THE EFFECTS OF $AlCl_3$ ADDITIONS ON RHIZOSPHERE SOIL AND FINE ROOT CHEMISTRY OF SUGAR MAPLE (*ACER SACCHARUM*)

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Abstract. Increased Al mobilization and Ca and Mg leaching have been linked to nutritional imbalances in sugar maple across the northeastern US and Canada. The susceptibility of sugar maple fine roots to Al stress is poorly understood, in part because roots respond to Al stress by altering the chemistry of the rhizosphere. $AlCl_3$ was applied to plots of sugar maple at the Hubbard Brook Experimental Forest, NH. After two years of treatment, we sampled fine roots of sugar maple, rhizosphere soil, and bulk soil in the Oa horizon and the upper 10 cm of the mineral soil. $AlCl_3$ treatments resulted in significantly less Ca (21%) and Mg (30%) in fine roots from the organic horizon, but had no significant effect on fine root Al. Fine root (Ca+Mg):Al ratios were 42% lower in $AlCl_3$ plots than in controls, though most roots had ratios above critical toxicity thresholds developed for hydroponically grown sugar maple seedlings. In the mineral horizon, roots differed only in Mg concentration, which was 22% lower in $AlCl_3$ plots. In the $AlCl_3$ treated plots, rhizosphere soil in the organic horizon had 47% greater Al and 29% less Mg than in controls. Combining data from both treatments we found significantly less Al and organically bound Al in rhizosphere soil than in bulk soil, possibly due to leaching of Al from the rhizosphere by organic acids released by roots. These results suggest that increased mobilization of Al in soil lowers (Ca+Mg):Al ratios in sugar maple fine roots, though roots may minimize Al stress by leaching Al from the rhizosphere.

Keywords: rhizosphere, aluminum, calcium, fine roots, Ca:Al ratios

1. Introduction

Strong acid inputs to northern hardwood forests increase the mobilization of phytotoxic Al and accelerate the leaching of essential plant nutrients such as Ca and Mg from the rooting zone (Tomlinson, 1990; Lawrence *et al*., 1995). Such changes in soil chemistry have been linked to nutritional imbalances in sugar maple (*Acer saccharum* Marsh.) resulting in decreased growth and increased mortality across the northeastern United States and Canada (Bernier and Brazeau, 1988; Kolb and McCormick, 1993; Horsley *et al*., 2000). For example, declining sugar maple stands have been positively correlated with low pH, high Al and low base cation concentrations in soil (Ouimet and Camire, 1995; Wilmot *et al*., 1995). Fertilizing declining stands with dolomitic lime (i.e. Ca and Mg) has also been shown to improve sugar maple health and vigor (Hendershot, 1991; Cote *et al*., 1995; Wilmot *et al*., 1996; Long *et al*., 1997). Moreover, sugar maple seedling biomass and Ca concentrations

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in foliage and roots decreased markedly in response to elevated Al in a hydroponic system (Thornton *et al*., 1986).

Fine roots (<1 mm diameter) are especially susceptible to Al stress in acidic forest soils (Joslin and Wolfe, 1989; Smith *et al*., 1995). High concentrations of Al in roots may directly impair cellular processes by binding to sensitive biomolecules (e.g., ATP, calmodulin) and cause decreased fine root biomass, fine root branching and terminal elongation (Cronan *et al*., 1989; Tomlinson, 1990). In general, however, Al transport is mostly inhibited at the root endodermis where it accumulates in the apoplast (Schlegal *et al*., 1992; Van Praag *et al*., 1997; Heim *et al*., 2000). Apoplastic Al indirectly impairs plant nutrition because Al is tightly adsorbed and thus interferes with Ca and Mg uptake (Cronan, 1991). Because root chemistry is believed to be the most sensitive indicator of Al stress (Cronan and Grigal, 1995), critical toxicity thresholds have been developed for roots of many species using hydroponically grown seedlings. Thornton *et al*. (1986) found that sugar maple seedling growth was adversely affected when Al concentrations in roots exceeded 0.27% and Ca concentrations were below 0.15%. Because of interactions between Al, Ca and Mg in the apoplast, Ca:Al and (Ca+Mg):Al ratios in fine roots have also been proposed as indicators of Al stress in mature trees (Hogberg and Jenson, 1994; Cronan and Grigal, 1995). Few studies have measured these ratios in sugar maple fine roots despite the fact that low Ca:Al and (Ca+Mg):Al ratios are well correlated with decline symptoms in Canadian forests (Adams and Hutchinson, 1992).

Recently a number of investigators have emphasized the importance of considering rhizosphere processes in assessing fine root responses to Al stress in forest soils (Smith and Pooley, 1989; Gobran and Clegg, 1996; Dieffenbach and Matzner, 2000; De Wit *et al*., 2001). In general, fine roots alter rhizosphere chemistry through ion uptake and exclusion, the release of organic and inorganic substrates, and enhancing the activity of soil microorganisms (Curl and Truelove, 1986; Smith, 1990; Marschner, 1995). Several studies found that strong acidic inputs affected the rhizosphere soil differently than bulk soil. Ruark *et al*. (1989) found that simulated acid precipitation treatments had a greater effect on rhizosphere pH than bulk soil pH. Other researchers found that ammonium sulfate additions had a pronounced effect on rhizosphere Ca and Mg (Clegg *et al*., 1997) and Al (Majdi and Bergholm, 1995), but little effect on these ions in bulk soil.

