Japanese larch seedlings (Kochenderfer et al., 1995). A 30-m wide buffer zone of mixed hardwood trees was left uncut along emphemeral and perennial streams on the watershed where some tree-ring chemistry sampling in sawtimber-sized black cherry (*Prunus serotina* Ehrh.) trees was conducted by DeWalle et al. (1995) in 1992. Applications of $(NH_4)_2SO_4$ on Clover Run began in April 1987 3 yr after larch were planted. Application amounts were 33, 101, and 33 kg ha^{-1} in March, July, and November of each year, respectively. Japanese larch trees which were approximately 12-yr old when sampled in August 1994 on an adjacent pair of 25 by 25 m treated and control plots on the upper slope of the treated Clover Run watershed.

On the Fernow Experimental Forest, $(NH_4)_2SO_4$ was added to WS3, a 34-ha forested watershed which had been clearcut to a 2.54 cm diam. at breast height lower limit in 1969 to 1970, except for a 3-ha forest streamside buffer zone that was clearcut in 1972. Climate, topography, soils, and geology on Fernow WS3 are similar to that found at Clover Run (Adams et al., 1994). The $(NH_4)_2SO_4$ treatments on Fernow WS3 began in January 1989, 2 yr later than at Clover Run, but the amounts and timing of applications were the same as at Clover Run (Adams et al., 1997) (Table 1). Large pole-sized trees approximately 25-yr old of three species, black cherry (*Prunus serotina* Ehrh.), yellow-poplar (*Liriodendron tulipifera* L.) and red maple (*Acer rubrum* L.) were sampled on Fernow WS3 and a nearby control area in July 1996. The control area for these measurements was a small clearcut (Compartment 39) within 0.5 km of Fernow WS3 that provided the necessary species and comparably-aged trees under similar soil and geologic conditions. Earlier dendrochemistry research reported by Tepp (1995) and DeWalle et al. (1995) was conducted in 1992 on Fernow WS3, with the adjacent watershed (Fernow WS7) with 26-yr-old forest used as a control.

In Maine, the $(NH_4)_2SO_4$ treatments were applied in six bimonthly increments to the 10.2-ha West Bear Brook watershed beginning in November 1989 (Norton et al., 1994; Kahl

Table 1. Experimental watersheds, treatments, and species sampled.

Name	Location	Area	$(NH_4)_2SO_4$ treatments: Begin-end dates	Amount	Timing	Tree species sampled	Date sampled	Reference
		ha		kg ha^{-1} yr^{-1}				
Clover Run, WV	39°9′ N 79°49′ W	55	April 1987–July 1994	167	3 applications/year	Japanese larch (*Larix leptolepis* Sieb. and Zucc.)	August 1994	Kochenderfer et al., 1995
Fernow WS3, WV	39°3′ N 79°41′ W	34	January 1989–present	167	3 applications/year	Red maple (*Acer rubrum* L.) Yellow-poplar (*Liriodendron tulipifera* L.) Black cherry (*Prunus serotina* Ehrh.)	July 1996	Adams et al., 1997
Bear Brook, ME	44°52′ N 68°6′ W	West 10.2 East 10.9	November 1989–present	125	6 bimonthly applications	Red spruce (*Picea rubens* Sarg.) Sugar maple (*Acer saccharum* L.) Yellow birch (*Betula alleghaniensis* Britton) American beech (*Fagus grandifolia* Ehrh.)	August 1994	Norton et al., 1994

et al., 1993). The adjacent 10.9-ha East Bear Brook watershed served as a control (Table 1). Spodosols at Bear Brook (Dixfield, Rawsonville, and Tunbridge types) are developed on 0 to 5 m thick glacial till overlying intermediate grade metapelites and some intruded granite near the summit. Trees on the Bear Brook watersheds were 40- to 60-yr-old mixed northern hardwoods with some conifers up to 80 to 100 yr old. Average annual air temperature at Bear Brook is 5°C and mean annual precipitation (25% snow) is 140 cm (Norton et al., 1994). Total relief on the Bear Brook watersheds is about 210 m. Representative trees of four species, red spruce, yellow birch (*Betula alleghaniensis* Britton), sugar maple (*Acer saccharinum* L.), and American beech (*Fagus grandifolia* Ehrh.) were sampled on East and West Bear Brook basins in August 1994. Sample trees were located from top to bottom of these watersheds, except for red spruce which were largely confined to the upper slopes.

Available soil chemical data for the three experimental watershed sites are given in Table 2. Data at Clover Run (Pickens, 1995) and Fernow (Tepp, 1995) taken 6 yr after treatments began show significant reductions in soil pH and exchangable Ca concentrations on treated plots in the soil A horizons on treated areas relative to control areas. No data comparing soils on treated and control basins were available for Bear Brook. Varying soil chemical extraction methods employed in these studies prevented direct comparisons of soil chemical properties among the three experimental sites.

Field Sampling and Laboratory Procedures

At all three field sites, wood cores were collected from each of five trees of each species in treated and control areas. Four cores, each 4 mm in diameter, were collected to the pith at breast height with Teflon-coated increment borers in quadrants around the circumference of the tree. Trees sampled were randomly selected from among dominants and codominants in each stand. Trees with major crown or bole damage or disease were avoided and extracted cores with obvious discoloration due to disease were discarded. Clean rubber gloves were worn when handling cores and increment borers were rinsed with deionized water after each extraction. Wood cores were rinsed lightly with deionized water after collection to remove bark particles and stored in plastic drinking straws on ice in a cooler in the field and later in a freezer.

