

10-2-2009

Evaluating the Influences of Soil Calcium and Aluminum Availability on Ecosystem Processes in the Northern Hardwood Forest

Homer Elliott
University of Vermont

Follow this and additional works at: <http://scholarworks.uvm.edu/graddis>

Recommended Citation

Elliott, Homer, "Evaluating the Influences of Soil Calcium and Aluminum Availability on Ecosystem Processes in the Northern Hardwood Forest" (2009). *Graduate College Dissertations and Theses*. Paper 75.

This Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks @ UVM. It has been accepted for inclusion in Graduate College Dissertations and Theses by an authorized administrator of ScholarWorks @ UVM. For more information, please contact donna.omalley@uvm.edu.

EVALUATING THE INFLUENCES OF SOIL CALCIUM AND ALUMINUM
AVAILABILITY ON ECOSYSTEM PROCESSES IN THE NORTHERN HARDWOOD
FOREST

A Thesis Presented

by

Homer L. Elliott

to

The Faculty of the Graduate College

of

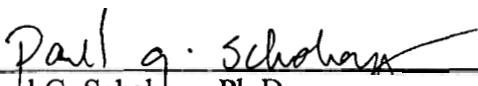
The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Master of Science
Specializing in Natural Resources

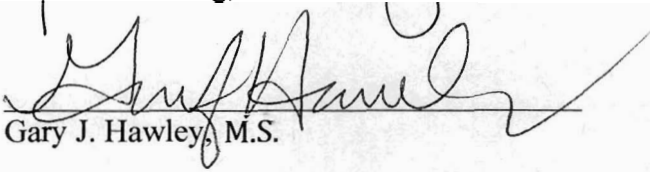
May, 2009

Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Master of Science, specializing in Natural Resources.

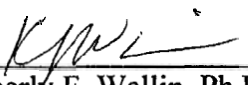
Thesis Examination Committee:



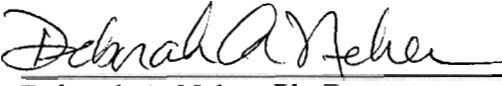
Paul G. Schaberg, Ph.D. **Advisor**



Gary J. Hawley, M.S.



Kimberly F. Wallin, Ph.D.



Deborah A. Neher, Ph. D. **Chairperson**



Patricia A. Stokowski, Ph. D. **Interim Dean, Graduate College**

Date: March 26, 2009

Abstract

Calcium (Ca) depletion and increased bioavailability of aluminum (Al) are potential consequences of soil acidification caused by acidic deposition and other anthropogenic factors. Tree declines are associated with base cation depletion and increased Al toxicity in forest soils in North America, Europe, and Asia. Changes in soil Ca and Al availability may lead to increased oxidative stress and disruptions in carbohydrate relationships in forest trees, as well as to substantial alterations in the capacity for enzymatically controlled processes of decomposition and mineralization in forest soils.

Assessments were made to determine if forest systems are prone to disruption associated with altered Ca and Al bioavailability. Foliar elemental concentrations, foliar antioxidant enzyme activities, foliar and woody shoot carbohydrates were measured in sugar maple (*Acer saccharum*, Marsh.), and soil extracellular enzyme activities (EEA) were assayed at a long-term nutrient perturbation study (NuPert) in the Hubbard Brook Experimental Forest, New Hampshire, USA. Treated plots received Ca to increase soil Ca above ambient depleted levels or Al to further reduce Ca availability. Additions of Ca to soil are associated with greater Ca concentrations in foliage compared to leaves from trees from control and Al-addition plots. Soil Al-additions are associated with lower foliar phosphorus concentrations in comparison with foliage from trees in Ca-addition plots. Additions of Al to soil are associated with higher antioxidant enzyme (glutathione reductase and ascorbate peroxidase) activities in foliage and lower shoot sugar (total sugars, sucrose, glucose and fructose) concentrations relative to trees in Ca-addition and control plots. Al accumulations in distal tissues likely triggered toxicity responses reported for leaves and stems. Soil EEA results highlight treatment-induced alterations to soil processes. Across soil enzyme systems, EEA levels are greatest in Al-addition soils in fall, but are elevated in Ca-addition soils in spring compared with ambient conditions. Seasonal differences in EEA levels suggest a differential influence of soil treatments on specific soil communities. Within this native, mature northern hardwood forest, early indications of response in foundation species to Ca and Al manipulation are detected including Al-induced oxidative stress and resulting carbohydrate irregularities in sugar maple trees, and substantial seasonal swings in soil EEA: processes that could foreshadow broader ecosystem alterations as anthropogenic disruptions of soil Ca and Al availability continue.

Acknowledgements

I would like to acknowledge the generous support of funds provided by the Northeastern States Research Cooperative (NSRC) Theme 2 and the Environmental Protection Agency that made this research, as well as my continued education, possible. The NSRC is a worthy collaboration of the USDA Forest Service, the Rubenstein School of Environment and Natural Resources (RSENr) at the University of Vermont, the University of New Hampshire, the Hubbard Brook Research Foundation, the Center for Research on Sustainable Forests at the University of Maine, and the State University of New York College of Environmental Science and Forestry fostering research for the future well-being of our forest resources.

I am grateful for the encouragement and support of my thesis committee members, including my advisor Paul G. Schaberg, Deb A. Neher, Gary J. Hawley, and Kimberly F. Wallin. I am also deeply indebted to the friends, students, and colleagues I have met along the way, without whom my work would have not had the same character and quality, including Paula Murakami, Josh Halman, Tom Weicht, Chris Hansen, Tom Saielli, Will Young, Brian Darby, Bret Huggett, Kendra Gurney, Chris Eagar, Ralph Perron, Ian Halm, the community of RSENr, and, of course the others I have forgotten to mention. I am also incredibly thankful for the support of my life partner, Tessa Izenour, who spurred me on my way and was the catalyst for this endeavor.

Table of Contents

	Page
Acknowledgements.....	ii
List of Tables	vi
List of Figures	vii
Chapter 1: Literature Review.....	1
1.1. Forest Ecosystem Impacts of Acidic Deposition.....	1
1.2. Calcium Depletion.....	2
1.3 Calcium Nutrition in Plant and Tree Physiology.....	3
1.3.1. Calcium and the Regulation of Plant Energy Relations	6
1.3.2. Calcium and Antioxidant Enzyme Activity.....	10
1.3.3. Poential Influence of Ca Depletion on Plant Health.....	11
1.3.4. Connections between Ca and Al Physiology.....	11
1.4. Aluminum Bioavailability in the Soil.....	12
1.5. Aluminum Toxicity in Plant and Tree Physiology	13
1.6. Soil Enzyme Systems and Extracellular Enzyme Activity Bioassay	16
1.7. Concluding Remarks	19
Chapter 2: Journal Article.....	22

2.1. Title Page.....	22
2.2. Abstract.....	23
2.3. Introduction	25
2.4. Methods	30
2.4.1. Study Site.....	30
2.4.2. Tree-based Measures	31
2.4.2.1. Sample Collection	31
2.4.2.2. Foliar Elemental Analysis	32
2.4.2.3. Foliar Antioxidant Activity	32
2.4.2.4. Carbohydrate Analysis	34
2.4.3. Soil-based Measures	35
2.4.3.1. Sample Collection	35
2.4.3.2. Extracellular Enzyme Activity Assays.....	36
2.4.4. Statistical Analyses.....	37
2.5. Results	38
2.5.1. Tree-based Measures	38
2.5.1.1. Foliar Elemental Analysis	39
2.5.1.2. Foliar Antioxidant Enzyme Systems.....	39
2.5.1.3. Foliar Carbohydrates	40
2.5.1.4. Woody Shoot Carbohydrates.....	41
2.5.2. Soil-based Measures of Extracellular Enzymes	41
2.5.2.1. September 2007 Assays.....	41
2.5.2.2. May 2008 Assays	42
2.5.2.3. Seasonal Fluxes in Soil EEA.....	42
2.6. Discussion.....	43
2.6.1. Tree-based Responses in a Forested Ecosystem.....	43
2.6.2. Soil-based Responses in a Forested Ecosystem.....	48

2.6.3. Conclusions.....	52
2.7. Acknowledgements	56
2.8. Literature Cited.....	57
Comprehensive Bibliography	80

List of Tables

Table	Page
Table 1: Dates and treatment applications in the long-term NuPert experiment.....	71
Table 2: Example ANOVA tables for the statistical analyses of tree-based and soil EEA data from the NuPert experiment.....	72
Table 3: Soil addition treatment differences in foliar element concentrations for sugar maple (<i>Acer saccharum</i>)	73
Table 4: Foliar carbohydrate concentrations for sugar maple (<i>Acer saccharum</i>).....	74

List of Figures

Figure	Page
Figure 1: Foliar glutathione reductase, ascorbate peroxidase, and catalase antioxidant enzyme activity for sugar maple (<i>Acer saccharum</i>)	75
Figure 2: Woody shoot carbohydrate concentrations of total sugars, sucrose, glucose, fructose, raffinose, xylose and starch for sugar maple (<i>Acer saccharum</i>).....	76
Figure 3: September extracellular enzyme activity in soil	77
Figure 4: May extracellular enzyme activity in soil	78
Figure 5: Seasonal change in extracellular enzyme activity in soil.....	79

Chapter 1: Literature Review

1.1. Forest Ecosystem Impacts of Acidic Deposition

Forest decline is described as a “global environmental problem” by Shigihara et al. (2008). Forest ecosystem health issues and tree decline in Europe, Asia, and North America have been associated with anthropogenic acidic deposition and resulting soil acidification. A number of ecologically and economically important tree species including *Acer saccharum*, *Picea rubens*, *Fagus sylvatica*, *Picea abies*, *Fagus crenata*, *Abies firma*, and *Pinus massoniana* have shown sensitivity to acidic deposition and soil acidification (Balsberg Pålsson 1990, DeHayes et al. 1999, Igawa et al. 2002, Hultberg and Ferm 2004, Jandl et al. 2004, Schaberg et al. 2006, Shigihara et al. 2008, Song et al. 2008). Symptoms of tree decline in these species can include loss of vigor, reductions in growth, crown dieback, and potentially increased mortality and reduced recruitment.

Increased inputs of protons into soils and associated reductions in soil pH are linked with shifts in soil buffering systems following acidic deposition (Chadwick and Chorover 2001). During soil acidification base cations such as calcium (Ca) and magnesium (Mg) are mobilized by protons from their exchange sites and released into the soil solution in a buffering process. Mobilized base cations are then vulnerable to subsequent leaching losses from the soil with anions (e.g., SO_4^-) from acidic deposition in a depletion process (Huntington et al. 2000). As these base cation stores are depleted, soil pH is then reduced and aluminum (Al) is increasingly solubilized, often to the point of toxicity, which, in turn, can be followed by iron (Fe) release (Bowman et al. 2008).

Intensifying Fe release can further aggravate symptoms of Al toxicity in trees (Nguyen et al. 2005).

1.2. Calcium Depletion

One of the most significant effects of acidic deposition is the increase in the rate of base cation loss from soils, especially the depletion of Ca stores. Evidence indicates that a variety of anthropogenic factors (most notably inputs of acidic deposition) are resulting in the net loss of cations from forested ecosystems throughout New England (Likens et al. 1996, Likens et al. 1998, Schaberg et al. 2001, Fernandez et al. 2003, Gbondo-Tugbawa and Driscoll 2003, Schaberg et al. 2006). Calcium depletion by acidic deposition is partially dependent on the capacity of the parent geology to replenish lost cations through weathering. A number of reports have documented Ca depletion in hardwood and coniferous forest ecosystems in other locations including Pennsylvania, the Southeast, the Canadian Shield, and parts of Europe, suggesting the widespread nature of this anthropogenic phenomenon (Huntington et al. 2000, Hultberg and Fern 2004, Jandl et al. 2004, Bailey et al. 2005, Duchesne and Houle 2006). Further evidence for the expanding extent of this phenomenon can now be found in Asia. A decrease in soil cation exchange capacity (CEC) and pH associated with acidic deposition has been reported for coniferous forests in China (Dai et al. 1998), while vulnerable soils and areas of extreme acidic deposition have been mapped (Tao et al. 2002, Wei and Wang 2005, Larssen et al. 2006).