Other studies have found large differences between rhizosphere and bulk soil chemistry (Table I), which may have implications for Al stress in trees. Fine roots influence rhizosphere pH by releasing H^+ or HCO_3^- depending on the relative uptake of cations versus anions (Nye, 1981; Haynes, 1990). In species like sugar maple that take up more NH_4^+ than NO_3^- (Rothstein *et al*., 1996), fine roots release H^+, which may increase Al solubility and decrease Ca and Mg availability in the rhizosphere (Smith and Pooley, 1989). Alternatively, increased H^+ in rhizosphere soil may ameliorate Al stress if H^+displaces Al on rhizosphere exchange sites, causing Al to leach below the rooting zone (Hogberg and Jensen, 1994; Marschner,

TABLE I
Comparison of rhizosphere and bulk soil chemistry around tree roots in several studies

Tree species (age)	Method for sampling the rhizosphere	Rhizosphere variables					Citation
		OM	Al	Ca	Mg	pH	
Red spruce (85 yrs)[a]	x-ray emission		−	ns			Smith and Pooley (1989)
Sessile oak (<1 year)[b]	adhering soil		+	ns	ns	ns	Bakker *et al*. (1999)
Douglas fir (<2 years)	adhering soil		+	+	+	−	Wang and Zabowski (1998)
Norway spruce (30 yrs)[c]	adhering soil	+	ns	+	+	ns	Gobran and Clegg (1996)
English oak (<1 year)	microlysimitry	+	+	−	−	ns	Gottlein *et al*. (1999)
Norway spruce (<1 year)	microlysimitry	ns	+	−	−	−	Wang *et al*. (2001)
European beech (<1 year)	microlysimitry	ns	+	−	−	−	Wang *et al.* (2001)
Norway spruce (145 yrs)	microlysimitry						Dieffenbach and Matzner (2000)
Mycorrhizal roots			+	ns	+	+	
Non-mycorrhizal roots			−	ns	ns	−	

[a]averaged over 0–1 mm distance from root.
[b]in A horizon only.
[c]in E horizon only; Ca and Mg inferred from base saturation.
Note. For each variable, letters and symbols represent rhizosphere values greater than (+), less than (−), or not significantly different from (ns) those in bulk soil. Blank spaces denote variables not measured.

1995). Rhizosphere acidification may also influence Al, Ca and Mg solubility by accelerating mineral weathering in the rhizosphere (April and Keller, 1990).

Fine roots protect trees from Al stress by releasing organic chelators (low molecular weight organic acids) to the rhizosphere (Jones, 1998). Complexation of Al results in the formation of organically bound Al (Al_o), which is less toxic to roots (Smith, 1990; Tomlinson, 1990) and more susceptible to leaching below the rooting zone (Driscoll *et al*., 1985; Stevenson and Vance, 1996). In Al-tolerant species, fine roots increase exudation in response to Al accumulation in the apoplast (Kochian, 1995; Jones and Kochian, 1996). Although sugar maple is considered moderately tolerant to Al stress relative to other northern hardwood tree species (Cronan *et al*., 1989), little is known about the influence of sugar maple roots on Al chemistry in the rhizosphere. Sugar maple roots primarily exude citric acid (Smith, 1976), which forms strong complexes with Al and has been shown to limit Al uptake in other tree species (Hue *et al*., 1986; Smith, 1990; Jones and Kochian, 1996). Increases in organically bound Al (Al_o) in the rhizosphere may reflect changes in organic acid exudation by fine roots under Al stress (Dieffenbach and Matzner, 2000).

At present, our understanding of sugar maple fine root response to Al stress is limited by our reliance on short-term studies of non-mycorrhizal seedlings grown hydroponically (Cronan *et al*., 1989; Hutchinson *et al*., 1999). In this study our objectives were to determine the response of fine roots of mature, *in situ* sugar

maple trees to experimental additions of Al by quantifying chemical changes in fine roots and adjacent rhizosphere soil.

2. Materials and Methods

2.1. Site description

This study was conducted in the upper reaches of the Falls Brook drainage at the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire (43 °56′N, 71 °45′W). Soils are primarily coarse loamy, moderately well-drained Aquic and Typic Haplorthods and Aquic Haplumbrepts developed from glacial till. The cation exchange capacity is due primarily to organic matter, as the clay content of these soils is low (Johnson *et al*., 1997). The chemical properties of soils at an adjacent site have been described in detail (Driscoll *et al*., 1985; Likens *et al*., 1998; Johnson *et al*., 2000).