In the laboratory, cores from each tree were dried, divided into year-class segments under low-level magnification and composited by segment and tree for chemical analysis. Tree-ring widths from cores within and among trees were used to cross date cores at each site to ensure that accurate year-class separations were conducted. Heartwood formation was not well defined in the relatively young trees at Clover Run and Fernow, and the position of the heartwood/sapwood boundary could not be reliably detected visually using dried cores from Bear Brook. The number of growth rings used in each segment to obtain the minimum mass of wood for chemical analysis varied inversely with growth rates of trees. Segment widths used were 1 to 4 yr for fast-growing larch at Clover Run, 4 to 5 yr for older, slower growing trees at Fernow and 5 to 7 yr for even older trees at Bear Brook.

Composited wood samples were analyzed for Ca, Mg, Mn, and Al concentrations at the Agricultural Analytical Services Laboratory, College of Agricultural Sciences, Penn State University using ICP emission spectroscopy (Dahlquist and Knoll, 1978). Coefficients of variation [(SD/mean) × 100] for repeated analysis of a replicate black cherry sample (n = 9) submitted with each batch of samples were 3.1, 1.3, and 7.6% for Ca, Mg, and Mn, respectively. Aluminum concentrations were too low for reliable detection by ICP, except in Japanese larch and yellow poplar. Comparisons of mean concentrations of elements in xylem between treatment and control areas at each site for each specific year-class were made using simple t-tests. A significance level of α = 0.05 is used in the paper unless otherwise stated.

RESULTS AND DISCUSSION

Normalization of tree-ring concentration data, by dividing concentrations in treated trees by concentrations in control trees by year segment, was used to examine responses of several species on the same time scale. The treated/control (T/C) concentration ratios would be expected to be less than unity or decline below unity in response to treatment due to depletion, while T/C ratios would be expected to exceed or gradually increase above unity due to their enhanced availability caused by soil acidification or mobilization.

Clover Run

Japanese larch at Clover Run in WV (Fig. 1) showed varying relative concentrations between treated and control plots for Ca and Mg, with T/C > 1, or enrichment, on treated plots up to 1990 and T/C < 1, or depletion, on the treated plots beginning in 1991. Although differences between treatment and control plots were significant only for 1996, trends in concentrations

Table 2. Soil chemical characteristics at three experimental watershed acidification experiments.

Site	Horizon	Depth	pH	Exchangeable cations: Ca	Mg	Mn	Al	Source
		cm		cmol kg^{-1}				
Clover Run, WV								
Control plots	A	2–10	4.4a†	0.62a	0.07a	0.03a	0.02a	Pickens,‡ 1995
Treated plots	A	2–10	3.9b	0.32b	0.03b	<0.01b	0.12b	
Fernow, WV								
Control basin WS7	A	0–6	4.7a	1.29a	0.28a	0.63a	0.63a	Tepp,§ 1995
Treated basin WS3	A	0–7	4.3b	0.54b	0.20a	0.52a	0.69a	
Control basin WS7	B	6–19	4.8a	0.60a	0.21a	0.18a	0.83a	
Treated basin WS3	B	8–22	4.6a	0.28a	0.15a	0.13a	0.83a	
Bear Brook (West)	Bhs	–	4.1	0.47	0.08	–	8.7	Norton et al.,¶ 1994
Tunbridge series	Bs	–	4.4	1.16	0.13	–	5.2	
	Bw	–	4.6	0.47	0.04	–	2.4	
	C	–	4.7	0.31	0.02	–	1.2	

† Means followed by different letter are significantly different between treatment and control at α = 0.05.
‡ Sampled in 1994, 6 yr after treatments began, 0.01 M $SrCl_2$ extraction for cations, pH 1:1 soil/water solution.
§ Sampled in 1995, 6 yr after treatments began, Mehlich three method for Ca and Mg, Mn and Al by ASTM D5435-93, pH 1:1 water/soil solution.
¶ Sampled prior to treatment, Tunbridge represents 31% of soil mapped area on Bear Brook West.