Although generalized cation loss has many potentially negative effects on forest system function the loss of Ca may be particularly consequential to forest health and productivity. Such evidence has been documented for red spruce (*Picea rubens* Sarg.) in New England (Schaberg and DeHayes 2000, Schaberg et al. 2002), and new research has implicated Ca depletion in the decline of sugar maple (*Acer saccharum* Marsh.) as well (Watmough 2002, Juice et al. 2006, Schaberg et al. 2006). Using the Till Source Model (TSM), Schaberg et al. (2006) were able to predict the Ca status of soils derived from glacial till as well as foliar Ca levels in Vermont maple forests where a significant relationship was found between low soil Ca and two symptoms of sugar maple decline: 1) elevated branch dieback, and 2) reduced basal area growth.

Once Ca is depleted from transient and long-term stores it is lost. In some forests of Europe (Sverdrup et al. 2006) and Asia (Nykvist 2000) Ca depletion is advanced to a point where forest harvest can no longer be considered sustainable without additional artificial inputs of Ca. In other forest stands with less advanced Ca depletion, some degree of harvest will be sustainable if good forest management practices (such as leaving cation-containing bark, branches, and foliage on site) and reductions in N deposition are achieved (Egli 1998).

1.3. Calcium Nutrition in Plant and Tree Physiology

Calcium is distinctly spatially and temporally compartmentalized in plant cells. Indeed, the great versatility of its physiological function is dependent upon this localized organization (Berridge et al. 2000, Rizzuto and Pozzan 2006). Even though

Ca is an essential micronutrient, large concentrations of its unbound form (Ca^{2+}) within the cytoplasm are damaging due to its propensity to form insoluble complexes with phosphate ions (Bush 1995, Knight 2000). In order to assure available phosphate and ATP for respiration, metabolism, and other processes, Ca^{2+} is actively transported out of the cytoplasm and sequestered in vacuoles, endoplasmic reticulum, and outside the plasma membrane, often as crystals of Ca oxalate (Fink 1991, Allen et al. 1995). Because Ca^{2+} is rapidly pumped out of the cytoplasm, it is immobile in the phloem, which is dependent on transport through the cytoplasm. In contrast to most other nutrient cations (except for Fe and boron), Ca cannot be redistributed within plants to surmount deficiencies because of this immobility (Salisbury and Ross 1992).

Concentrations of Ca in tissues and cells support two broadly defined and important functions in plants: 1) Ca adds to the structural stability membranes and plant cells, and 2) Ca^{2+} has a fundamental role in the regulation of cellular biochemistry, especially in mechanisms that allow cells to sense and respond to environmental stimuli and change (Marschner 2002). Calcium is a key component of the middle lamella of cell walls where its divalent charge helps to bind proximal cells together, strengthening the apoplast (Salisbury and Ross 1992). Calcium also influences and regulates membrane stabilization, permeability, and the gating of channels by joining carboxylate and phosphate groups of phospholipids, enzymes, and proteins (Palta and Li 1978, Legge et al. 1982, Davies and Monk-Talbot 1990). Regardless of its role in basic cell structure, Ca has its greatest influence on plant physiology through its influence on biochemical regulation (Marschner 2002).

One of the most important roles of Ca is its function as a second messenger in the perception of environmental change and stress and the transduction of these signals (Bush 1995, Sanders et al. 1999, Roos 2000, Pandey et al. 2000, Pandey et al. 2004, Abbasi et al. 2004). In a response cascade, environmental stimuli interact with membrane-bound Ca and transiently alter the permeability of the plasma membrane. Because of the low concentrations of cytoplasmic Ca^{2+} , Ca^{2+} then streams into cells through a steep concentration gradient (Allen et al. 1995, Sanders et al. 1999). Once it has entered the cytoplasm, Ca^{2+} quickly attaches to Ca-specific messenger proteins such as Ca-dependent protein kinases (CDPK's) and calmodulins (CaM's), and initiates a chain of physiological events (e.g., changes in enzyme activity, gene transcription) that help cells adapt to changes in environmental conditions. This entry of Ca^{2+} into the cytoplasm appears to be an essential first step in cellular processing of environmental information and initiating a broad range of physiological responses in plants that allows adjustment to environmental change or defense against pests and pathogens. The wide array of stimuli and stresses plants respond to include low temperature (Monroy et al. 1993, DeHayes et al. 1997, DeHayes et al. 1999, Abbasi et al. 2004), drought, salinity (Sheen 1996, Cheong et al. 2003), insect infestations (McLaughlin and Wimmer 1999), fungal infections (Hebe et al. 1999, Gaulin et al. 2006), oxidative stress (Jiang and Huang 2001, Schmitz-Eibeger et al. 2002), and touch (Takezawa et al. 1995).

Transient hyper-polarity of plant cellular membranes is caused in part by rapid influx of Ca^{2+} into the cytoplasm and efflux of K^{+} and Cl^{-} , this polarity is associated

with electrical signals that pulse through the symplast in response to environmental stimuli, such as leaf exposure to flame (Fromm and Lautner 2007). Furthermore, signal strength is significantly reduced in *Populus* with low ambient Ca, even at a point before trees show symptoms of deficiency (Fromm and Lautner 2007). Trees with low ambient Ca have much less ability to respond to environmental stresses and stimuli in a signaling continuum through the whole plant. In addition to its generalized influence on stress signaling, a growing body of literature indicates that Ca is particularly important to the basic regulation of plant energy relations.

1.3.1. Calcium and the Regulation of Plant Energy Relations

Free and bound Ca are essential in the signaling processes of plants (Medvedev 2005), and these processes are important to plant energy relations. Much of the recent inquiry into Ca-dependent plant physiology concentrates on this area of research. Four main families of Ca-binding proteins have been identified in plants (Ludwig et al. 2004), including CDPK's, CaM's, calmodulin-like, and calcineurin-B-like proteins. The CDPK's in *Plantae*, *Chlorophyta*, and *Protista* function in a manner that differs from the Ca-signaling proteins in the other branches in the phylogeny of life, in that CDPK's are able to respond directly to cytoplasmic Ca^{2+} (Ca_{cyt}) instead of relying on signal mediation by CaM (Abbasi et al. 2004). Calcineurin B-like proteins are unique to plants and have been shown to modulate abscisic acid (ABA) sensitivity and synthesis (Pandey et al. 2004, 2008). The largest group of Ca-binding proteins in plants are CDPK's, which

function in signaling through phosphorylation and polarity changes (Ludwig et al. 2004, Medvedev 2005).

Calcium is required for the transcription and reproduction of the genetic mechanics of chloroplasts, which, among other things, provides evidence supporting the theory that the chloroplasts of land plants are descended from cyanobacteria-like organisms (Tozawa et al. 2008). Calcium is also required for the function and structure of the oxygen evolving complex (OEC) in the oxidation of water, and likely needed for all S-state transitions in photosynthesis (the removal of the four e^- from two H_2O for the e^- transport chain) (Miqyass et al. 2007). Notably, evidence continues to build for the importance of Ca in multiple roles in photosynthesis, such as: 1) providing necessary binding sites and connections between important photosynthetic structures (i.e. PSII, CP29), proteins, and pigments (Jegerschöld et al. 2000), 2) control of lumen pH and thylakoid proton gradients and control of the xanthophyll cycle (Pan and Dilley 2000, Dilley 2004), 3) stomatal control and regulation of gas exchange, especially that of CO_2 , but also controlling stomatal response to light, ozone, reactive oxygen species (ROS), water status and humidity by controlling ABA signaling and ion channels (Vahisalu et al. 2008, Young et al. 2006), and 4) the transfer of e^- from PSII to PSI (Semin et al. 2007). Pan and Dilley (2000) and Dilley (2004) give evidence that Ca in and near the thylakoid membrane regulates adenosine triphosphate (ATP) formation, photoprotection and the xanthophyll cycle: in part by 1) controlling the pH of the thylakoid membrane, and by 2) Ca gating of proton (H^+) channels in the thylakoid membrane. If conditions favor photosynthesis, H^+ 's go to ATP production, whereas if photoinhibition begins to occur

Ca gates open and the H^+ fluxes to the lumen acidify it. This acidity and concurrent activation of violaxanthin deepoxidase by Ca drive the xanthophyll cycle as ATP production is down regulated.

The precursor of ATP synthase is made functional in a Ca-dependent manner as it binds at the outer mitochondrial membrane (von Stedingk et al. 1999). Adenosine triphosphate is essential in the reactions forming the building blocks of carbohydrates, and many other reactions such as the formation of sucrose from glucose and fructose (hexoses)(Salisbury and Ross 1992). Calcium and ATP are required for the function of Ca-ATPases, which are important Ca channels in biosynthesis processes, as well as in related Ca signaling (Medvedev 2005). A CDPK (CPK1) binds to a Ca-ATPase at low Ca_{cyt} , phosphorylating the serine component. When Ca_{cyt} levels are higher, such as during Ca^{2+} influx, CaM can then bind to the Ca-ATPase and remove the autoinhibition. This is due to the preferential binding of Ca_{cyt} to the CDPK over CaM, for which Ca_{cyt} has a lesser affinity. In this way Ca is in part regulating the use of ATP, as well as the active transport of Ca (Medvedev 2005).

Sucrose synthase (SUS) activity has been shown to be CDPK dependent (Hardin et al. 2004), so it is likely that alterations in Ca nutrition can affect SUS activity as well. Sucrose surplus favors the tetramer form of SUS by CDPK-regulated signaling and phosphorylation of the enzyme and, thereby, the activity and products of SUS (Duncan and Huber 2007). Sucrose surplus occurs when photosynthesis production is efficient, or when sucrose cannot be utilized or transported. In fact, sucrose utilization by SUS is affected by Ca nutrition (Bhuja et al. 2004). SUS in its dimer form recycles sucrose or

produces hexoses that can be used for starch synthesis, while in its tetramer form it provides raw materials for cellulose synthesis (Duncan and Huber 2007).

In addition to detailed mechanistic studies of Ca influences on the biochemistry of plants, research at the whole plant level also suggests that the processes of photosynthesis are sensitive to changes in Ca nutrition. Decreases in photosynthetic efficiency due to Ca deprivation are observed in *Populus* long before symptoms of deficiency appear (Lautner et al. 2005), as is the case in a pot study of tomato where nutrient levels were strictly monitored and controlled (Schmitz-Eiberger et al. 2002). In both of these studies fluorescence yield was used to give early indication of decrease in photosynthetic efficiency. Lautner et al. (2005) also found that stomatal conductance and stomatal response to leaf flaming were reduced in trees with less foliar Ca before the onset of other deficiency symptoms. Furthermore, St. Clair et al. (2005) were able to connect foliar nutrient imbalances of low Ca and Mg, and high Al and Mn concentrations with significantly lower photosynthesis, stomatal conductance, and chlorophyll (a and b) content in sugar maple trees showing symptoms of decline.

The downstream products of carbon (C) capture also reflect the influence of Ca nutrition at the whole plant level. Calcium nutrition has been found to have a significant influence on wood formation in trees of *Populus* spp. where the basal area increments (BAI) are significantly less in deficient trees (Lautner et al. 2007). Glucose, fructose and sucrose accumulated in the leaves of Ca deficient trees, which appeared to have impaired mechanisms of carbohydrate utilization and transport. Further evidence of this was supported by the loss of starch storage in leaves, as well as lower concentrations of

glucose, fructose, and sucrose in other tissues, and reduced cellulosic and hemicellulosic content in the apoplast (Lautner et al. 2007).

1.3.2. Calcium and Antioxidant Enzyme Activity

Calcium initiates and coordinates the plant physiological responses of antioxidant systems to oxidative stresses (Wise and Naylor 1987). Oxidative stresses generate reactive oxygen species (ROS). Antioxidant systems help to mitigate the impacts of ROS, such as those produced by environmental stresses, including photooxidative damage at low temperatures (Wise and Naylor 1987, Polle and Rennenberg 1994, Becana 2007). Unscavenged ROS can result in cellular and mitochondrial dysfunction (Foyer et al. 1994, Yamamoto et al. 2002). Calcium is a major constituent of the pathways that support antioxidant activity and increases the efficiency of many antioxidant enzymes, including ascorbate peroxidase (APX) (Jiang and Huang 2001). Schmitz-Eibeger et al. (2002) and St. Clair et al. (2005) also explored the relationship between foliar antioxidants and their postulated protection of the photosynthetic apparatus in plants with cation imbalances. Schmitz-Eibeger et al. (2002) found impaired antioxidant activity as indicated by significantly reduced superoxide dismutase (SOD) activity and increased peroxidase (PO) activity in the foliage of Ca deficient tomato exhibiting blossom end rot. St. Clair et al. (2005) used the antioxidant activities of APX and glutathione reductase (GR) as biochemical markers of oxidative stress in foliage, and observed higher activity of these antioxidants in nutrient imbalanced trees.