Eight plots were established in northern hardwoods (730 and 760 m elevation) dominated by overstory sugar maple (60–80% of basal area; 70–85% of all stems). The plots measured 45 m × 45 m. Four plots were treated with $AlCl_3$ and four plots served as controls. Aluminum was applied from early spring of 1996 to late spring of 1997 with a backpack sprayer (Berger *et al*., 2001). Over the two-year period, a total of 0.167 mol/m^2 (45 kg/ha) of Al was added which represents a chemically equivalent dose (on a charge basis) of the estimated loss of exchangeable Ca over the last 30 years due to acidic deposition (Likens *et al*., 1998). This study is part of larger one looking at the effects of changing Ca and Al concentrations in soil on sugar maple growth, mortality and nutrition.

2.2. Field methods

Due to the destructive nature of root and soil sampling, our sampling was restricted to the outer 10 m of each plot. In October of 1997, we selected one dominant or co-dominant sugar maple tree from each of the four edges within each plot. These trees were all greater than 18 cm in diameter at breast height. We excluded trees within 3 m of plot boundaries to insure that the fine roots were subjected to the treatment.

Within 2 m of each tree, we collected fine roots (<1 mm in diameter) from two soil pits on opposite sides of the tree. Fine roots were collected separately in the O+A and upper 10 cm of the mineral soil (B horizon). Rhizosphere soil was operationally defined as soil adhering to the roots after gentle shaking in the field following the method of Wollum (1994). Bulk soil (root-free soil) was defined as the soil removed from roots after shaking. We combined root and soil samples from the O+A horizon (hereafter referred to as the organic horizon) because of the absence of a pronounced Oa in some soils and the difficulty in demarcating in the

field the Oa/A boundary in others (Federer, 1982). When a pronounced E horizon was present, fine roots and soil samples from the mineral horizon were collected mostly in the B horizon due to the scarcity of fine roots in the E horizon. Root and bulk soil samples were kept in a cooler until they were brought to the lab. All root and soil sampling was completed within two weeks.

2.3. Laboratory methods

Rhizosphere soil was carefully removed from sugar maple fine roots with a shed-free paintbrush and tweezers (Kirlew and Bouldin, 1987). Sugar maple roots were identified by small bead-like contractions on distal branches visible through a dissecting microscope. Dead roots and the roots of other species were discarded.

Roots were cleaned in a water bath and sonicated for two minutes to remove adhering soil particles while minimizing the loss of ions from roots (Clemensson-Lindell and Persson, 1992). They were then oven dried at 65 °C. Oven-dry roots were ground in a Wiley Mill (20 mesh), dry oxidized at 470 °C for 12 h, digested with 6 N HCl and the extract filtered through Whatman 42 paper filters (Bickelhaupt and White, 1982). Concentrations of Al, Ca, and Mg were determined by inductively coupled plasma emission spectrophotometry (ICP-AES). Citrus leaves from the U.S. National Institute of Standards and Technology analyzed using the same procedure were within 10% of certified values for all elements.

Rhizosphere and bulk soil samples were air-dried and sieved through a 1 mm screen to minimize root contamination in rhizosphere samples (Norvell and Cary, 1992). Exchangeable Ca, Mg and Al were extracted with 1 N KCl (10:1 solution:soil), by shaking samples for 1 h, centrifuging at 4000 rpm for 20 min and filtering through a 0.2 μm polycarbonate membrane syringe filter (Bickelhaupt and White, 1982). Due to the small volume of rhizosphere soil, pH was measured in the 10:1 KCl extracts of both rhizosphere and bulk soil. Organically bound Al (Al_o) was determined by a sequential metal-extraction procedure (Miller and McFee, 1983; Driscoll *et al*., 1985). In this procedure, the residue from the KCl extraction was washed with 10 mL of distilled deionized water, and the resulting solution discarded. Next, 15 mL of 0.1 N $Na_4P_2O_7$ (15:1 solution:soil) was added, followed by shaking for 16 h and centrifuging at 4000 rpm for 20 min. The extract was then filtered through a 0.4 μm polycarbonate membrane filter in order to limit colloidal interference (Bertsch and Bloom, 1996). Elemental concentrations were determined by ICP-AES. Subsamples of all soil samples were oven dried at 85 °C and ashed to estimate percent organic matter.

2.4. Statistical analyses

To test for treatment effects on soil (rhizosphere and bulk) and fine root chemistry, an analysis of variance was used to examine differences between mean ion

concentrations in $AlCl_3$ and control plots (4 replicate plots per treatment). We used linear contrasts to assess differences between treatment and control plots for each soil fraction (i.e. rhizosphere organic, bulk organic, rhizosphere mineral and bulk mineral soil) and root type (organic and mineral). (Ca+Mg):Al ratios in soils and roots were computed from the mean concentration from each plot rather than as a mean concentration from each sample because the number of rhizosphere samples per plot varied due to the need to composite samples with insufficient mass for chemical analysis (19% of all rhizosphere samples).

To compare rhizosphere and bulk soil chemistry, samples from $AlCl_3$ and control plots were combined because we found no significant interaction between soil fraction and treatment for any measured variables. We analyzed for chemical differences between rhizosphere and bulk soil samples two ways. First, we compared means from each plot ($n = 4$) and used a Fisher's Protected LSD test ($\alpha = 0.05$). Secondly, we paired individual rhizosphere and bulk soil samples (i.e. from the same soil pit) and used a paired t-test ($\alpha = 0.05$) to examine differences between means irrespective of treatment and soil horizon. Composited rhizosphere samples (i.e. taken from more than one soil pit) were excluded from the paired t-test.