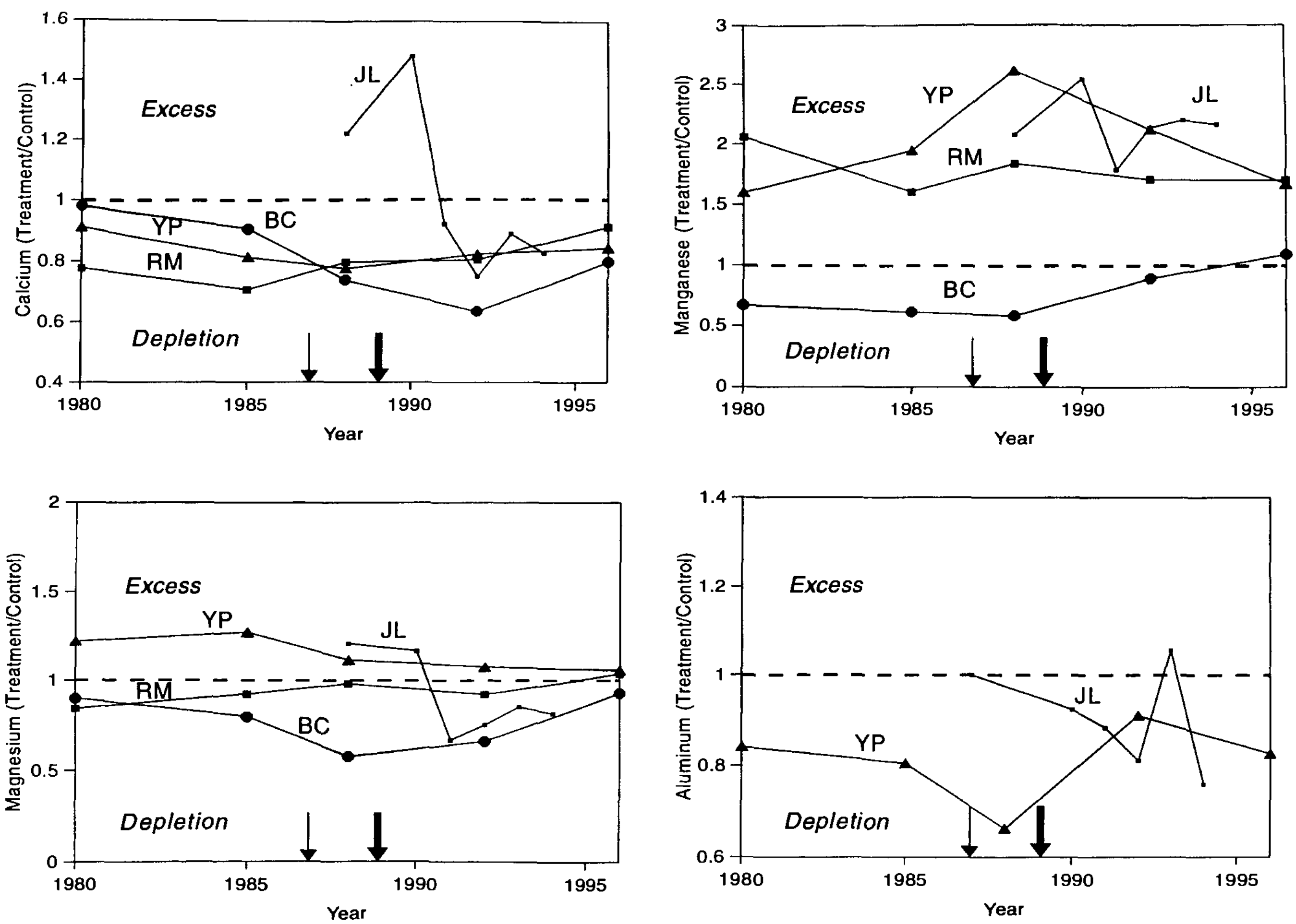

Fig. 1. Mean ratios of treatment to control concentrations for Ca, Mg, Mn, and Al in tree rings formed during 1980 to 1996 at Clover Run and Fernow WS3 sites in West Virginia. Species: Clover Run JL = Japanese larch; Fernow WS3 BC = black cherry, YP = yellow-poplar, and RM = red maple. Treatments began in 1987 at Clover Run (arrow) and 1989 at Fernow WS3 (bold arrow).

over time suggested that Ca and Mg were mobilized in larch sapwood rings in the first 5 yr of treatment and were depleted thereafter until the time of sampling after 8 yr of treatment. Manganese concentrations were greater in rings of treated than control larch trees (i.e., T/C > 1) for all years and were significantly different for 1992, 1993, and 1994. Treatment/control ratios for Al were generally less than unity, (i.e., Al was higher in control trees) and differences between treatments and controls were not significant.

Both Ca/Mn and Mg/Mn ratios in Japanese larch were significantly lower in treated plots compared to controls in all age segments (Fig. 2). The Ca/Mn and Mg/Mn ratios became increasingly different between treatment and control trees with time as Mn continued to increase and Ca and Mg were gradually depleted. In 1994, Ca/Mn and Mg/Mn ratios in control trees exceeded those in treated trees by 200 to 300%. In contrast, Ca/Al ratios were not significantly different between treatment and control plots in any age segment in Japanese larch (Fig. 2). Lack of signficant differences in Ca/Al ratios in Japanese larch between treatment and control was caused by lower Al concentrations that offset Ca reductions in rings of treated trees (see Fig. 1).

The Ca/Mn and Mg/Mn ratios did not preserve a pattern suggesting mobilization followed by depletion as did Ca or Mg concentrations alone. Manganese concentrations were increased during mobilization along with Ca and Mg during the first 5 yr of treatment (Fig. 1) and then remained at elevated levels due to soil acidification during the onset of Ca and Mg depletion. Thus, Mg/Mn and Ca/Mn ratios in larch tree rings were not useful in detecting the transition from mobilization to depletion.

Tree-ring chemistry trends in this study appear generally consistent with evidence from other studies at Clover Run. Support for early mobilization of cations is given by DeWalle et al. (1995) who reported significantly increased Ca concentrations in wood formed over the first 4 yr of treatment in mature black cherry trees growing in the treated riparian zone compared to a nearby control area on Clover Run. Pickens et al. (1995), working on Clover Run 6 to 7 yr after treatments began, found reduced larch foliage concentrations of Mg and increased foliage Mn and Al levels in treated areas compared to controls. Pickens et al. (1995), using Sr Cl_2 extraction, also found reduced soil exchangable concentrations of Ca, Mg, and Mn and increased exchangable concentrations of Al in the A horizons after 9 yr of treatment (Table 2). Kochenderfer et al. (1995) also