1.3.3. Potential Influence of Ca Depletion on Plant Health

The importance of Ca in plant sensing and signaling cannot be overemphasized. CDPK's, in part, function as the sensors of abiotic and biotic stresses and fluctuations in environmental conditions for plants (Sangawan et al. 2001, Ludwig et al. 2004, Yang et al. 2004, Rodríguez et al. 2006). Influxes of Ca²⁺ interacting with numerous CDPK's are the front line of how plants respond to stress and stimuli, and initiate a chain of events that up- or down-regulate the genes controlling plant metabolism and secondary compound production (Sangawan et al. 2001, Abbasi et al. 2004, Ludwig et al. 2004, Liu et al. 2005, Rodríguez et al. 2006). Given the fundamental role Ca plays in plant metabolic regulation and response systems, it is possible that depletions of biologically available Ca could suppress the ability of plants to adequately sense and respond to changes in their surroundings and make them more vulnerable to decline.

1.3.4. Connections between Ca and Al Physiology

Especially of interest considering global trends of increasing soil acidification that both decrease Ca and increase Al bioavailability in soils, physiological responses to Al have been shown to be Ca dependent, including the up-regulation of 1) specific CDPK's, and 2) genes involved in Ca-dependent signal transduction in Al-tolerant plants (Zhang et al. 2007). In addition, greater internal stores of Ca and carbohydrates are present in Al-tolerant cultivars, while Al-sensitive cultivars do not have the same capacity (Giannakoula et al. 2008).

Interactions between Ca deficiency and Al toxicity were identified early in the study of Al toxicity, where soil Ca-additions appeared to alleviate symptoms of Al toxicity (see review by Delhaize and Ryan 1995). However, Ca-addition does not always ameliorate plant response to Al toxicity. In fact, Al has been shown to impede Ca uptake by plants and cause Ca deficiency, even before the point of Al toxicity (Bruce et al. 1988). These interactions between Ca and Al bioavailability are not only present in plant and tree physiology, these interactions originate within the soil matrix.

1.4. Aluminum Bioavailability in the Soil

Aluminum is exceedingly widespread as the third most common element and the most common metal of the Earth's crust (Rudnick and Gao 2003). The soil solutions in most temperate forests at soil pH values above 4.5 are buffered by base cations (van Breemen et al. 1983). However, acid deposition has shifted some forest soils previously buffered by base cations in Europe and North America to be buffered by Al as cation stores are depleted (de Vries et al. 1995, DeHayes et al. 1999, Driscoll et al. 2001, Bowman et al. 2008). Aluminum bioavailability in the soil is intrinsically connected to soil acidification (Kinraide 1991). In acidic soil conditions (below pH 5) Al is found predominately as disassociated Al^{3+} , which is considered the most bioavailable form of Al. At pH levels below 4.5, Al^{3+} can dominate the acid buffering reactions of the soil solution (Bowman et al. 2008). At higher pH values Al occurs as less available hydroxy (e.g., $\text{Al}(\text{OH})_4^-$) or solid phase (e.g., $\text{Al}(\text{OH})_3$) forms (Wagatsuma and Kaneko 1987, Kinraide and Parker 1990, Kinraide 1991). Organic, organometallic and other Al

complexes, such as with phosphorus (P) (e.g., AlHPO_4^+ , $\text{AlH}_2\text{PO}_4^{2+}$), can also influence the bioavailability of Al (Ma et al. 2001, Nguyen et al. 2003).

Aluminum released during the weathering of soil parent materials and anthropogenic soil acidification effectively competes with soil Ca and can greatly reduce the availability of Ca on soil exchange sites (Huntington et al. 2000). Aluminum greatly depresses the cation exchange capacity (CEC) of forest soils (Rampazzo and Blum 1992). Protonation may occur concurrently with Al^{3+} competition for exchange sites, and H^+ may deplete Al at extremely low soil pH (as soil pH falls below 3.2 to 3.5) (Bowman et al. 2008). Soils on schist and granite are especially likely to release Al^{3+} to the soil solution during soil acidification (Merino et al. 2000). Soil Ca:Al ratios have been used as indicators of stress levels in forested ecosystems and have been corroborated by Ca:Al in plant tissues (Cronan and Grigal 1995). As soils are increasingly acidified by anthropogenic means in Europe, Asia, and North America, agriculture and forestry are further burdened by the increase in bioavailable and toxic Al^{3+} in soil (Weber-Blaschke and Rehfuss 2002, Courchesne et al. 2005, Fenn et al. 2006, Guo et al. 2007, Zhang et al. 2007, Zhen et al. 2007, Houde and Diallo 2008).

1.5. Aluminum Toxicity in Plant and Tree Physiology

Bioavailable Al^{3+} is widely considered the most toxic form of Al (Delhaize and Ryan 1995). Aluminum is not known to be a plant nutrient (Salisbury and Ross 1992), and has long been associated with plant root damage and reductions in root growth on acid soils (Hartwell and Pember 1918). It has been estimated that more than half of the

land on Earth suitable for cultivation is acidic, and that acid deposition and widespread use of acidifying fertilizers and soil amendments are quickly increasing the extent of soil acidification and associated Al toxicity (von Uexkull and Mutert 1995). Since at least the 1980's, Al toxicity in soils acidified by acidic deposition has been implicated as a major factor in forest decline (McLaughlin 1985).

Two predominant mechanisms are proposed in plant adaptation to Al toxicity, these are 1) exclusion and 2) tolerance (Kochian et al. 2005). The majority of Al-adapted plants use the exclusion mechanism (Kochian et al. 2005). Plant species such as *Polygonum* spp. and *Pinus taeda* exhibit both mechanisms (Ma et al. 2001, Nowak and Friend 2006). The exclusion mechanism works primarily by the exudation at root apices of carboxylate organic acids that form organometallic complexes (ligands) with Al such as with oxalate, citrate, and malate that detoxify Al in soil adjacent to the root as well as in the extracellular environment (de la Fuente et al. 1997, Tesfaye et al. 2001, Ma et al. 2001, Nguyen et al. 2003, Kochian et al. 2005). In Al-adapted plants this form of exclusion greatly increases organic exudation into the rhizosphere and adjacent soils (Poschenrieder et al. 2008). Aluminum tolerant plants also detoxify Al through the formation of carboxylate ligands within plants that can accumulate in vacuoles; often in the root but sometimes in other organs including stems and leaves (Ma et al. 2001, Nguyen et al. 2003). As confirmation of the above, accumulation of Al on or within the root occurs for a number of plant species, including many forest trees, (Thornton et al. 1986a, Thornton et al. 1986b, Schaedle et al. 1989, Nguyen et al. 2003), and root cell

walls contain many potential sites for Al binding and detoxification through root accumulation (Poschenrieder et al. 2008).

A third mechanism by which plants adapt to Al toxicity is through an induced defense strategy. Aluminum tolerant plants are known to up-regulate the transcription of genes encoding antioxidant enzyme and organic acid production (Houde and Diallo 2008). Aluminum toxicity increases the production of reactive oxygen species (ROS) and elicits the production of antioxidant enzymes in adapted plants (Richards et al. 1998, Boscolo et al. 2003, Panda et al. 2003). Those plants not adapted to Al exposure are likely subject to greater lipid peroxidation and cellular damage by ROS (Yamamoto et al. 2001), especially in the vicinity of the photosynthetic apparatus (Becana 2007). Al toxicity may also impair mitochondria, causing excessive ROS production during cellular respiration, and resulting in the depletion of ATP (Yamamoto et al. 2002).

Alterations in root morphology and reductions in shoot and root biomass have commonly been reported in plant and tree response to Al toxicity (Thornton et al. 1986*a*, Thornton et al. 1986*b*, Schaedle et al. 1989, Delhaize and Ryan 1995, Nowak and Friend 2006). Potential mechanisms for this Al toxicity response in biomass reduction are many, but could include factors such as 1) overall reduction in metabolism through the reported down-regulation of the production of enzymes which drive the Krebs cycle (Zhang et al. 2007), 2) potential increased demand for photosynthate from increases in organic root exudation (Ma et al. 2001), 3) decreased photosynthesis (Chen et al. 2005*b*), 4) decreased root surface area for uptake (Delhaize and Ryan 1995), 5) alterations in carbohydrate synthesis and allocation (Tabuchi et al. 2004, Hossain et al. 2005, Giannakoula et al.

2008), 6) inhibition of active transport through the plasma membrane (Ahn et al. 2001), and 7) increased oxidative stress and photoinhibition resulting in inefficient photosynthesis (Chen et al. 2005a).

1.6. Soil Enzyme Systems and Extracellular Enzyme Activity Bioassay

Beyond the considerable influences of Ca nutrition and Al toxicity on plant function and form, Ca deficiencies and excess Al availability also influence biological processes in the soil matrix. Calcium concentrations were found to significantly affect enzyme activity in an agricultural soil (Acosta-Martínez and Tabatabai 2000). It is the presence of anions from acidic deposition, such as SO_4^- , in the soil solution that make Ca^{2+} cations in the soil solution susceptible to depletion by leaching (Huntington et al. 2000). In addition to disrupting soil and plant Ca and Al relationships, acidic deposition, including S and N deposition, can have strong impacts on soil enzyme activity. The presence of excess SO_4^- anions in the soil solution is known to inhibit the activity of arylsulphatase, the primary sulphatase in forest soils (Prietzel 2001), involved not only in the S cycle, as in the release of organic-bound S which predominates in forest soils, but also in other mineral cycles as well (e.g., the P cycle). Nitrogen deposition is associated with significant impacts on hydrolytic soil enzymes and decomposition (Saiya-Cork et al. 2002, DeForest et al. 2004, Sinsabaugh et al. 2004, 2005). In the northern hardwood forest at the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, SO_4^- anions are known to persist in the organic soil horizons and continue to impact a range of biogeochemical cycles, even though S deposition has decreased

(Likens et al. 2002). Nitrogen deposition in the Northeast, on the other hand, has not shown a decreasing pattern, and it is estimated that N levels in soil have increased to five to ten times above their concentrations preceding industrialization (Galloway et al. 2003, Aber et al. 2003).

The soil, itself, has many characteristics of a living organism. An example is the presence of a plethora of enzymes catalyzing a great number of biochemical reactions and processes, enabling the interconnection of forest strata through the medium of the soil. It has been suggested that the bulk of enzymes in soil are not found within the roots, microbes and fauna of the soil, but rather in the soil solution itself as exuded by all of these organismal strata to facilitate the biochemical reactions upon which these organisms rely for their existence (Brady and Weil 2002).

Calcium has a role in the functionality of many enzymes, sometimes by serving as a structural component of the enzyme molecule (e.g., as in some arylsulphatases), by being an important secondary messenger in the signal transduction process, or by improving the efficiency of the chemical reactions that are catalyzed by enzymes (e.g., as with foliar L-asparaginase activity) (Sieciechowicz et al. 1988). L-asparaginase is also an extracellular enzyme product of soil bacteria. Studies of bacterial enzymes have shown increased activity or activation of many of these enzymes in the presence of Ca (Bernard et al. 1998, Barwe et al. 2001, Konno et al. 2002, Reggiani and Bertani 2003, Berteau et al. 2006).