To test which rhizosphere and bulk soil variables most influenced fine root (Ca+Mg):Al ratios, we used various properties of rhizosphere soil, bulk soil and fine roots (taken from the same soil pit) in a step-wise multiple linear regression. Independent variables were concentrations of Al, Ca, Mg, (Ca+Mg):Al, pH and Al_o in rhizosphere soil and bulk soil and the dependent variable was the fine root (Ca+Mg):Al ratio. All statistical analyses were performed using SAS version 6.12 (SAS Institute Inc., 1996).

3. Results

3.1. Treatment effects on fine root and soil chemistry

Most treatment effects on fine roots were restricted to the organic horizon (Table II). Aluminum chloride treatment resulted in roots in the organic horizon with 21% less Ca and 30% less Mg than in untreated plots, but no significant effect on root Al. Fine root (Ca+Mg):Al ratios were significantly lower (42%) in $AlCl_3$ plots than controls in the organic horizon. In the mineral horizon, treatment effects differed significantly only for root Mg which was 22% lower in $AlCl_3$ plots. The treatment induced no significant differences in fine root (Ca+Mg):Al ratios in the mineral horizon.

In soil, additions of $AlCl_3$ induced significant changes in both rhizosphere and bulk soil (Table III). In $AlCl_3$ treated plots, exchangeable ions followed the expected pattern of greater Al and less Ca and Mg, though significant differences were detected only in the rhizosphere. In the organic horizon, $AlCl_3$ treatments resulted in 47% greater Al in rhizosphere soil and 35% greater Al in bulk soil. Treated plots had 29% and 42% less Mg in the rhizosphere in the organic and mineral horizons,

TABLE II

Treatment effects on sugar maple fine root chemistry (ash-free dry weight %) in organic and mineral horizons

Treatment	Al	Ca	Mg	Ca:Al	(Ca+Mg):Al
			Organic horizon		
Control	0.21 (0.05)a	0.19 (0.01)a	0.10 (0.01)a	1.35 (0.30)a	2.05 (0.52)a
$AlCl_3$	0.22 (0.01)a	0.15 (0.01)b	0.07 (0.01)b	0.76 (0.07)b	1.12 (0.08)b
			Mineral horizon		
Control	0.33 (0.06)a	0.16 (0.01)a	0.09 (0.01)a	0.60 (0.10)a	0.90 (0.17)a
$AlCl_3$	0.34 (0.03)a	0.15 (0.01)a	0.07 (0.01)b	0.53 (0.09)a	0.77 (0.12)a

Note. Treatment means (and standard errors) of each element within a soil horizon are compared to means of controls ($n = 4$). Different letters within a column and horizon indicate means differ significantly ($p < 0.05$) by Fisher's Protected LSD test.

respectively, compared to controls. In the $AlCl_3$ treatments, (Ca+Mg):Al ratios in rhizosphere soil of the organic horizon were 54% lower than ratios in control plots. In bulk soil, (Ca+Mg):Al ratios were 39% lower than in control plots, though this difference was not statistically significant.

3.2. Differences between rhizosphere and bulk soil

In samples from both $AlCl_3$ and control plots, the chemistry of rhizosphere soil differed from that of the bulk soil (Table IV). In the organic horizon, rhizosphere soil had 23% less Al and 24% more Mg than bulk soil. (Ca+Mg):Al ratios in rhizosphere soil were 60% greater than those in bulk soil in the organic horizon. Differences between rhizosphere and bulk soil OM, Ca, pH and Al_o in organic horizons were not statistically significant. In mineral horizons, OM and Mg in rhizosphere soil were significantly greater (22% and 42%) than in bulk soil and pH was significantly lower (0.1 pH units). (Ca+Mg):Al ratios in rhizosphere soil mineral horizons were 46% greater than those in bulk soil. Paired rhizosphere and bulk soils from all plots differed as well (Figures 1 and 2). We found significantly less Al and Al_o in rhizosphere soil, and significantly more Mg and OM. Rhizosphere soil was also more acidic and had greater (Ca+Mg):Al ratios than the bulk soil.

3.3. Soil and fine root interactions

We used a step-wise multiple linear regression to assess which variables in rhizosphere and bulk soil most influenced fine root (Ca+Mg):Al ratios. In the organic horizon, the two most influential single variables were (Ca+Mg):Al ratios in rhizosphere soil (adjusted $r^2 = 0.74$; $p < 0.0001$) and (Ca+Mg):Al ratios in bulk soil (adjusted $r^2 = 0.53$; $p < 0.0001$). Using three variables in the model, Ca in