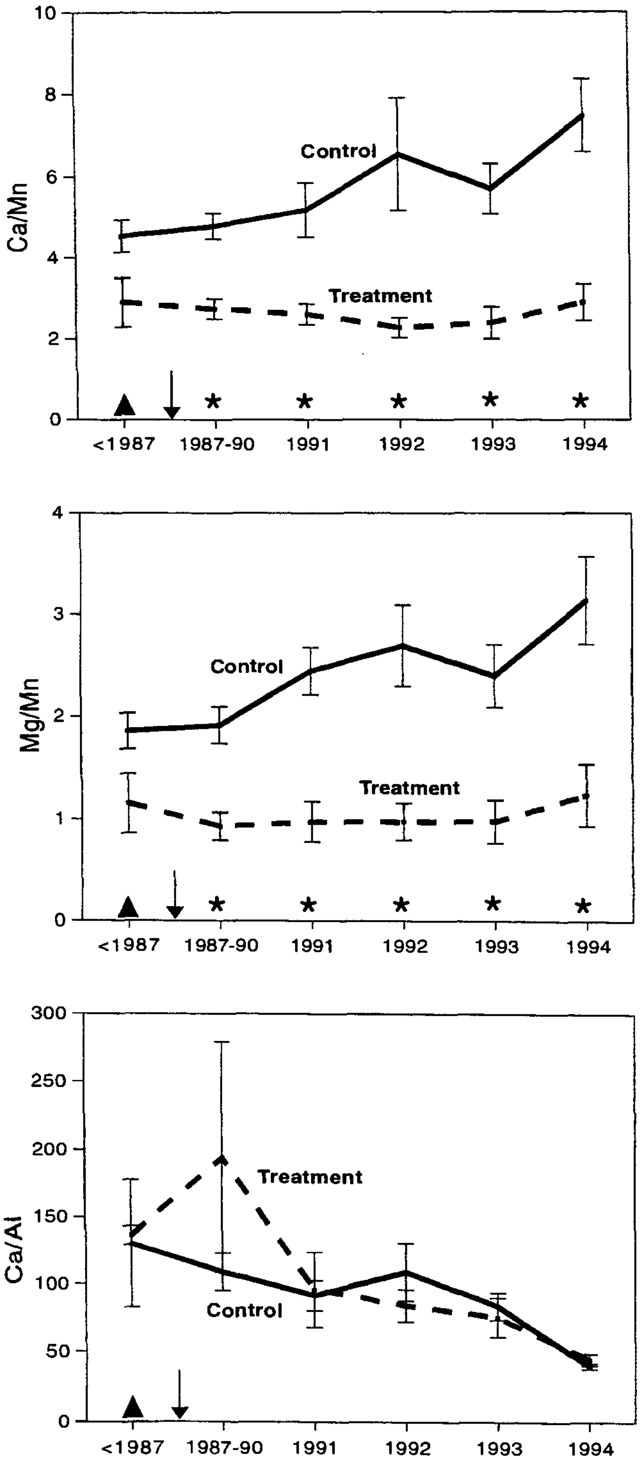

Fig. 2. Mean molar ratios of Ca/Mn, Mg/Mn, and Ca/Al concentrations (± SE) in Japanese larch tree rings on treated and control areas at Clover Run, WV. An arrow indicates start of treatments in 1987 (↓) and an asterisk or triangle indicates a significant difference between treatment and control means for that time interval at the α = 0.05 or α = 0.1 levels, respectively.

reported elevated larch foliar Mn concentrations on treated plots, but no Ca, Mg, or Al foliar concentration differences between treatments and controls. However, Kochenderfer et al. (1995) did not find exchangable soil cation depletion after 7 yr of treatment on Clover Run.

Fernow

Dendrochemical response to treatment in three tree species at Fernow was generally similar to that at Clover Run. Fernow species showed roughly 20% Ca depletion (Fig. 1), in rings on the treated watershed. Calcium concentration differences between treatment and control tree-rings were signficant in yellow-poplar for 1981 to 1985, 1986 to 1988, and 1993 to 1996 segments; in red maple for 1976 to 1980, 1981 to 1985, 1986 to 1988, and 1989 to 1992 segments; and in black cherry for 1986 to 1988, 1989 to 1992, and 1993 to 1996 segments. Although Mg differences at Fernow were not as great as at Clover Run, black cherry and red maple generally did show lower Mg concentrations in rings of treated trees. Magnesium differences were significant only for the 1986 to 1988 segment in black cherry. Even though yellow-poplar tree rings showed Ca depletion, Mg concentrations always were higher in rings of treated trees and appeared to be only gradually declining on treatment relative to control.

Unlike Japanese larch at Clover Run, tree species at Fernow did not show a pattern of Ca or Mg concentrations that suggested mobilization followed by depletion. Depletion of Ca and Mg were reflected in the oldest wood sampled (1976–1980 segments), which showed that current conditions were reflected in wood formed 9+ yr before treatment began.

Tree-ring chemistry results generally agreed with the response to $(NH_4)_2SO_4$ treatment reported by others working at Fernow. After 3 yr of treatment at Fernow WS3, Adams et al. (1993) reported increased export of NO_3 and Ca in streamflow and after 5 yr of $(NH_4)_2SO_4$ treatments Adams et al. (1997) reported continuing increases in concentrations of Ca, Mg, and NO_3 in stream and soil water. Gilliam et al. (1996) also showed that foliar Ca and Mg concentrations, forest floor concentrations of Ca, and soil pH were reduced on Fernow WS3 after 3 yr of treatment. However, Adams and Angradi (1996) in a study of litter decomposition rates, reported no difference in soil exchangable Ca and Mg concentrations (0–15 cm depth) between treated basin WS3 and control basin WS7 after 3 yr of treatment. Overall, results of other studies generally support the occurrence of base cation depletion found in tree rings on Fernow WS3 relative to nearby untreated areas.

Manganese concentrations at Fernow were higher in treated tree rings for both yellow-poplar and red maple; differences between treated and control were signficant for all age segments in red maple and 1986 to 1988, 1989 to 1992, 1993 to 1996 segments for yellow-poplar. In black cherry, Mn T/C ratios were initially <1, but rose to >1 by 1996. Aluminum concentrations always were lower in rings of treated than control yellow-poplar trees, but never significantly so.