Chitinase is an enzyme prevalent in soils where fungi are an important part of the decomposition process (such as in northern hardwood forest soils), and is exuded by

bacteria, fungi, soil invertebrates and plant roots (Paul 2007). Chitin is a polysaccharide and the prominent component of the cell walls of most fungal organisms within the soil (Raven et al. 1999). Insects in the order Collembola are known to consume the hyphae of soil fungi, and the symbiant *Bacillus* spp. in their gut are known to produce chitinase (König 2006). Chitinase is involved in plant root and cell wall defense mechanisms against penetration by pathogenic fungal haustoria from organisms such as *Phytophthora* (Hodge et al. 1995, Gaulin et al. 2006). Chitinase is also required in the initiation and maintenance of the symbiotic relationships between mycorrhizae and plant roots, such as the relationship between *Picea abies* and the ectomycorrhizal (EM) fly agaric mushroom *Amanita muscaria* (Sauter and Hager 1989). Several EM plant symbiants were recently reported to release chitinase in a defensive response to pathogenic infection of their plant hosts by *Rhizoctonia solani* (Tang et al. 2008). Calcium is also necessary in these relationships between plants and mycorrhizae, as well as in symbiant relationships with microbes (Macció et al. 2001, Chen et al 2007). Barwe et al. 2001 found the activity of chitinase in cucumber cotyledons to be increased six-fold by external application of Ca chloride and increased five-fold by Ca ionophores after chitinase induction with zeatin, a Ca-sensitive kinase, in response to a likely Ca-modulated cytokinin signal. In a study of a limed agricultural soil, Ca application was found to increase soil chitinase activity (Acosta-Martínez and Tabatabai 2000) as well as activity of soil proteases such as L-asparaginase, and hydrolases such as arylsulphatase, β -glucosidase, but diminish the activity of acid phosphatase.

β -glucosidase (a cellobiosidase) acts on hemicellulose and cellulose, polysaccharide components of plant cell walls. β -glucosidase is important in plant growth and facilitates cell wall expansion (Salisbury and Ross 1992). It is also produced by fungal plant pathogens as haustoria penetrate cell walls and likely plays a role in disease and symbiont recognition in plants. β -glucosidase is also generated by the fungal decomposers of leaf litter and soil O horizons prevalent in forest soils (Paul 2007). Under Ca deficiency in cultured carrot cells, β -glucosidase production increased over a thirty day period and production of extracellular protein exudates increased three-fold while cell growth was impaired (Konno et al. 2002). This suggests the involvement of Ca in a feedback system associated with plant growth and β -glucosidase production. Similar feedback mechanisms involving Ca bioavailability are likely at work in the soil pool of extracellular enzymes.

1.7. Concluding Remarks

The majority of studies of Al toxicity in plants are pot studies of acute Al exposure to plants under artificial conditions (Thornton et al. 1986a, Thornton et al. 1986b, Schaedle et al. 1989, Balsberg Pahlsson 1990, Boscolo et al. 2003, Hossain et al. 2005). In contrast, evidence from a long-term pot study indicates that chronic and comparatively lower levels of Al exposure can induce Al toxicity responses in *Picea abies* (Heim et al. 2003). Long-term study in the field, in particular in mature, native forests dominated by sugar maple (a potentially vulnerable species; Schaberg et al. 2006), will be of even greater value than pot studies in understanding Al toxicity and global

trends in increased bioavailability of Al under ambient conditions. Such field study would also provide a broader perspective into the understanding of the interactions between forest trees and the other biological strata of forest ecosystems, especially soil flora and fauna because the complexity of the soil matrix cannot be truly duplicated in confined, artificial containers.

Further understanding of the impacts of acid deposition and subsequent soil acidification, Ca depletion, and increased bioavailability of toxic Al species in forest soils is needed if we are to continue to rely on forest resources and ecosystem services. Knowledge of alterations to carbohydrate relationships in trees brought about by progressive anthropogenic soil acidification and associated reductions in available Ca and increases in bioavailable Al in native forests will be key to understanding potential impacts to C sequestration as well as to forest productivity. Calcium depletion can disrupt plant energy relations and reduce tree woody growth. In addition, Al toxicity can divert C stores toward protective measures (e.g., antioxidant production and activity) and away from biomass accrual. These responses to altered Ca nutrition and Al toxicity may be pertinent to all forest trees when meaningful thresholds in Ca and Al availability are crossed. However, given that ecologically dominant trees generally have greater access to nutrients, water, light, and substrate within forests (Whittaker 1953), the influence of acid-induced soil cation perturbations may be especially meaningful for this class of trees because they can dominate fluxes in many ecosystem services (e.g., C sequestration, water cycling, etc.). These dominant trees are ecosystem foundation species upon which a diversity of species relies. If greater stress is detected among these trees, this could

implicate acid deposition in significant physiological disruptions and potentially in broader alterations of ecosystem integrity and function. In addition, other measures of forest ecosystem function are needed to bring about a better, more integrated understanding of such impacts to all ecosystem strata at their confluence in the soil matrix. Bioassays of extracellular soil enzymes provide such a tool, because enzymes are the products of genetic and environmental interactions across many ecosystem strata, and are likely to be influenced by Ca and Al dynamics. New England, and especially the Hubbard Brook Experimental Forest, where inputs of acid deposition and soil Ca depletion are well documented, provides fertile ground (by literally being the opposite) to test the nature and extent of physiological and ecological disruptions associated with acid-induced perturbations in cation relationships. Science and society need to better understand this potential threat to ecosystem function and stability if we are to safeguard the many ecosystem services provided by the Northern Forest.

Chapter 2: Journal Article

SOIL CALCIUM AND ALUMINUM ADDITIONS ALTER ECOSYSTEM PROCESSES IN NORTHERN HARDWOOD FOREST

Homer L. Elliott,¹ Paul G. Schaberg,² Gary J. Hawley,¹ Paula F. Murakami,²
Deborah A. Neher,³ and Christopher Eager⁴

¹*The University of Vermont, Rubenstein School of Environment and Natural Resources,
Burlington, Vermont 05405 USA*

²*Forest Service, U.S. Department of Agriculture, Northern Research Station, South
Burlington, Vermont 05403 USA*

³*The University of Vermont, Department of Plant and Soil Science, Burlington,
Vermont 05405 USA*

⁴*Forest Service, U.S. Department of Agriculture, Northern Research Station, Durham,
New Hampshire 03824 USA*

2.2. Abstract

Calcium (Ca) depletion and increased bioavailability of aluminum (Al) are potential consequences of soil acidification caused by acid deposition and other anthropogenic factors. These changes in Ca and Al availability have been shown to alter some physiology in tree species. However, little analysis has been conducted on impacts in mature hardwood forests, including soluble carbohydrates and antioxidants in dominant canopy trees, and enzymes in forest soils involved in mineralization and decomposition. We measured foliar elemental concentrations, foliar antioxidant enzyme activities, foliar and woody shoot carbohydrates in sugar maple (*Acer saccharum*), and soil extracellular enzyme activities (EEA) at a long-term nutrient perturbation study (NuPert) at the Hubbard Brook Experimental Forest, New Hampshire, USA. Treated NuPert plots received Ca to increase soil Ca above ambient depleted levels or Al to compete with Ca and further reduce Ca availability. Additions of Ca to soil are associated with greater Ca concentrations in foliage compared to leaves from trees in control and Al-addition plots. Soil Al-additions are associated with lower foliar phosphorus concentrations in comparison with foliage from trees in Ca-addition plots. Although Al concentrations in leaves appeared unaffected by soil Al-treatment, additions of Al to soil are associated with higher antioxidant enzyme (glutathione reductase and ascorbate peroxidase) activities in foliage and lower shoot sugar (total sugars, sucrose, glucose and fructose) concentrations relative to trees in Ca-addition and control plots. We propose that Al accumulations in distal (likely root) tissues triggered toxicity responses that we report for leaves and stems. Soil EEA results highlight treatment-

induced alterations to soil processes. Across soil enzyme systems EEA levels are greatest in Al-addition plots in fall, but are elevated in Ca-addition plots in spring compared with ambient conditions, suggesting impacts to specific soil communities. Within this native, mature northern hardwood forest, we detect early indications of ecosystem response to Ca and Al manipulation including Al-induced oxidative stress and resulting carbohydrate irregularities in sugar maple trees, and substantial seasonal swings in EEA: processes that could foreshadow broader ecosystem alterations as anthropogenic disruptions of soil Ca and Al availability continue.

Key words: acidic deposition; soil acidification; calcium depletion; aluminum bioavailability; sugar maple; antioxidants; GR; APX; CAT; carbohydrates; EEA

2.3. Introduction

Calcium (Ca) is a biologically necessary nutrient, the presence and availability of which in forested ecosystems is governed through the interplay of numerous natural processes, including atmospheric additions, mineral weathering, soil formation, plant uptake and growth, forest stand dynamics, and leaching losses (Likens et al. 1998). Mounting evidence indicates that a variety of anthropogenic influences, including inputs of acid deposition (Likens et al. 1996), nitrogen (N) saturation (Aber et al. 1998, 2003), forest harvesting (Federer et al. 1989, Hultberg and Ferm 2004), changing climatic conditions (Tomlinson 1993), soil aluminum (Al) mobilization (Lawrence et al. 1995), and declines in atmospheric base cation deposition (Hedin et al. 1994) are altering biogeochemical cycles and depleting Ca from terrestrial ecosystems. Chief among these drivers of Ca loss is the pollution-induced acidification of the soil matrix and through-fall leaching of aboveground strata causing an imbalance between the rate of Ca entry into ecosystems and the loss of exchangeable Ca (Likens et al. 1996, 1998, Driscoll et al. 2001, Hultberg and Ferm 2004). Net Ca depletion occurs when the rate of Ca loss exceeds the supply of Ca into the ecosystem. Although many regions of the world are susceptible to acidification-induced Ca depletion (including parts of North America, Europe and Asia) New England is among the regions uniquely vulnerable because, 1) regional soils and geology, often have little capacity to release Ca to the soil matrix through weathering (Hornbeck et al. 1997, Fenn et al. 2006), and 2) the region is downwind of population centers that include a number of coal-fired power plants and other stationary and mobile fossil fuel combustion sources which contribute to region-

wide inputs of acidic deposition (National Atmospheric Deposition Program or NADP data, annual data summaries 1997-2007). By altering the bioavailability of Ca, and also Al, which competes with Ca in biological systems, acidic deposition is likely altering the presence and function of Ca in forest systems (DeHayes et al. 1999, Schaberg et al. 2001).

Calcium is an essential element in physiology, and its relatively unique qualities give the molecule many roles throughout the phylogeny of life. For example, Ca has a primary role in supporting plant structure and cellular membrane integrity (Bangerth 1979). Perhaps more importantly, Ca's unique binding and electrochemical properties give its ionic form Ca^{2+} a plethora of functions in biochemical pathways and feedback systems. These include enzymatic pathways (Pan and Dilley 2000, Tozawa et al. 2008), evolution of oxygen and other components of photosynthesis (Dilley 2004, Miqyass et al. 2007), and signaling, sensing, and response to environmental factors and stressors (Abbasi et al. 2004, Medvedev 2005). Calcium is also critical in the biosynthesis and transport of the structural carbohydrate components that comprise wood and much of the other biomass in forests (Lautner et al. 2007, Duncan and Huber 2007). Thus, among other influences, adequate Ca stores are necessary to support the ability of the forest to sequester carbon (C) and to produce carbon-intensive forest products, which have direct relevance to overall C cycling and climate change. Because of its biological importance in so many processes, depletion of Ca could have serious ecological and economic impacts on forest health, productivity, and reliant ecosystem services.

In addition to displacing exchangeable Ca via protonation and, thereby, increasing Ca leaching and loss, acidic deposition mobilizes soil Al (Shamrikova et al. 2005). In acidified soils, Al often enters the soil solution as the cation Al^{3+} . In soil, Al^{3+} is shown to compete with Ca^{2+} for uptake by plants, exacerbating Ca deficiencies (Bruce et al. 1988, Rengel and Elliott 1992). Aluminum as Al^{3+} can also be directly phytotoxic (Kinraide 1991). To limit potential Al toxicity, plants have evolved various carbon-intensive compensatory mechanisms, especially those which exclude Al from roots, or hyperaccumulate Al within roots limiting Al transport and toxicity to above-ground tissues (Marschner 2002, Kochian et al. 2005, Poschenrieder et al. 2008). Nonetheless, Al can be transported to stem and leaf tissues and trigger initial signs of toxicity, including 1) oxidative stress eliciting antioxidant response, (Yamamoto et al. 2002, Boscolo et al. 2003, Panda et al. 2003, Ezaki et al. 2005); and 2) alterations in carbohydrate physiology (Balsberg Pålsson 1990). Interest in the importance of soil Al mobility and Al toxicity in plants is increasing worldwide as soils are increasingly anthropogenically acidified (Weber-Blaschke and Rehfues 2002, Courchesne et al. 2005, Zhang et al. 2007, Zhen et al. 2007, Houde and Diallo 2008).