TABLE III

Treatment effects on rhizosphere and bulk soil chemistry

	Rhizosphere soil				Bulk soil			
Treatment	Al	Ca	Mg	(Ca+Mg):Al	Al	Ca	Mg	(Ca+Mg):Al
				Organic horizon				
Control	10.2b (1.2)	10.8a (2.2)	3.4a (0.6)	1.81a (0.51)	13.9a (1.3)	9.1a (2.0)	2.2a (0.4)	1.06a (0.25)
$AlCl_3$	15.0a (1.6)	7.9a (1.1)	2.4b (0.2)	0.76b (0.11)	18.7a (1.0)	7.6a (1.6)	2.1a (0.3)	0.62a (0.14)
				Mineral horizon				
Control	32.3a (3.3)	12.8a (3.2)	4.5a (1.3)	0.56a (0.15)	38.6a (5.9)	12.1a (3.3)	3.0a (0.6)	0.38a (0.07)
$AlCl_3$	25.1a (2.6)	8.6a (1.7)	2.6b (0.4)	0.48a (0.1)	31.8a (3.1)	9.2a (2.1)	2.4a (0.4)	0.36a (0.06)

Note. Treatment means (and standard errors) of exchangeable Al, Ca and Mg ($cmols_+\ kg_o^{-1}$) within a soil horizon are compared to means of controls ($n = 4$). Different letters within a column and horizon indicate means differ significantly ($p < 0.05$) by Fisher's Protected LSD test.

TABLE IV

Differences between rhizosphere and bulk soil chemistry

Soil fraction	%OM	Al	Ca	Mg	(Ca+Mg):Al	pH	Al_o
			Organic horizon				
Rhizosphere	36.6a (2.9)	12.3b (1.0)	10.7a (0.9)	2.9a (0.2)	1.71a (0.35)	3.44a (0.04)	1.9a (0.5)
Bulk	31.4a (2.4)	16.0a (0.9)	9.9a (0.8)	2.3b (0.1)	1.07b (0.17)	3.51a (0.04)	2.2a (0.3)
			Mineral horizon				
Rhizosphere	14.7a (0.9)	33.3a (3.4)	12.8a (1.6)	3.7a (0.4)	0.57a (0.07)	3.72b (0.04)	2.5a (0.3)
Bulk	12.0b (0.8)	35.5a (1.5)	10.7a (1.1)	2.6b (0.2)	0.39b (0.04)	3.83a (0.03)	2.9a (0.4)

Note. Mean concentrations (and standard errors) of percentage organic matter (%OM), exchangeable Al, Ca, Mg ($cmols_+\ kg_o^{-1}$), (Ca+Mg):Al ratios, pH and Al_o ($g\ kg^{-1}$) in rhizosphere and bulk soil ($n = 4$). Different letters within a column and horizon indicate means differ significantly ($p < 0.05$) by Fisher's Protected LSD test.

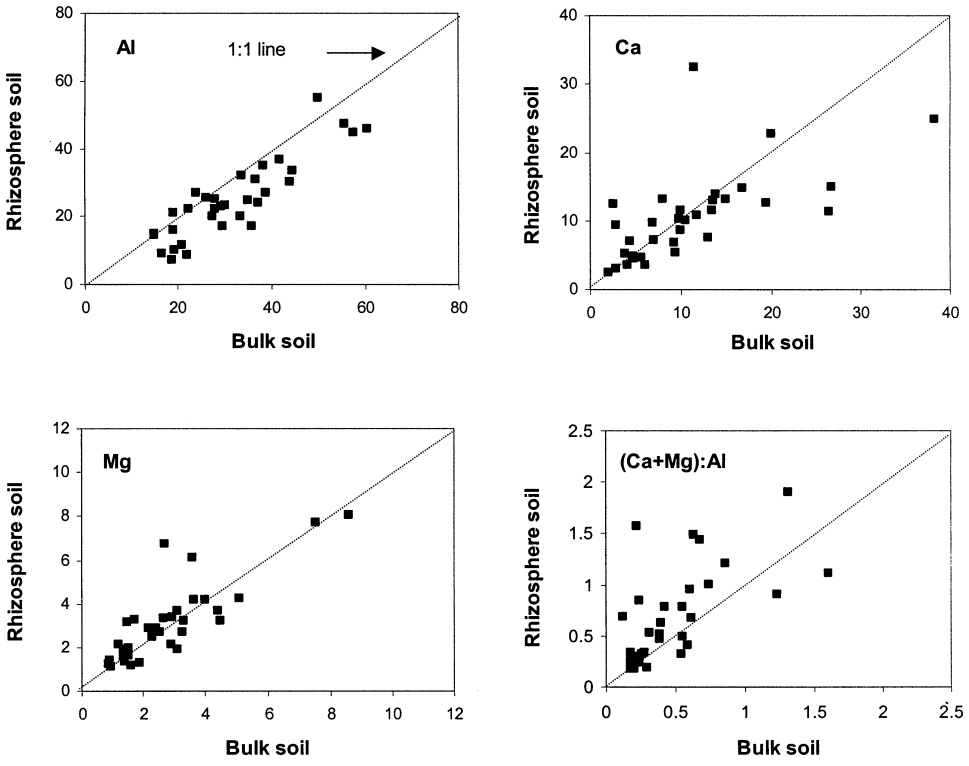

Figure 1. Relationship between rhizosphere and bulk soil exchangeable Al, Ca, Mg, and (Ca+Mg):Al molar ratios in paired rhizosphere and bulk soil samples from organic and mineral horizons. Significant differences between rhizosphere and bulk soil samples were determined using a paired t-test ($p < 0.05$)