Red maple also showed Ca/Mn and Mg/Mn ratios that were signficantly lower in treatment compared to control tree rings in all age segments (Fig. 3). Tree-ring Ca/Mn and Mg/Mn ratios were also lower in treated yellow-poplar trees at Fernow at all times, but the ratio differences were not always significant. Black cherry at

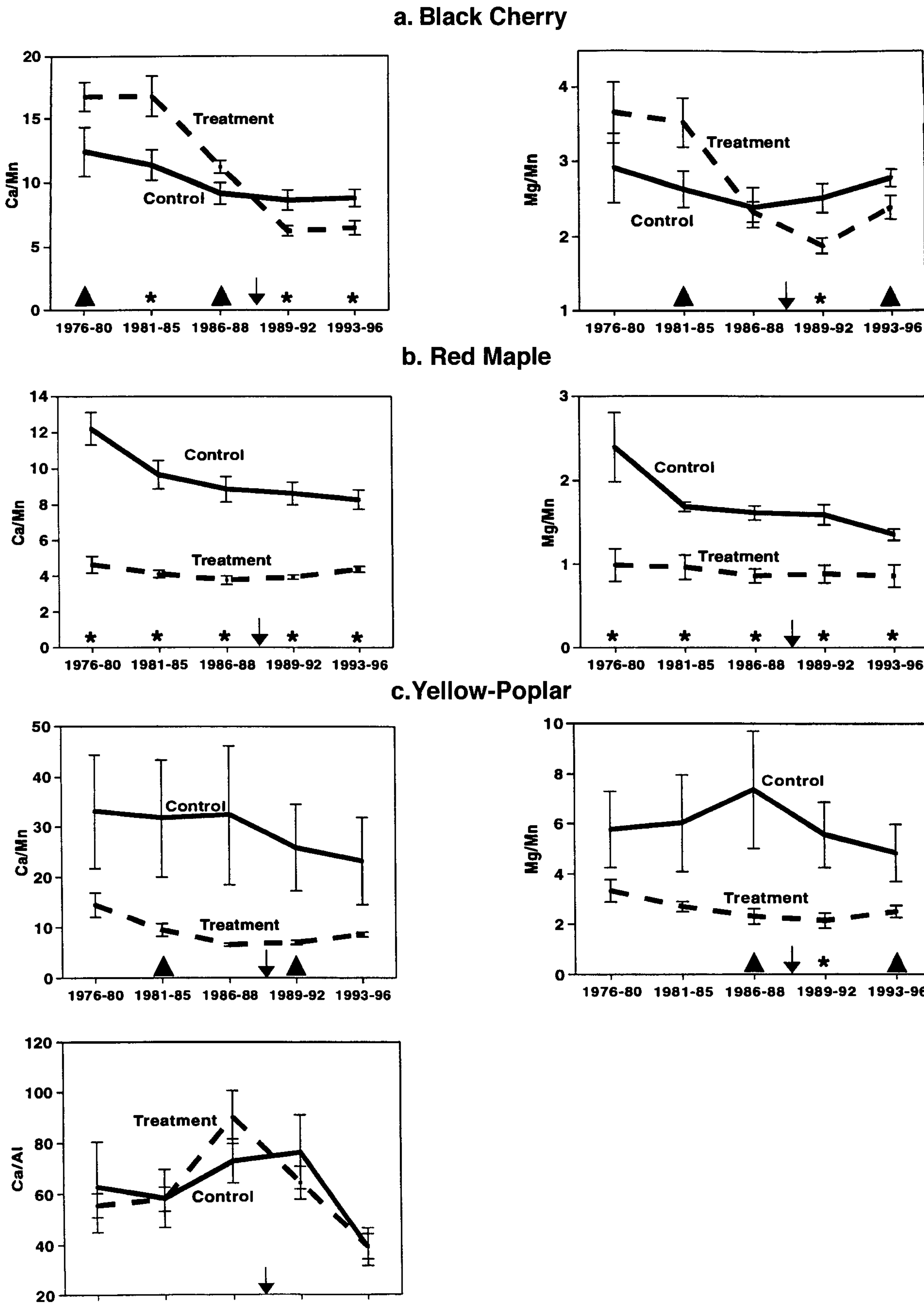

Fig. 3. Mean molar ratios of Ca/Mn, Mg/Mn, and Ca/Al concentrations (± SE) in tree rings on treated and control areas at Fernow, WV. Al concentrations were only above detection limits for yellow-poplar. An arrow indicates start of treatments in 1989 (↓) and an asterisk or triangle indicates a significant difference between treatment and control means for that time interval at the $\alpha = 0.05$ or $\alpha = 0.1$ levels, respectively.