Although changes in ecosystem Ca and Al availability may be broadly pertinent to the health and productivities of forests, some species are likely to be particularly susceptible to perturbations in the cycling of these cations (DeHayes et al. 1999, Schaberg et al. 2001). For example, sugar maple (*Acer saccharum* Marsh.), a tree of ecological and economic importance, is among the species found to be sensitive to Ca and Al levels found in acidified forest soils (Thornton et al. 1986a). Indeed, imbalances

of soil Ca and Al concentrations are implicated in declines of sugar maple in the Northeast and adjacent Canada (Ouimet et al. 2001, Duchesne et al. 2005, Fenn et al. 2006, Schaberg et al. 2006).

Throughout much of the Northern Harwood Forest sugar maple is a dominant keystone species (Horsley et al. 2002). Therefore, Ca and Al-induced alterations to the physiology and health of sugar maple are likely to extend beyond this one species and influence the myriad of species found with it that rely on C inputs associated with rapidly decomposing litter and the intensive Ca cycling of the species (Dijkstra and Smits 2002, Dijkstra 2003). For example, in Québec beneath healthy sugar maple trees on soils with Ca addition and associated reductions in available Al, the diversity of Collembola (and microbial biomass) markedly increased as compared to beneath trees with ambient conditions of little exchangeable Ca and greater available Al in the soil (Chagnon et al. 2001).

The Nutrient Perturbation Experiment (NuPert) at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire is an ideal location to explore the relationship between disturbances of bioavailable Al and Ca and potential impacts on forest ecosystem health and function. At HBEF, Ca depletion and acidic deposition are well documented, and it is estimated that half of the exchangeable Ca has been depleted from the soil profile by acidic deposition (Likens et al. 1998). The NuPert experiment is dominated by sugar maple, has long-term replicated additions of soil Ca and Al, and is proximal to NADP data collection at HBEF. Recent work at NuPert provides evidence of

lessened decline symptoms, improved tree health and growth due to Ca-addition, although no signs of Al toxicity had been noted to date (Huggett et al. 2007).

Here, we report on new research at NuPert that builds upon past measures of tree Ca nutrition and physiology to broaden measures of Ca deficiency and explicitly test for early signs of potential Al toxicity in the dominant sugar maple trees at this site. Furthermore, to expand assessments beyond previous tree-based measures, we also present assessments of extracellular enzyme activity (EEA) in soil to evaluate the influence of Ca and Al-treatments on part of the soil matrix. The utility of using EEA to monitor the responses in functional capacity (C decomposition and mineralization especially) of fungi and bacteria to anthropogenic changes has been demonstrated in response to N deposition in sugar maple forests (Carreiro et al. 2000), but to our knowledge EEA has not been evaluated in response to anthropogenic changes in Ca and Al availability. Extracellular enzymes in the soils at NuPert originate from microbes (here, bacteria, micro-fungi, and similar organisms), mycorrhizae and other symbionts, saprotrophs, grazers, predators, micro and macroinvertebrates, Plantae, and other flora and fauna. Contributions of enzymes to soil come through many processes including root growth, root exudation, litterfall and decomposition, floral and faunal death, and deposition of faunal fecal material. As such, EEA measures provide an integrated analysis of the influence of Ca and Al-treatment on many biological strata that converge in the soil matrix.

2.4. Methods

2.4.1. Study Site

The HBEF is part of the White Mountain National Forest near West Thornton, New Hampshire, and exists on metasedimentary and granitic geologies within the Northern Hardwood Forest. The HBEF is an LTER (Long Term Ecological Research) site, where acid-induced Ca depletion in soils is studied intensively (Likens et al. 1996, 1998). Calcium addition in 1999 to an experimental watershed at the HBEF has been associated with lessened sugar maple decline symptoms within that watershed, and improved colonization of mycorrhizal symbionts in sugar maple (Juice et al. 2006). Soil Ca-addition has been made over a longer term at the NuPert experiment at the HBEF. NuPert consists of twelve randomly selected elevationally similar (716 m to 762 m) 45 m² plots, with four replicates of three soil fertilization treatments: Ca-addition, control (no treatment and representative of ambient Ca-depleted conditions), and Al-addition. Calcium was applied as CaCl₂ from 1995 until 1998, and afterwards as wollastonite (CaSiO₃) (Table 1). Wollastonite forms through the contact metamorphism of interbedded limestone and silicate sand-derived geologies, and provides a long-term release form of Ca. Calcium additions approximate Ca availability levels in the soil before anthropogenic atmospheric deposition depleted Ca pools at HBEF (Likens et al. 1998). Aluminum additions were included in the NuPert experiment primarily to reduce Ca availability through competitive uptake where Al occupies Ca exchange sites. Secondarily, additions of AlCl₃ at NuPert contribute to acidity (Lewis acid) and reflect

the increased Al bioavailability associated with progressive soil acidification and Ca depletion through time.

2.4.2. Tree-based Measures

2.4.2.1. Sample Collection. Five dominant or co-dominant sugar maple trees were used for all tree-based measures in each NuPert plot. Branches of sugar maple (woody shoots and foliage) were collected from sun-lit portions of the canopy using a shotgun in August 2006. Due to technical problems with the shotgun, only three of the four treatment replications at NuPert were sampled. Twelve leaves free of fungal damage, herbivory, or decay, and four 5 mm x 20 mm woody shoots were collected from each tree for foliar and shoot measures. Samples were placed into resealable plastic bags and frozen on dry ice in the field for transport. Once in the laboratory, all were kept at -80° C until further analyses were conducted. Leaf samples were analyzed to measure sugar, starch, and elemental concentrations, and were used to assay the activities of three antioxidant enzymes. Shoot tissues were analyzed to measure sugar and starch concentrations. All tree-based measures were based on well-established protocols.

2.4.2.2. Foliar Elemental Analysis. In the laboratory, sugar maple leaves from NuPert and NIST (National Institute of Standards and Technology) apple and peach leaves as standards were oven-dried at 70° C and ground using a Wiley Mill to pass through a 2 mm screen. Using the block digest methods of Issac and Johnson (1976), 0.25 g of dried, ground leaves of samples and standards were placed into digestion tubes.

Following this, the foliage was then run through a series of H₂SO₄ acid and H₂SeO₃ digestions where two Teflon™ boiling chips and 7 ml of Se / sulfuric acid digest solution were added to each tube, and the contents brought to 400° C. After this temperature was reached 3 mL of 30% H₂O₂ was added to each tube and heated for 30 minutes. After cooling, each sample was brought to 75 ml adding de-ionized distilled water and homogenized with a vortex mixer. Cation concentrations of Al, Ca, K, Fe, Mg, Mn and P were then analyzed by Varian Vista (Varian Inc., Palo Alto CA) inductively coupled plasma atomic emission spectrometry (ICP-AES). Tissue standards were within 5% of NIST certified values for each element analyzed.

2.4.2.3. Foliar Antioxidant Enzyme Activity. Enzyme activity was assayed of ascorbate peroxidase (APX, E.C. 1.11.1.11), glutathione reductase (GR, E.C. 1.6.4.2), and catalase (CAT, E.C. 1.11.1.6). Extraction procedures, were adapted from the methods of Pell et al. (1999), where leaf blade tissues, exclusive of larger vascular tissues, were frozen in liquid N and ground with mortar and pestle. Homogenization of 0.2 g of frozen tissue for 30 s in a 2 mM buffer of 90 mM potassium phosphate (pH 7.8), 1.0 mM ethylenediaminetetraacetic acid (EDTA), 5.0 mM ascorbate, 4% polyvinylpolypyrrolidone (PVPP), 1.5% polyvinylpolypyrrolidone (PVP-40T), and 8% glycerol used a homogenizer (Brinkman Instruments, Inc., Westbury NY), and was followed by centrifugation (Sorvall Ultra 80 centrifuge, DuPont Co., Wilmington, DE) at 15,850 g for 15 min. at 4° C. Processed samples were then frozen at -80° C until each of the antioxidant enzyme activity assays were performed. For all antioxidant enzyme

systems, measurements used for linear calculations were from the dynamic ranges of calibration curves to estimate enzyme activities.

Ascorbate peroxidase activity was determined using the methods of Nakano and Asada (1981). Briefly, APX activity ($\mu\text{mol ascorbate min}^{-1} \text{mg}^{-1}$) was calculated from spectrophotometric measurement (DU800 UV/VIS spectrophotometer, Beckman-Coulter, Inc., Fullerton, CA) of the linear decrease in absorbance at 290 nm (extinction coefficient $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) of a 1 mL reaction mixture of 50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.15 mM H_2O_2 , and 10 μL of sample extract, with final activity corrected by subtraction of ascorbate activity and non-enzymatic ascorbate breakdown.

Glutathione reductase activity ($\mu\text{mol TNB min}^{-1} \text{mg}^{-1}$) was quantified according to the methods of Smith et al. (1988) and Pell et al. (1999). Here the 1mL reaction mixture consisted of 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), 0.5 mM 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), 0.2 glutathione oxidoreductase (GSSG), and 50 10 μL of sample extract. Linear increases in absorbance of DTNB reduced to GSH were measured at 412 nm (extinction coefficient $14.15 \text{ mM}^{-1} \text{ cm}^{-1}$) for 120 s.

Catalase activity ($\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$) was ascertained using the methods of Aebi (1984) from a reaction mixture containing 50 mM potassium phosphate (pH 7.0), 10 mM H_2O_2 , and 10-30 μL of sample extract. Linear reductions in absorbance were measured at 240 nm (extinction coefficient $43.6 \text{ mM}^{-1} \text{ cm}^{-1}$) for 30 s.

2.4.2.4. Carbohydrate Analysis. Soluble carbohydrates were measured using methods adapted from Hinesley et al. (1992). Carbohydrate concentrations were expressed as mg / cm³ dry weight for leaves and as mg / g dry weight for woody tissue. Pith, bark, phloem and cambium from woody tissue samples were removed prior to other sample processing. After sample preparation, soluble carbohydrates were extracted into 80% ethanol and centrifuged (Sorvall Ultra 80 centrifuge, DuPont Co., Wilmington, DE) to separate the ethanol supernatant containing the carbohydrates from the pellet. Pellets from the woody tissue samples were saved for starch analysis. For leaf tissue, chlorophyll was removed from the soluble sugar ethanol supernatant using a C18 Sep-Pak Plus Cartridge (Waters Corporation, Milford, MA). Sub-samples of the filtered supernatant from the woody and foliar samples were dried at 37° C in limited volume inserts, reconstituted in 200 µl 0.1 mM Ca EDTA and filtered through a 0.45 µm syringe filter. Then, samples were analyzed by high performance liquid chromatography (HPLC) for glucose, fructose, sucrose, stachyose, raffinose and xylose using a Waters HPLC with a Sugar-Pak column (Waters Corporation, Milford, MA). The column was maintained at 90° C and 0.1 mM Ca EDTA was used as the solvent at a flow rate of 0.6 ml min⁻¹. Sugar concentrations were quantified using Waters Millennium™ 2000 software.

After being separated from the ethanol supernatant, the pellets from the woody tissue samples were gelatinized with 0.2 N KOH, boiled for 30 minutes in a water bath, and neutralized with 1 M acetic acid. Solubilized starch from these samples was then hydrolyzed to glucose with amyloglucosidase (E.C. 3.2.1.3, #10115, Fluka Chemical Co., Ronkonkoma, NY) in 0.1 M acetate buffer (pH 4.5), incubated at 55° C for 30 min, and

the reaction terminated by boiling for 4 min. This step was followed by centrifugation for 10 min at 3000 rpm, and the starch content quantified from the supernatant by assaying for glucose (glucose assay #115-A, Sigma Chemical Co., St. Louis, MO) as described by Hendrix (1993). Samples and glucose standards were read with an EL_X 800_{UV} universal microplate reader (BioTek Instruments, Inc., Winooski, VT) at 492 nm. Starch concentrations were calculated using glucose standard curves.

2.4.3. Soil-based Measures

2.4.3.1. Sample Collection. Soil cores, 2.5 cm across and 10 cm deep, were collected with a soil core sampler (Oakfield Apparatus Corporation, Goleta, CA) in September 2007 and May 2008. The 10 cm depth was chosen to sample both the O and the proximal portion of the A or E horizons of the spodosol, and the range of collection dates covered the most active enzyme activity periods for Northern Hardwood Forest soil profiles (Myers et al. 2001, Sinsabaugh et al. 2004). Three cores were randomly collected from each treatment plot interior well away from peripheral influences, providing a total of 36 cores per collection date. Results from preliminary tests using nine soil cores per plot for the EEA assays indicated that three cores per plot would be sufficient to account for the variability within individual plots for hydrolytic and proteolytic enzyme activities within individual plots during summer (when tests were conducted; data not shown).