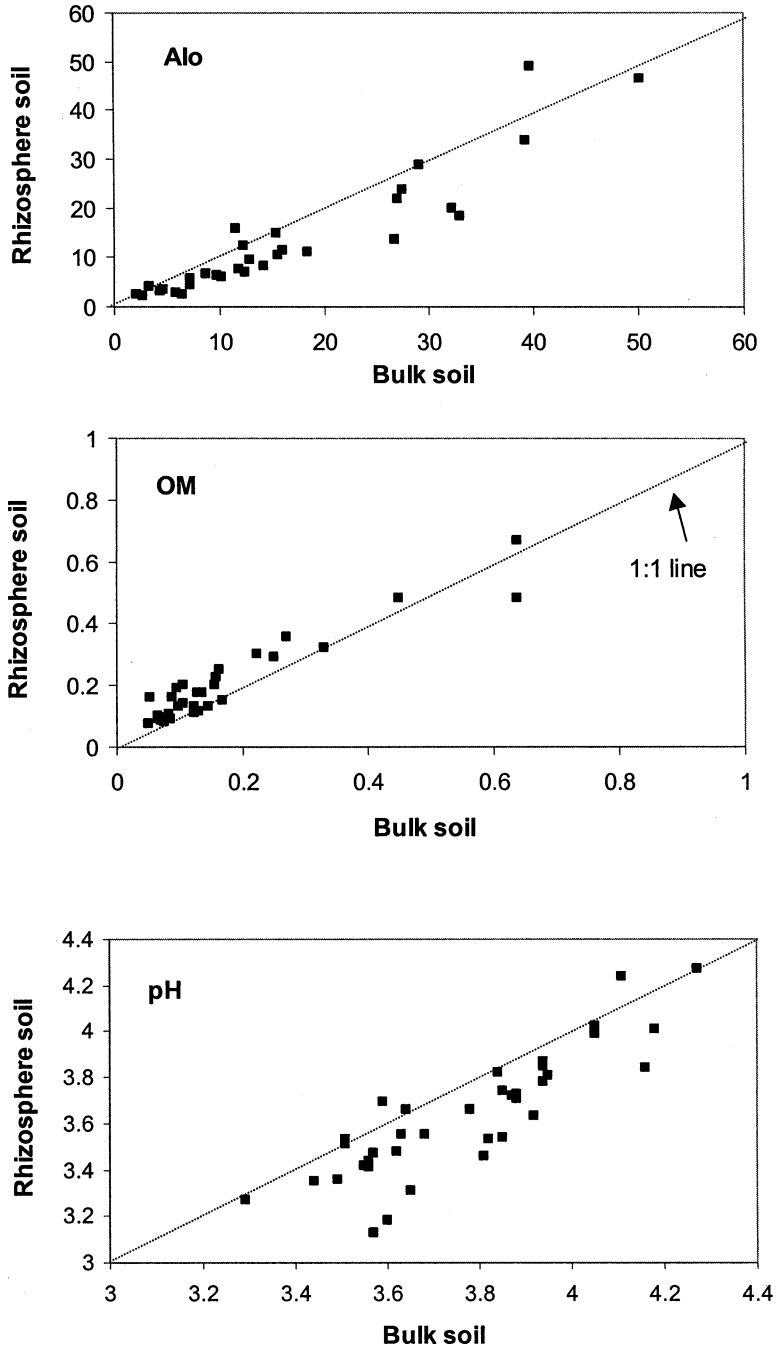

Figure 2. Relationship between rhizosphere and bulk soil organically bound Al (Al_o – g/kg_o), percentage organic matter (OM) and pH in paired rhizosphere and bulk soil samples from organic and mineral horizons. Significant differences between rhizosphere and bulk soil samples were determined using a paired *t*-test ($p < 0.05$).

bulk soil and Al and Mg in rhizosphere soil explained over 92% of the variation in fine root (Ca+Mg):Al ratios in the organic horizon. In mineral horizons, only the (Ca+Mg):Al ratios in rhizosphere soil ($r^2 = 0.13$; $p < 0.05$) significantly predicted (Ca+Mg):Al root ratios. Including additional rhizosphere and bulk soil variables from mineral horizons in the model resulted in only marginal increases in the coefficient of determination.

4. Discussion

4.1. Treatment effects on soil and fine root chemistry

We added substantial amounts of $AlCl_3$ to our plots over a three-year period. The added Al (0.17 mol/m^2) was almost 3 times the size of the total exchangeable Al pool in the Oa (0.06 mol/m^2; Johnson *et al*., 2000) and 52% of the CEC in the Oa (Johnson *et al*., 1997). Treatment effects on soil chemistry may have been small because of high within-treatment variability of Al and Ca. Using an inverse *t*-test, we calculated that changes in soil Ca greater than 71% in the rhizosphere and 94% in bulk soil would have been needed to detect significant treatment effects. Treatment effects may have also been difficult to detect because of varying amounts of mineral soil in the organic horizons. Although we reduced variation by presenting concentrations on an organic matter basis (i.e. $cmols_+\ kg_o^{-1}$), variation in Al and Ca concentrations may have resulted from the difficulty in separating A and B horizons in the field. Exchangeable Al concentrations are 2–3 fold greater in mineral horizons than organic horizons (Table III), and thus small amounts of mineral soil in organic horizon samples may have a large effect on variability in soil Al.