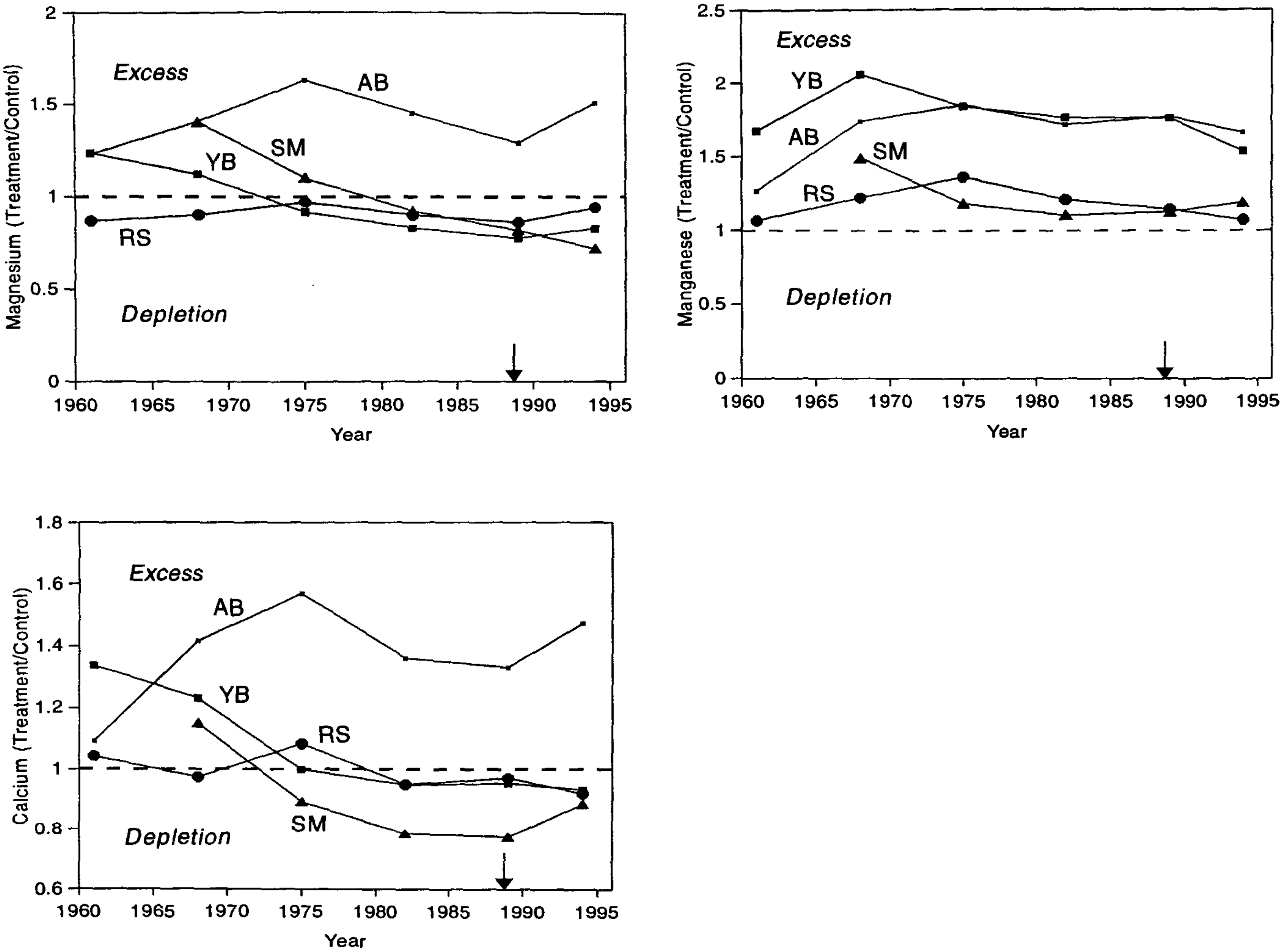

Fig. 4. Mean ratios of treatment to control concentrations for Ca, Mg, and Mn in tree rings formed during 1960 to 1995 at Bear Brook, ME. Treatments began in 1989 (↓). Species: AB = American beech, RS = red spruce, SM = sugar maple, YB = yellow birch.

Fernow showed Ca/Mn and Mg/Mn ratios that generally were significantly higher in treatment trees for wood formed before about 1988 to 1989, but generally were signficantly lower for wood formed after 1988 to 1989 (Fig. 3). By 1993 to 1996, Ca/Mn and Mg/Mn ratios at Fernow ranged from 14 to 80% lower in treated trees compared to control trees, with the greatest differences appearing in yellow-poplar. The significant differences in Ca/Mn and Mg/Mn ratios between treated and control areas for species at Fernow noted in Fig. 3 were primarily related to higher Mn concentrations in treated areas for black cherry and both higher Mn and lower Ca concentrations for yellow-poplar and red maple (see Fig. 1).

Black cherry Ca/Mn and Mg/Mn ratios showed a unique cross-over pattern at Fernow (Fig. 3) suggesting a switch from Ca and Mg mobilization to depletion between the 1986 to 1988 and 1989 to 1992 segments. However, the switch began in the 1986 to1988 tree-ring segment formed just prior to treatment initiation in 1989 suggesting that sapwood formed prior to treatment was being affected.

Comparisons with earlier tree-ring chemistry data (Tepp, 1995; DeWalle et al., 1995) at Fernow also suggest that a base cation mobilization and depletion stage has occurred in response to treatment. In 1992 after 4 yr of treatment, Tepp (1995) and DeWalle et al. (1995) reported significantly higher concentrations of Ca and Mg in tree rings from black cherry and yellow-poplar, but not red maple, on the treated watershed (WS3) relative to a control watershed (WS7). These results contrast with results of this study where in 1996 after 8 yr of treatment we found significantly lower concentrations of Ca and Mg in tree rings of all three species on WS3 compared to a control area. If mobilization of base cations occurred initially, it appears that red maple may have begun to show cation depletion effects even by 1992 or after 3 yr of treatment. Unfortunately, none of the species sampled at Fernow appeared to preserve an accurate record of the change from mobilization to depletion.

Bear Brook

In all species at Bear Brook, except American beech, Ca and Mg T/C ratios declined below 1 indicating depletion of about 10 to 30% in wood formed after about 1990 (Fig. 4). The Ca and Mg differences between treat-