Upon collection, sample cores were placed into Nasco Whirl-Pak® collection bags, sealed and packed with dry ice in a cooler for transport to the laboratory. Samples

were stored at -80° C until EEA assays were performed. This storage temperature kept the enzyme activity of the soil samples suspended prior to analysis (Bélangier et al. 1997).

2.4.3.2 Extracellular Enzyme Activity Assays. Thirty-six samples were collected on each of two sampling dates, generating 72 total samples for EEA analysis. A suite of substrates, including methylumbelliferone salts and L-serine-7-amido-4-methylcoumarin (Sigma Chemical Co., St. Louis, MO) was selected for fluorometric measurement of hydrolytic and proteolytic enzymes involved in the soil processes of bacterial (here referring to organisms such as Archaea, Bacteria, and Eucarya) and fungal decomposition and mineralization (Coleman et al. 2004, Coleman 2008). EEA can be interpreted as a quantitative measure as well as a qualitative measure of the functionality of these processes in soils (Sinsabaugh et al. 2008). In addition, enzyme systems for which Ca either directly or indirectly plays a role in their efficiency, production, or structure were preferentially assessed (Bernard et al. 1998, Acosta-Martínez and Tabatabai 2000, Berteau et al. 2006). The enzyme activities of phosphatase (E.C. 3.1.3.2), sulphatase (E.C. 3.1.6.1), β -glucosidase (E.C. 3.2.1.21), chitinase (E.C. 3.2.1.14), and xylosidase (E.C. 3.2.1.37) were measured in fall and spring. β -cellobiosidase (E.C. 3.2.1.91) activity was assayed in fall only, while the measurement of serine protease (E.C. 4.3.1.17) activity was only assayed in the spring.

Overall EEA methodology followed well-established protocols (Saiya-Cork et al. 2002, Sinsabaugh et al. 1993, Sinsabaugh et al. 2002, Sinsabaugh et al. 2004). Frozen soil cores (-80° C) were thawed and immediately processed for EEA. For sample

processing, 1 g of each homogenized sample was placed in 100 mL of 50 mM sodium acetate buffer solution titrated with acetic acid to the average ambient pH of the samples (determined separately, data not shown). The solution was kept agitated, and 200 μL of the solution was micropipetted into sterile, black 96-well polystyrene plates (NUNC TM, Fisher Thermal-Scientific, Rochester, NY) and combined with the appropriate 50 μL of 200 μM MUF or methylcoumarin substrate (16 wells per sample, 3 samples per plate), or 50 μL 10 μM MUF or methylcoumarin (8 wells per sample) standard, the remaining 24 wells each with 250 μL of buffer (8 wells), buffer and standard (8 wells), or buffer and substrate (8 wells) for assay. Control plates with 8 wells each per sample consisting of 200 μL of the buffer sample mixture and an additional 50 μL buffer were used to provide comparisons needed for activity calculations. After 3 hrs of incubation at 20° C, enzyme activity was assayed fluorometrically (BioTek Instruments, Inc., Winooski, VT). Enzyme activity ($\mu\text{mol hr}^{-1} \text{ g}^{-1}$) was calculated from 16 subsamples per sample, extraneous parameters and bulk density adjusted for, and averaged to obtain 36 data points per assay.

2.4.4. Statistical Analyses

Analysis of variance (ANOVA) was conducted using the fit model procedure in the JMP 5.1 software package (SAS Institute, Cary, North Carolina) to test for treatment differences in tree-based and soil-based measures associated with Ca and Al addition. For tree-based data, significance tests utilized a fully nested design in which treatment differences were tested with the mean square for plot within treatment, and plot

differences were tested with the mean square for tree within plot (Montgomery 2001). Examples of ANOVA tables for this analysis appear in Table 2 (A and B). Soil-based EEA data for individual enzyme systems were also tested using a fully nested design in which treatment differences were tested with the mean square for plot within treatment, and plot differences were tested with the mean square for sample within plot. A mixed model was employed to test for differences among EEA means from all enzyme systems attributable to treatment, enzyme system, and the interaction of treatment and enzyme system within the otherwise nested design at NuPert (Montgomery 2001). Examples of ANOVA tables for this analysis appear in Table 2 (C and D). The impact of seasonal change in EEA across enzyme systems ($\Delta = \text{May activity} - \text{September activity}$) was also evaluated using this mixed model design. Here, negative activity values indicate greater EEA activity in September and positive values indicate greater EEA activity in May. Specific differences among treatments for all ANOVA were tested using Tukey's HSD post hoc test. When necessary, tree-based and soil EEA data were transformed ($\text{Log}_{10}y$, y^4 , or $1/y$) prior to statistical analyses to fulfill assumptions of ANOVA. Correlation analysis was used to assess the possible mechanistic association among tree-based measures and better understand physiological responses to treatment. Results were considered significant if $P < 0.05$, unless noted otherwise.

2.5. Results

2.5.1. Tree-based Measures

2.5.1.1. Foliar Elemental Analysis. Trees in the Ca-addition plots had increased foliar Ca concentrations relative to trees in the Al-addition and control plots (Table 3). Resulting concentrations in the foliage from trees on Ca-addition plots was above the sufficiency threshold of 5000 $\mu\text{g/g}$ documented for sugar maple trees (Kolb and McCormick 1993). Aluminum treatment was associated with reductions in concentrations of foliar P in trees relative to trees in the Ca-treatment (Table 3). The critical level of P in foliage for health in sugar maple has been estimated to be between 1000 $\mu\text{g/g}$ and 1200 $\mu\text{g/g}$ (Ouimet and Camiré 1995). Foliar concentrations of P were well above this threshold in the trees from the Ca-addition plots ($1390 \pm 50 \mu\text{g/g}$), while the P concentrations in foliage of trees from the control plots were just above this estimate ($1210 \pm 30 \mu\text{g/g}$). In trees from Al-addition, the P concentrations in foliage may have been deficient or were barely meeting the sufficiency threshold ($1140 \pm 30 \mu\text{g/g}$). No differences in foliar nutrition attributable to soil treatments were detected for foliar Mg, Mn, and Al. Even for trees on Al-addition plots, foliar Al concentrations were below the 32 $\mu\text{g/g}$ to 60 $\mu\text{g/g}$ reported for healthy sugar maple trees (Kolb and McCormick 1993).

2.5.1.2. Foliar Antioxidant Enzyme Systems. In two of the three enzyme systems assayed (Fig. 1A and B, GR and APX), Al-treatment was associated with higher foliar antioxidant activity relative to trees in the other treatment groups. Glutathione reductase activity (Fig. 1A) in the foliage of trees from the Al-addition soils was more than six times the activity of the trees from the control and Ca-addition treatments ($F_{2,6} =$

29.75, $P < 0.001$). For APX (Fig. 1B), antioxidant activity was lowest in the leaves of trees from the Ca-treatment, intermediate activity levels were found in leaves of trees from control plots, and the greatest APX activity was measured in the foliage of trees from the Al soil treatment ($F_{2, 6} = 19.54$, $P = 0.002$). No differences in CAT activity associated with treatment were found (Fig. 1C, $F_{2, 6} = 1.62$, $P = 0.275$), which is a response similar to that reported for maize following Al-treatment (Boscolo et al. 2003). Several significant but slight correlations between the activities of foliar antioxidant enzyme systems and foliar Ca concentrations were detected for all trees regardless of treatment. In general, greater concentrations of foliar Ca were associated with decreased GR ($r = -0.31$, $P = 0.046$) and APX ($r = -0.42$, $P = 0.005$) activities, and with increased CAT activity ($r = 0.46$, $P = 0.002$).

2.5.1.3. Foliar Carbohydrates. No significant differences in foliar carbohydrates attributable to treatment were found, although there tended to be greater glucose accumulations within the leaves of trees from the Al-treatment ($P = 0.088$, Table 3). When all trees regardless of treatment were evaluated, a weak positive correlation was found for the concentrations of foliar sucrose/total sugars and Ca ($r = 0.36$, $P = 0.018$), and a weak negative correlation existed between the concentrations of foliar fructose/total sugars and Ca ($r = -0.38$, $P = 0.012$). In general, the concentrations of sucrose increase, and the concentrations of fructose and glucose decrease in foliage as Ca concentrations rise. Similarly, weak correlations between foliar Al and soluble carbohydrate concentrations exist. Foliar Al and sucrose/total sugar concentrations were negatively

correlated ($r = -0.33$, $P = 0.030$), and foliar Al and fructose/total sugar concentrations were positively correlated ($r = 0.38$, $P = 0.012$).

2.5.1.4. Woody Shoot Carbohydrates. Consistent treatment differences were detected in shoot sugar concentrations. Lower concentrations of sugars were found in shoots of trees from Al-treatment plots relative to trees from other treatments for total sugars, sucrose, and fructose (Fig. 2A, B, and D). Trees in Al-treated plots have smaller glucose concentrations in their shoots than trees from control plots, while trees in Ca plots show intermediate (but statistically indistinguishable) concentrations of glucose (Fig. 2C). Concentrations of raffinose, xylose, and starch were similar among treatments (Fig. 2E, F, and G). Numerous linear correlations between foliar Al and shoot carbohydrate concentrations exist when data from all trees were analyzed. For example, wood glucose was correlated positively with foliar Al concentration ($r = 0.50$, $P < 0.001$), while woody total sugar, sucrose, and xylose concentrations had slight negative correlations with foliar Al ($r = -0.39$, $P = 0.011$; $r = -0.48$, $P = 0.001$, $r = -0.35$, $P = 0.023$, respectively).

2.5.2. Soil-based Measures of Extracellular Enzymes

2.5.2.1. September 2007 EEA Assays. Across all enzyme systems in fall, enzyme activity measurements from the Al-treatment are higher than those from control plots, with measurements from the Ca-treatment being intermediate and indistinguishable from those of the Al-treatment (Fig. 3A; $F_{2, 8} = 11.68$, $P = 0.004$). Five of the six

individual enzyme system assays exhibited this same general trend in activity. However, only for the assays of β -glucosidase and xylosidase assays (Fig. 3C and E; $P = 0.097$ and $P = 0.084$, $df = 2, 6$) did these patterns approach statistical significance.

2.5.2.2. May 2008 EEA Assays. For enzyme activity across all enzyme systems, means differed among all three treatments (Fig. 4A, $F = 146.87_{2, 8}$, $P < 0.001$), with activities highest for samples from the Ca-treatment, lowest for samples from control plots, and intermediate for samples from the Al-treatment. All six of the individual EEA assays in May (Fig. 4 B-G) showed a similar pattern among treatment means as seen for EEA measurements across enzyme systems with activity levels higher for samples from Ca-addition plots and lower for samples from Al-addition and control plots. This pattern was distinguishable for two of the six individual assays. Phosphatase activity was higher for samples from Ca-addition plots relative to measurements from the other treatments (Fig. 4B, $F_{2, 9} = 6.58$, $P = 0.017$). In addition, β -glucosidase activity was higher for samples from the Ca-addition relative to samples from control plots, with enzyme activity of samples from Al plots being intermediate and indistinguishable from levels measured for the other treatments (Fig. 4C, $F_{2, 9} = 6.91$, $P = 0.012$).

2.5.2.3. Seasonal Fluxes in Soil EEA. Seasonal changes ($\Delta = \text{May activity} - \text{September activity}$) across all enzyme systems ($F_{2, 8} = 20.66$, $P < 0.001$) were observed among treatments (Fig. 5). Negative values in Fig. 5A indicate greater activity in September, while positive values indicate greater activity in May. Enzyme activity levels

among soil cores from Ca-addition plots increased from September to May, whereas levels decreased from September to May for samples from the Al-addition plots (Fig. 5B). Samples from the control plots showed relatively little change in activity levels through time, especially as compared to the samples from Ca-addition plots (Fig. 5B).