We hypothesized that rhizosphere chemistry would respond differently than bulk soil chemistry to elevated Al because of fine root influences on the rhizosphere. In our study, $AlCl_3$ treatments caused losses of Mg from rhizosphere soil but not from bulk soil (Table III). The displacement of Mg in the rhizosphere may be the result of differences in organic matter exchange properties between rhizosphere and bulk soil. Organic matter, which likely provides a greater fraction of the total CEC in rhizosphere soil than bulk soil, has a greater relative affinity for trivalent Al than inorganic exchange sites (Stevenson and Vance, 1996). This argument has been used to explain differences in exchangeable Al and base cations in rhizosphere and bulk soils from an acidic almond orchard (Chung *et al*., 1994). Aluminum may have also displaced Mg and not Ca from rhizosphere exchange sites because of the greater hydrated radius of Mg (Marschner, 1995; Van Praag *et al*., 1997). Greater displacement of rhizosphere Mg suggests that the rhizosphere may be more poorly buffered than bulk soil despite greater organic matter and CEC (Gobran and Clegg, 1996).

We detected no significant response of sugar maple fine root Al to $AlCl_3$ additions, despite significant effects on soils. The lack of a treatment effect on root Al may have resulted from Al tolerance mechanisms in sugar maple. Sugar maple is

considered moderately tolerant to Al stress relative to some other hardwood species (Cronan *et al*., 1989). In general, Al tolerance in plants results from internal cellular tolerance and Al exclusion (Heim *et al*., 2000). A proposed exclusion mechanism is root exudation of organic acids (Jones, 1998), whereby organic chelators released by roots form soluble Al complexes that leach to the subsoil (Hue *et al*., 1986). Although we did not measure either mechanism directly, Al exclusion and accelerated leaching seem possible given the lack of a treatment effect on root Al and strong depletion of Al and Al_o around fine roots. Although such an exclusion mechanism has been described for ectomycorrhizal trees (Cumming *et al*., 2001), few investigations have demonstrated the importance of this mechanism in arbuscular-mycorrhzal trees (Lux and Cumming, 2001).

Some investigators have proposed that critical thresholds in fine roots might be useful indicators of Al stress for several tree species, including sugar maple (Cronan and Grigal, 1995). Thornton *et al*. (1986) found that sugar maple seedling growth was adversely affected when Al concentrations in roots were as high as 0.27% and Ca concentrations as low as 0.15% (no data were presented for Mg). In our experiment, these critical thresholds were exceeded for Ca (60% of samples) and Al (57% of roots) in mineral horizons of $AlCl_3$ and control plots. In organic horizons from $AlCl_3$ treated plots, 62% of roots had Ca concentrations lower than the threshold as opposed to 13% of roots from control plots. However, these thresholds should be applied with caution because they were developed for non-mycorrhizal seedlings grown in hydroponic systems, and may not reflect native soil conditions or chemical changes in roots with age and soil depth (DeWit *et al*. 2001). These thresholds also do not include root Mg or (Ca+Mg):Al ratios which may be especially important given the greater sensitivity of root Mg to elevated Al (Van Praag *et al*., 1997) and the link between Mg deficiency and sugar maple decline (Horsley *et al*., 2000). More research is needed to evaluate whether thresholds developed for sugar maple seedlings are relevant for mature trees, and whether separate thresholds need to be developed for different soil horizons.

4.2. Fine root influences on rhizosphere chemistry

We hypothesized that Ca and Mg would be depleted around fine roots due to uptake, and rhizosphere Al would be greater than in bulk soil due to rhizosphere acidification and exclusion by roots (Kirlew and Bouldin, 1987; Gottlein *et al*., 1999; Wang *et al*., 2001). However, we found that Al (but not Ca and Mg) was depleted in the rhizosphere suggesting that differences between rhizosphere and bulk soil Al are probably not due to root uptake. Aluminum depletion was particularly surprising given rhizosphere pH was generally lower (Figure 2) and pH changes in the rhizosphere of 0.1 pH unit can increase Al solubility twofold (Smith, 1990). This suggests that factors other than pH (e.g. Al_o) and root uptake must influence concentrations of Al in the rooting zone (Smith and Pooley, 1989; Lawrence *et al*., 1995).