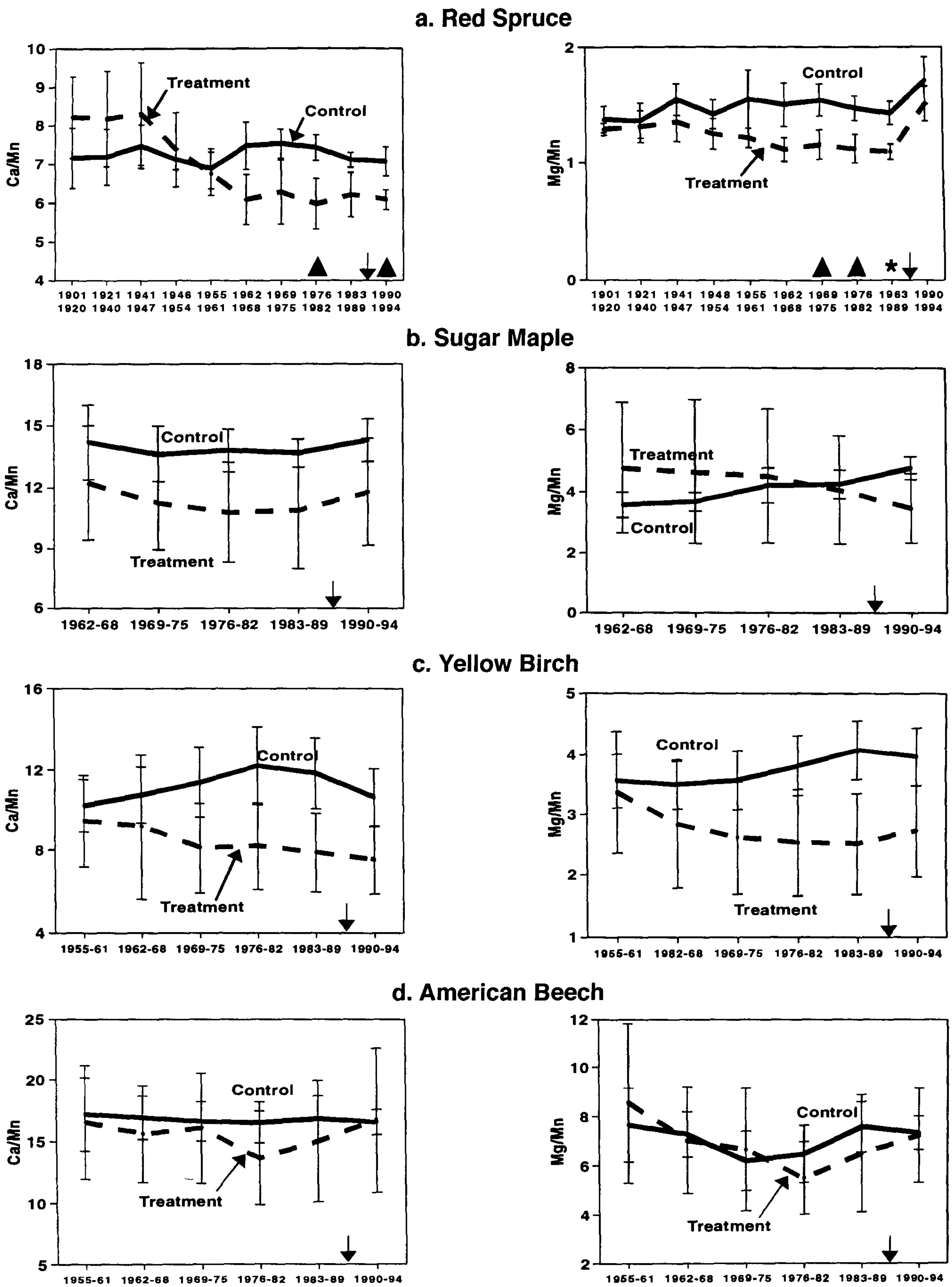

Fig. 5. Mean molar ratios of Ca/Mn and Mg/Mn concentrations (± SE) in tree rings for four tree species on treated and control areas at Bear Brook, ME. An arrow indicates start of treatments in 1989 (↓) and an asterisk or triangle indicates a significant difference between treatment and control means for that time interval at the $\alpha = 0.05$ or $\alpha = 0.1$ levels, respectively.

ment and control tree rings generally were not significant at Bear Brook, although for sugar maple, Ca concentrations were signficantly lower in treated trees for the latter 3-yr segments and for Mg for the last year segment. Anomalous response was found in American beech tree rings, where Ca and Mg concentrations were enriched rather than depleted in all age segments sampled, with enrichment reaching up to 50%. Differences between treatment and control concentrations in beech for Ca were significant for 1969 to 1975 and for Mg were significant for 1969 to 1975 and 1990 to 1994.

Manganese T/C ratios were uniformly above unity for all species and all age segments at Bear Brook (Fig. 4). Manganese concentration differences between treatment and control areas were large, about 20 to 80% greater on the treated watershed. Manganese differences between treatment and control were signficant for red spruce for the 1976 to 1982 and 1969 to 1975 age segments and for American beech for all segments from 1969 to 1994. Aluminum concentrations were too low in species sampled at Bear Brook to permit comparisons between treatment and control watersheds.

Kahl et al. (1993) reported that stream NO_3 increases in response to $(NH_4)_2SO_4$ treatment occurred rapidly at Bear Brook, and Norton et al. (1994) reported that stream export of Ca, Mg, and Al significantly increased after treatment. Depletion of Ca and Mg in tree rings of red spruce, sugar maple, and yellow birch found in this study are consistent with such changes in stream chemistry.

At Bear Brook, tree-ring element ratio patterns were similar to those found in West Virginia, with lower Ca/Mn and Mg/Mn ratios generally occurring in rings of treated trees compared to control trees (Fig. 5). Cation ratio differences between treated and control watersheds at Bear Brook were smaller than in West Virginia and commonly were not significantly different. Signficantly lower Ca/Mn and Mg/Mn ratios in treatment relative to control trees were found in red spruce for wood formed after about 1975 (Fig. 5). The Ca/Mn and Mg/Mn ratios in control and treatment tree rings in yellow birch were not significantly different, but xylem in treated trees always exhibited lower ratio values (Fig. 5). Sugar maple at Bear Brook showed a crossover pattern of Mg/Mn ratios from higher to lower ratios in treated trees relative to control trees over time, similar to that in black cherry at Fernow (Fig. 3), but with no significant differences. The Ca/Mn ratios were always lower in treated sugar maple trees, but again not significantly different. American beech showed erratic differences in Ca/Mn and Mg/Mn ratios between treated and control trees, with treated trees generally showing lower ratios.