2.6. Discussion

2.6.1. Tree-based Responses in a Forested Ecosystem

Foliar elemental analysis indicates that soil treatments alter foliar Ca and P concentrations in mature sugar maple trees (Table 2). In particular, foliar Ca levels in trees from the Ca-treatment were at least 2800 $\mu\text{g/g}$ above the species-specific deficiency threshold of 5000 $\mu\text{g/g}$ for sugar maple trees (Kolb and McCormick 1993). In addition, foliar P was lower in trees from Al-addition plots relative to those from Ca-addition plots. Past experiments have show that Ca fertilization can increase (Moore et al. 2000, Wilmot et al. 1996), decrease (Moore and Ouimet 2006, Ouimet and Fortin 1992), or not alter (Long et al. 1997) the P concentrations of sugar maple leaves. Differences in sugar maple foliar P nutrition in these other experiments may have occurred because of other factors in addition to the Ca-treatment, such differences in the source of Ca-treatment (e.g., dolomitic lime which may contain other cations also influencing nutrition), the amount of Ca-treatment, parent geology and soil, or other factors. Furthermore, although soil Al-treatment typically increases the P content of roots, including sugar maple roots, it

decreases the P content of aboveground plant tissues (Thornton et al. 1986a, b, Liang et al. 2001, Quartin et al. 2001). The unequal distribution of P in plants exposed to bioavailable Al results from the formation of Al and P organic complexes in and exuded from roots, which inhibit P availability and transport to shoots (Hodson and Evans 1995, Liang et al. 2001, Nguyen et al. 2003). Whatever the causes, in our study foliar P levels associated with Al-addition ($1140 \pm 30 \mu\text{g/g}$) were at the lower limit of sufficiency or may have been at deficiency levels ($1100 \pm 100 \mu\text{g/g}$) for sugar maple trees (Ouimet and Camiré 1995). Other studies have shown that the health and productivity of sugar maple trees may be limited by disruptions to P nutrition associated with acidic deposition, particularly N deposition (Gradowski and Thomas 2006).

Previously at NuPert, Ca-treatment was associated with increased wound closure, increased annual basal area increment growth, and greater crown vigor, as well as decreased branch dieback for intermediate crown class sugar maple trees relative to trees in Al-addition and control plots (Huggett et al. 2007). No increases in foliar Al or changes in growth and health were associated with Al treatment by Huggett et al. (2007). In contrast, in the current study the Al-treatment is associated with significant changes in plant physiology. In particular, Al-treatment is associated with a six-fold increase in the activities of two of the three foliar antioxidant enzyme systems analyzed (GR and APX, Fig. 1A, B). We interpret these increases in APX and GR activity as an indication of elevated oxidative stress in trees from Al-addition plots. Aluminum toxicity is known to heighten oxidative stress and cause the accumulation of reactive oxygen species (ROS) in plant tissues, and instigates the production of antioxidant enzymes that serve as protective

defense compounds (Panda et al. 2003, Chen 2006, Becana 2007, Houde and Diallo 2008). Aluminum treatment to soil also results in significantly lower concentrations of fructose, sucrose, and total sugars in woody stems in comparison to the concentrations of these carbohydrates found in trees exposed to ambient conditions (control) or soil Ca treatment (Fig. 2A-D).

Differences in Al concentrations in foliage due to soil Al treatment were not detectable, yet physiological differences in trees due to soil Al treatment were evident. These physiological influences include increased antioxidant enzyme activity and reductions in sugar concentrations in woody shoots. We looked for patterns across all sampled trees in order to more thoroughly examine the relationship between Al concentrations in foliage and physiological differences. Correlation analyses further highlight the potential associations between Al-treatment and plant C relations at NuPert. Foliar Al concentrations were negatively correlated with foliar sucrose/total sugar concentrations and were positively correlated with foliar fructose/total sugar concentrations. These correlations may reflect an association between greater Al in leaves and a reduced capacity for sucrose production from component hexoses in leaves, and impaired transport of sucrose to stems and labile stores of other soluble carbohydrates. This mechanistic explanation is consistent both with the literature (e.g., in Lautner et al. (2007) where a similar pattern was observed in *Populus* spp. and further associated with reductions in wood formation). A mechanistic association between Al and carbohydrate concentrations is also consistent with our treatment-based results of low concentrations of woody stem carbohydrates (total sugars, sucrose, and fructose) in trees

from Al-addition plots relative to trees in Ca-addition and control plots. These treatment-based differences occurred even though these foliar Al concentrations were a third to half of the lower range limit (32 $\mu\text{g/g}$) of foliar Al levels reported for healthy sugar maple trees (Kolb and McCormick 1993). A definable threshold of Al toxicity in sugar maple trees in the field has yet to be determined. However, our report of increased oxidative stress for trees in Al-treated plots and reduced stem carbohydrates for trees with foliar Al concentrations lower than the range reported for healthy sugar maple trees suggest that Al toxicity may occur at foliar Al concentrations previously thought to be safe. Schaberg et al. (2006) also found that foliar concentrations of Al below those reported by Kolb and McCormick (1993) were associated with toxicity, here as reduced growth of sugar maples in the field.

There are many reasons why Al-treatment could reduce tissue sugar storage. Prominently, Al toxicity is known to reduce the quantity of photosynthetic pigments and decrease gross and net photosynthesis in a range of plant species (see reviews by Roy et al. 1988, Chen 2006). In addition, because Al-treatment reduces C assimilation more than it does light absorption capacity, it can also increase the risk of photooxidative damage in leaves (Chen et al. 2005a, Becana 2007). The resulting increase in photooxidative stress triggers the production of antioxidant enzymes including APX and GR that partially protect sensitive tissues (Chen et al. 2005b). Notwithstanding this protective influence, reductions in soluble carbohydrates often follow protracted Al-treatment (Chen 2006). Furthermore, increases in oxidative stress and associated reductions in C relations likely extend beyond those associated with Al-induced

alterations of plant photosystems. Yamamoto et al. (2002) found that Al-treatment instantaneously suppresses mitochondrial activity and that, after about 12 h, this triggers the production of ROS, a decrease in cellular respiration, a depletion of ATP, and a cessation of growth.

At NuPert, the increases in antioxidant activity (oxidative protection) and associated reductions in shoot sugar concentrations may result from Al-induced alterations in oxidative stress and photosystem functions that have been commonly reported for a wide-range of plant species and tissue types (see review by Chen 2006). However, increased Al availability is also known to reduce P concentrations in leaves and shoots, thereby affecting ATP cycling and ATP-dependent H⁺ transport (Liang et al. 2001), which is a critical biochemical component of cell wall growth and extension (Salisbury and Ross 1992). Because the foliage of Al-treated trees has P concentrations that at most barely meet sufficiency standards for sugar maple, it is possible that low P availability contributes to the more direct influences of Al on cell ATP and energy relations already under strain from physiological stress. Validation of these hypotheses at NuPert awaits experimental analysis.

The combined effects of Al-treatment that we report are the first signs that protracted Al-treatment is now altering the physiology and health of sugar maple trees at NuPert. Changes in foliar physiology were not accompanied by detectable alterations in foliar Al concentrations. This may be expected because many plants preferentially sequester Al in below-ground sinks; by forming carbohydrate intensive organic soil complexes with Al formed through the exudation of organic acids from root apices; or

through hyperaccumulation and detoxification of Al in root cell walls (Ma et al. 2001, Kochian et al. 2005, Poschenrieder et al. 2008). Furthermore, the most dramatic effects of Al toxicity are often seen as morphological changes of roots (Delhaize and Ryan 1995, Hossain et al. 2005). Thus, it is possible that biologically relevant concentrations of Al may exist within plant roots but not be evident at the leaf level. Indeed, our EEA analyses indicate that the soil treatments at NuPert influence belowground processes.

2.6.2. Soil-based Responses in a Forested Ecosystem

Extracellular enzyme activity is interpreted as a direct measure of the potential functionality of soil processes (Carreiro et al. 2000, Sinsabaugh et. al 2008). Here, we assay the activities of extracellular enzymes that are involved in fundamental ecosystem processes including mineralization (phosphatases and sulphatases), hydrolytic organic decomposition (β -glucosidase, β -cellobiosidase, and xylosidase) and the breaking of bonds in more recalcitrant or amide-bonded substances (chitinase and L-serinase protease). The enzymes we assayed are involved in the cycling of C, N, P and S in soil (Acosta-Martínez and Tabatabai 2000). Soil Ca and Al-additions at NuPert have various influences on EEA, most notably causing decided seasonal swings in enzyme activities across enzyme systems (see Figs. 3A, 4A, and 5).

Aluminum is known to cause considerable increases to the organic acid exudation from plants into the soil matrix (Ma et al. 2001) potentially influencing the formation of soil aggregates and providing hydrolytic substrate for decomposition and mineralization (Coleman 2008). Organic acid leakage could also represent a shunting of

carbohydrates away from tree growth to the soil as a defense mechanism (Poschenrieder et al. 2008), and our results for woody stem carbohydrates are consistent with this hypothesis. Protective organic-metal complexes form which may simultaneously limit Al toxicity and consume available P (Hodson and Evans 1995, Nguyen et al. 2003, Coleman et al. 2004). Aluminum also causes an accumulation of P in plant roots (Roy et al. 1988), increasing P competition among ecosystem strata in the adjacent soil. Alone or in combination, these Al-induced changes likely have a notable impact on the enzyme activities in the rhizosphere and proximal soils. Our measure of low foliar P nutrition in the Al-treatment plot samples relative to those from Ca-addition is consistent with the possibility of reductions in soil P availability with Al-addition. The potential for P immobilization would be greatest in soil horizons with high organic contents similar to the ones sampled for this study.

Our measure of greater EEA of phosphatase in the Ca-addition plots relative to the other plots is consistent with higher potential P mineralization in these plots in spring, this may be influential in providing the greater foliar P nutrition seen in the sugar maple trees growing on these plots. It is also likely that the greater P concentrations in sugar maple leaves from Ca-addition plots are also found in the litterfall from these trees, this potential source of P in substrate may, itself, cause the greater EEA of phosphatase in the soils of Ca-addition plots.

Greater EEA of β -glucosidase in the Ca-addition plots in spring indicates that greater rates of decomposition are occurring in these plots in spring (Carreiro et al. 2000, Sinsabaugh et al. 2004). β -glucosidase is particularly important in the break-down of

cellulose from leaves (Carreiro et al. 2000). The low activity of β -glucosidase on control plots may indicate that decomposition could be impaired here (as well as in ambient conditions) in spring. We document greatest enzyme activities across enzyme systems within the Al-addition plots as compared to ambient soils in fall, and a greater upsurge of enzyme activity in the Ca-addition soils relative to the Al-addition and control treatments in spring. In other studies, fall and spring are peaks of enzyme activity and of associated bacterial/fungal biomass in a range of healthy northern hardwood forests where sugar maple trees dominate (Myers et al. 2001, Sinsabaugh et al. 2004). The relative lack of activity or seasonal change in EEA across enzyme systems (Δ = May activity –September activity, Fig. 5) in the untreated plots relative to the activities in the Al- and Ca-additions is notable, and may indicate a substantial inhibition of soil enzyme systems under ambient conditions at NuPert, with neither fungal nor bacterial components dominating soil flora. A peak of EEA activity in the fall is associated with an increase in fungal activity in the hardwood forest at that time (Myers et al. 2001, Sinsabaugh et al. 2004).

Our measure of September enzyme activity across enzyme systems (Fig 3A) indicates a higher activity in fall in the Al-addition plots as compared to soils in control plots. We speculate that the higher EEA across enzyme systems in fall (Fig 3A) could indicate that Al-treatment favors the fungal contingent of the soil. Soil fungi are known to be considerably more tolerant of acidity than the majority of soil bacteria (Coleman et al. 2004). In our study, the lowest pH values (determined separately, data not shown) were found in the Al-addition plots, which is consistent with the speculation that Al-addition to soil favors the fungal community over the bacterial community. Even though

pH was lowest in the soils with Al-addition in fall as compared to the other soil treatments, Al-addition plots had the highest enzyme activities in fall. This potentially indicates that the effect on activity was more likely attributable to differences in soil community composition rather than to a direct effect of pH on enzyme activity. This may reflect influence of pH on community composition. Other possible influences of Al-addition to soil on sources of extracellular enzymes include Al toxicity to roots and microbes, and through Al-induced changes in root exudation, as from Al-induced plant exudation of carboxylates and resulting changes in soil flora (Weisskopf et al. 2008). Typically, the spring increase of EEA activity in the hardwood forest is associated with the soil bacterial and bacterial grazer communities, potentially favored by wetter spring conditions. Bacteria and associated soil fauna continue the processes of decay, mineralization, mobilization and immobilization begun by the fungi in fall (Myers et al. 2001, Sinsabaugh et al. 2004). We note greater EEA activity in spring in the plots with soil Ca-addition. Our results (Fig. 4A, B, C) are consistent with greater activity of the bacterial community in the spring in the Ca-addition plots as compared to Al-addition plots. However, enzyme activity across enzyme systems in control plots seems impaired relative to enzyme activity in Al-addition plots in spring (Fig 4A), suggesting a complex response to Ca and Al soil additions as well as partially inhibited mineralization and decomposition under ambient conditions. The intensive Ca cycling in sugar maple dominated forests (Dijkstra and Smits 2002, Dijkstra 2003), may be an important factor influencing this complexity. Increases in bacterial biomass have been associated with Ca-addition and associated reductions in available Al beneath sugar maples in Québec

(Chagnon et al. 2001), but the capacity of these soils for enzyme-driven processes was not investigated. Phosphatase and β -glucosidase are enzymes that catalyze reactions involved in mineralization and decomposition (Carreiro et al. 2000, Sinsabaugh et al. 2008). Our individual EEA assays of phosphatase and β -glucosidase in May (Fig. 4 B, C) are consistent with greater capacity for spring mineralization in Ca-addition plots relative to control and Al-addition plots, and greater potential spring decomposition in the Ca-addition plots as compared to control soils. Validation with PLFA (phospholipid fatty acid) analyses could help determine the nature, state of stress, and composition of the soil communities at work in the NuPert experiment.