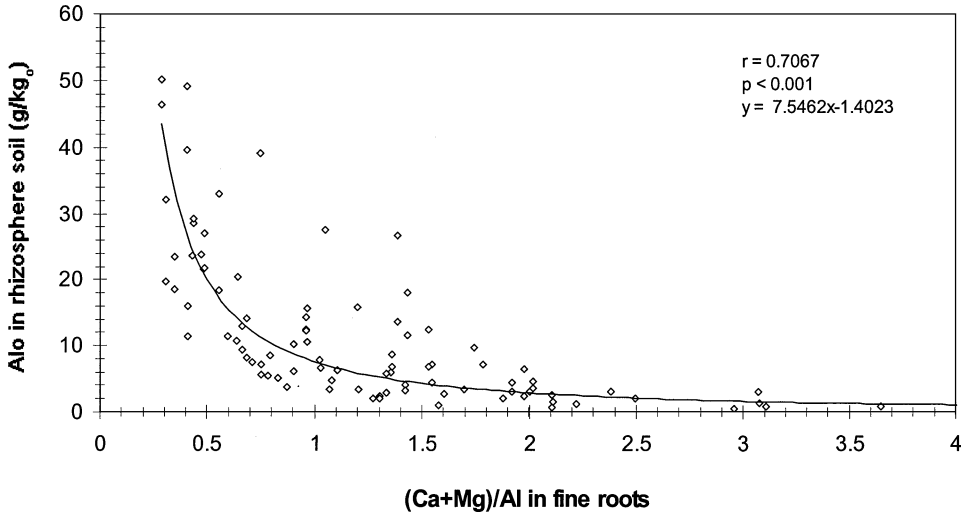

Figure 3. Relationship between fine root (Ca+Mg):Al molar ratios and organically bound Al (Al_o – g/kg_o) in the rhizosphere from organic and mineral horizons.

Aluminum depletion may have resulted from accelerated leaching from the rhizosphere due to root release of organic ligands in response to Al stress (Smith and Pooley, 1989; Jones, 1998). Chelation of Al by low molecular weight organic acids is the primary mechanism of Al exclusion by Al-tolerant plants (Kochian, 1995; Jones, 1998). We observed that high rhizosphere Al_o in the organic horizon was highly correlated with low fine root (Ca+Mg):Al ratios, which might be due to exudation of organic acids by roots under Al stress (Figure 3). However, we cannot rule out a simple equilibrium between Al_o and Al in the rhizosphere, such that the relationship between rhizosphere Al_o and fine root (Ca+Mg):Al ratios might be an artifact of the relationship between rhizosphere Al and fine root (Ca+Mg):Al ratios.

4.3. Limitations of rhizosphere studies

Few studies have examined rhizosphere soil in mature trees because sampling the rhizosphere is time-consuming and fraught with methodological and statistical limitations. The amount of soil adhering after gentle shaking may be influenced by soil moisture content, root diameter and the researcher's subjective view of what constitutes a "gentle" shaking. Ortas (1997) estimated that soil adhering after gentle shaking ranged from 0.4 to 0.6 mm from the root surface, and may or may not capture the full extent of the root's influence. Perhaps a bigger problem is the small amount of soil obtained by this laborious technique, which severely limits sample size and statistical power. We paired rhizosphere and bulk soil samples, which may

better characterize the net effects of rhizosphere processes in spatially variable soils. In this study, mean concentrations of rhizosphere Al were not significantly less than bulk soil concentrations in organic horizons (Table IV), but differences were significant using paired samples (Figure 1). In addition, element ratios could be quite sensitive to the amount of soil collected because concentration gradients vary among elements (Marschner, 1995). Finally, root contamination of the rhizosphere sample is an inevitable cost of mechanically separating soil from roots and may influence the exchange chemistry of rhizosphere soil (Norvell and Cary, 1992).

We did not consider the effects of rhizosphere microbes and mycorrhizae on Al chemistry in the rhizosphere. Rhizosphere microbial activity is generally greater than in bulk soil due to the release of exudates from roots, and rhizosphere microbes release organic acids that complex and detoxify Al (Devevre *et al*., 1996; Stevenson and Vance, 1996). In ectomycorrhizal Norway spruce, mycorrhizae exert a large influence on Al chemistry in the rhizosphere (Dieffenbach and Matzner, 2000). Sugar maple roots are vesicular arbuscular mycorrhizal, and the effects of the mycorrhizae on rhizosphere chemistry are presently unknown (Lux and Cumming, 2001). Aluminum has been detected in mycorrhizal hyphae of sugar maple seedlings growing on acidic soils (McQuattie *et al*., 1998), though it is unclear whether the Al is transferred to the root, immobilized in the hyphae or released to the rhizopshere. Moreover, changes in rhizosphere chemistry may influence the percentage of sugar maple roots infected by mycorrhizae. Hutchinson *et al*. (1999) and Ouimet and Camire (1995) reported decreased percent mycorrhizal infection in mature sugar maple exposed to elevated soil Al. Clearly more research is needed on the interactions between roots, mycorrhizae and rhizosphere microbes, and their effects on Al chemistry in acidic forest soils.

5. Conclusions

Aluminum chloride treatment decreased Mg from rhizosphere exchange sites resulting in lower Mg and (Ca+Mg):Al in sugar maple roots. The treatment did not, however, increase Al in fine roots suggesting that roots may respond to changes in (Ca+Mg):Al by chelating Al in the rhizosphere. The strong depletion of Al_o in the rhizosphere suggests that most of the chelated Al may have leached from the rhizosphere to mineral soil horizons below the rooting zone. Thus, the response of sugar maple fine roots to elevated Al may depend, in part, on how roots influence the chemistry of the rhizosphere. Sampling protocols that focus exclusively on bulk soil may fail to account for fine root influences on rhizosphere Al and Mg.

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