The cross-over pattern with time from higher to lower Ca/Mn ratios for red spruce and Mg/Mn ratios for sugar maple (Fig. 5) also suggests a staged response to treatment from mobilization to depletion, but the time of cross-over occurred in rings formed 10 to 30 yr prior to treatment initiation. As part of an acid irrigation plot study conducted adjacent to the Bear Brook watersheds, Rustad et al. (1996) reported a relatively rapid transition from base cation mobilization to depletion in the soil within the first 4 yr of treatment. It is probable that a mobilization phase did occur due to $(NH_4)_2SO_4$ treatments at Bear Brook, but both red spruce and sugar maple did not appear to accurately record the timing of such soil changes in this study.

The relative lack of statistical significance of differences in dendrochemistry between treatment and control at Bear Brook compared to Fernow and Clover Run are not known, but may be due the residual effects of base cation mobilization. Sampling at Bear Brook was done after only 5 yr of treatment, while sampling at both Fernow and Clover Run occurred after 8 yr of treatment. Thus, the transition from base cation mobilization to depletion may still have been occurring at Bear Brook at the time of sampling.

Implications for Dendrochemical Research

Results largely suggest that much of the wood existing prior to treatment was affected by treatment and that the timing of chemical changes in rings cannot be used for dating. Regardless, several of the species studied show promise of usefulness in dendrochemistry. Japanese larch showed trends in tree-ring chemistry that suggested Ca and Mg concentrations can be used to track mobilization followed by depletion in the years after the initiation of treatment. Cutter and Guyette (1993) have recommended *Larix* spp. for use in dendrochemistry research based on wood permeability and other factors. Black cherry also showed some promise that Ca/Mn and Mg/Mn ratios may be useful in establishing a chronology of change. Red maple, yellow-poplar, yellow birch, red spruce, sugar maple, and American beech all showed that chemical changes occurred in wood formed up to about 20+ yr prior to treatment initiation and did not appear useful in dating times of soil chemical changes. These latter species make it very difficult to detect changes like sequential cation mobilization and depletion caused by watershed acidfication, since cation content of the entire sap conducting region can shift from higher concentrations during mobilization to lower concentrations during depletion in as little as a 4-yr period. American beech also exhibited the tendency to undergo a longer period and red maple a shorter period of cation mobilization than other species at their respective sites, for unknown reasons.

Use of Ca/Mn or Mg/Mn ratios in tree rings to detect overall effects of $(NH_4)_2SO_4$ treatments produced significant differences much more frequently than use of Ca, Mg, or Mn concentrations alone, and where comparisons could be made, Ca/Mn and Mg/Mn ratios were superior to Ca/Al ratios. Higher frequencies of significant effects for Ca/Mn and Mg/Mn ratios occurred because the opposing effects of Ca and Mg decreases and Mn increases in tree rings due to acidification (Guyette et al., 1992) make the ratios more sensitive to soil changes than concentrations alone. Concentrations of Ca, Mg, and Mn are still of interest when considering the processes of cation mobilization and depletion, since at least for Japanese larch the Ca/Mn and Mg/Mn ratios remained relatively constant and did not indicate a marked shift as soil Ca and Mg mobilization ended and depletion began.

Calcium/Manganese and Mg/Mn ratios in tree rings were preferable to using Ca/Al for Japanese larch and yellow-poplar, the only two species with detectable levels of Al in this study. $(NH_4)_2SO_4$ treatment did not increase tree-ring Al concentrations and in fact appeared to reduce Al concentrations slightly. Cronan and Grigal (1995) also have pointed out the computational difficulty of using a tree-ring cation ratio, such as Ca/Al, where the concentration of Al is much lower than the concentration of Ca. Manganese levels in tree rings exceeded Al levels by an order of magnitude and using Ca/Mn or Mg/Mn ratios caused no computational problems.

In dendrochemical studies, small adjacent treatment and control plots such as those at Clover Run were clearly preferable as an experimental design than sampling across entire watersheds as at Bear Brook or Fernow. The ideal design for dendrochemical studies probably would be random fertilization of soil around single-trees within the same stand with adequate buffer zones around treated and control trees.

Even in species such as Japanese larch, we currently are limited to detecting change by comparing treatment to control areas. Until natural variations in xylem chemistry are better understood, the full potential for dendrochemistry to enable detection of environmental change and stress even in selected tree species cannot be achieved.

CONCLUSIONS

Experimental watershed acidification or N saturation using applications of $(NH_4)_2SO_4$ equivalent to twice the normal annual wet plus dry N and S deposition caused base cation mobilization followed by depletion that was detectable in sapwood xylem after about 8 yr of treatment. Sapwood tree-ring Ca, Mg, and Mn concentrations and Ca/Mn and Mg/Mn molar ratios were sensitive indicators of the response of trees to experiemental treatments. Timing of tree-ring chemical mobilization and depletion response of individual tree species to treatments appeared to vary among species at a site. Aluminum concentrations in tree rings were not increased by experimental soil acidification and Al concentrations were at or below detection levels in most species, thus Ca/Al molar ratios were not useful in detecting treatment effects. Periodic sampling of tree-ring chemistry is recommended to help assess current response of trees to soil acidification. However, with the exception of Japanese larch and possibly black cherry, sapwood tree rings did not preserve a reliable chemical chronology of past soil changes caused by experimental acidification treatments.

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