2.6.3. Conclusions

Our study is novel in documenting Al toxicity in dominant trees in a soil cation manipulation study in a mature hardwood forest. This is in contrast to numerous pot and greenhouse-based studies of Al toxicity in seedlings, including sugar maple (Thornton et al. 1986*a*, Thornton et al. 1986*b*, Schaedle et al. 1989, Weber-Blaschke and Rehfuss 2002, Naik et al. 2009). Furthermore, our measures of Al toxicity due to soil Al-addition (increases in foliar antioxidant activity and significantly lower concentrations of soluble sugars in woody shoots) exist despite no leaf-based evidence of increased Al uptake. Indeed, alterations in physiology associated with the Al-addition treatment occur at foliar Al concentrations that are low compared to many sugar maple trees (Kolb and McCormick 1993). This apparent disparity may result from the disproportionate concentration of Al in root tissues, or in organic exudate complexes in the rhizosphere

(Ma et al. 2001, Marschner 2002, Poschenrieder et al. 2008). It is possible that root accumulations of Al can trigger a more systemic toxicity, including physiological alterations in distal tissues that do not directly accumulate toxic levels of Al. Further analysis of sugar maple root P and Al concentrations at NuPert would confirm this speculation. Our results for foliar antioxidants likely indicate that foliar Al levels previously reported as normal (Kolb and McCormick 1993) are actually toxic, as is consistent with other reports for sugar maple (Schaberg et al. 2006). Furthermore, our results for woody shoot carbohydrates indicate that C assimilation and transport from leaves are impaired by increases in soil Al. Although some indications of Al toxicity appear to be ameliorated by soil Ca-addition, such as foliar APX activity, this is not apparent in foliar GR activity or Al-induced changes in shoot sugar concentrations. A lack of consistent ameliorating influence of Ca on Al toxicity symptoms highlights the importance of direct Al toxicity relative to indirect protection or damage due to the competitive interactions of Ca and Al. Furthermore, treatment differences in soil EEA measures that we document suggest that manipulations of soil Al and Ca nutrition have ecosystem implications beyond influences on tree physiology, health, and productivity. However, because treatment differences in soil EEA vary with season, it is clear that influences on these measures (and the life forms that generate these enzymes) are complex and require additional and more detailed analyses.

Our study hints at the relationships between the keystone species of sugar maple and soil communities, but these relationships require further clarification. Soil treatments at NuPert are changing the Ca and P concentrations in foliage, primarily expressed as

improved Ca and P nutrition in foliage from Ca-addition plots. Litterfall from sugar maple trees from Ca-addition plots likely also reflect the improved nutrition of living foliage, especially for Ca because it is functionally immobile in plants and remains in leaves through autumn senescence. Greater EEA of phosphatase activity in the Ca-addition plots suggests a correlation between foliar P and enzyme activity in the soil. It is possible that the litterfall also retains the improved P nutrition measured in the living leaves of these trees. Chagnon et al. (2001) found increased bacterial biomass, and increased diversity and change of dominance in Collembola genera associated with increased Ca nutrition of litterfall in response to liming two years after treatment in a sugar maple forest (where reductions in soil available Al also occurred). At HBEF, Fisk et al. (2006) found that Collembola abundance decreased for the first two years after soil treatment with Ca in watershed 1, but rebounded in the third year after treatment with a change in dominance in elevations dominated by sugar maple (e.g., *Folsomia* no longer dominant). NuPert, with its longer history of soil treatments, would likely better inform our understanding of impacts of anthropogenic soil alterations of soil Ca- and Al-additions on soil faunal communities. Our measure of greater EEA of β -glucosidase activity in Ca-addition plots as compared with control plots is likely associated with bacteria producing this enzyme, and these bacteria are also found in the gut of Collembola (König 2006), so it is possible that there is a relationship between the greater β -glucosidase activity and populations of Collembola. Other strata may benefit from changes in the nutritional status of sugar maple foliage. Foliar herbivores may benefit from this improved nutrition, and in Québec declines in populations of leaf-mining

Lepidoptera have been associated with sugar maples in decline with poor Ca nutrition (Martel and Mauffette 1997). Likewise, avian populations of “canopy snatchers” and “canopy foliage gleaners” (birds that consume such insects) have also shown a negative relationship with a decline index for sugar maple in Québec (Darveau et al. 1997). Declines in sugar maple as a keystone species and alterations in foliar nutrition have implications for other ecosystem strata below and aboveground.

Calcium depletion and widespread soil acidification with associated increases in bioavailable Al have serious implications for global forests. After approximately 12 years of intermittent soil amendment, we have found evidence of oxidative stress and carbohydrate imbalance in dominant trees of a keystone species, and disturbance of forest soil enzyme systems at HBEF associated with treatment, especially Al-addition. However, because a common symptom of advanced Al toxicity in a range of plant species is a reduction in whole plant biomass and growth, both above and below ground, (Thornton et al. 1986*a*, Thornton et al. 1986*b*, Schaedle et al. 1989, Schaberg et al. 2000, Weber-Blaschke and Rehfues 2002, Nguyen et al. 2003, Nowak and Friend 2006), the perturbations that we note may be the precursors of more consequential alterations of tree physiology, forest function and productivity. As such, continued anthropogenic soil acidification that reduces available Ca and increases available Al could further impair ecosystem health beyond the measures we document, and notably diminish forest carbon sequestration by trees at a time when it is most needed to buffer atmospheric C increases.

2.7 Acknowledgements

We acknowledge the generous support of funds provided by the Northeastern States Research Cooperative (NSRC) Theme 2, the Environmental Protection Agency and the McIntire Stennis research program that made this research possible. We are thankful for the editorial and technical support and encouragement so freely given by Kimberly Wallin, Josh Halman, Tom Weicht, Chris Hansen, Tom Saielli, Will Young, Brian Darby, Bret Huggett, Kendra Gurney, Ralph Perron, Ian Halm, and Tim Fahey.

2.8. Literature Cited

- Abbasi, F., H. Onodera, S. Toki, H. Tanaka, and S. Komatsu. 2004. OsCDPK13, a calcium-dependent protein kinase gene from rice, is induced by cold and gibberellin in rice leaf sheath. *Plant Molecular Biology* 55:541-552.
- Aber, J.D., C.L. Goodale, S.V. Ollinger, M-L. Smith, A.H. Magill, M.E. Martin, and R.A. Hallet, J.L. Stoddard. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? *BioScience* 53:375-389.
- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems. *BioScience* 48:921-934.
- Acosta-Martínez, V., and M.A. Tabatabai. 2000. Enzyme activities in a limed agricultural soil. *Biology and Fertility in Soils* 31:85-91.
- Aebi, H. 1984. Catalase *in vitro*. Pages 121-126 in S.P. Colowick and N.O. Kaplan, editors. *Methods of enzymology*. Academic Press, New York, NY, USA.
- Balsberg Pålsson, A-M. 1990. Influence of aluminium on biomass, nutrients, soluble carbohydrates and phenols in beech (*Fagus sylvatica*). *Physiolgia Plantarum* 78:79-84.
- Bangerth, F. 1979. Calcium-related physiological disorders of plants. *Annual Review of Phytopathology* 17:97-122.
- Becana, M. 2007. Oxidative stress as a response to salinity and aluminum. Consortium LOTASSA workshop. October 25-26. Porto Alegre, Brazil.
- Bélanger, C., B. Desrosiers, and K. Lee. 1997. Microbial extracellular enzyme activity in

- marine sediments: extreme pH to terminate reaction and sample storage. *Aquatic Microbial Ecology* 13:187-196.
- Bernard, B.K., S. Tsubuku, and S. Shioya. 1998. Acute toxicity and genotoxicity studies of a microbial transglutaminase. *International Journal of Toxicology* 17:703-721.
- Berteau, O., A. Guillot, A. Benjdia, and S. Rabot. 2006. A new type of bacterial sulfatase reveals a novel maturation pathway in Prokaryotes. *The Journal of Biological Chemistry* 281:22464-22470.
- Boscolo, P.R.S., M. Menossi, and R.A. Jorge. 2003. Aluminum-induced oxidative stress in maize. *Phytochemistry* 62:181-189.
- Bruce, R.C., L.A. Warrell, D.G. Edwards, and L.C. Bell. 1988. Effects of aluminum and calcium in the soil solution of acid soils on root elongation of *Glycine max* cv. Forrest. *Australian Journal of Agricultural Research* 39:319-338.
- Carreiro, M.M., R.L. Sinsabaugh, D.A. Repert, and D.F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359-2365.
- Chagnon, M., D. Paré, C. Hébert, and C. Camiré. Effects of experimental liming on collembolan communities and soil microbial biomass in a southern Quebec sugar maple (*Acer saccharum* Marsh.) stand. *Applied Soil Ecology* 17:81-90.
- Chen, L-S. 2006. Physiological responses and tolerance of plant shoot to aluminum toxicity. *Journal of Plant Physiology and Molecular Biology* 32:143-155.
- Chen L-S., Y-P. Qi, B.R. Smith, and X-H. Liu. 2005a. Aluminum-induced decrease in

- CO₂ assimilation in citrus seedlings is unaccompanied by decreased activities of key enzymes involved in CO₂ assimilation. *Tree Physiology* 25:317-324.
- Chen, L-S., Y-P. Qi, and X-H. Liu. 2005*b*. Effects of aluminum on light energy utilization and photoprotective systems in citrus leaves. *Annals of Botany* 96:35-41.
- Coleman, D.C. 2008. From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. *Soil Biology and Biochemistry* 40: 271-289.
- Coleman, D.C., D.A. Crossley, and P.F. Hendrix. 2004. *Fundamentals of soil ecology*. Elsevier Academic Press, Burlington, MA, USA.
- Courchesne, F., B. Côte', J. W. Fyles, W. H. Hendershot, P. M. Biron, A. G. Roy, and M-C. Turmel. 2005. Recent changes in soil chemistry in a forested ecosystem of southern Québec, Canada. *Soil Science Society of America Journal* 69:1298-1313.
- Darveau, M., J. Martel, J-L DesGranges, and Y. Mauffette. 1997. Associations between forest decline and bird and insect communities in northern hardwoods. *Canadian Journal of Forest Research* 27:876-882.
- DeHayes, D.H., P.G. Schaberg, G.J. Hawley, and G.R. Strimbeck. 1999. Alteration of membrane-associated calcium leads to membrane destabilization and foliar injury in red spruce. *Bioscience* 49:789–800.
- Delhaize, E., and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiology* 107:315-321.
- Dijkstra, F.A. 2003. Calcium mineralization in the forest floor and surface beneath

- different tree species in the northeastern US. *Forest Ecology and Management* 175:185-194.
- Dijkstra, F.A., and M.M. Smits. 2002. Tree species effects on calcium cycling: The role of calcium uptake in deep soils. *Ecosystems* 5:385-398.
- Dilley, R.A. 2004. On why thylakoids energize ATP formation using either delocalized or localized proton gradients – a Ca^{2+} mediated role in thylakoid stress responses. *Photosynthesis Research* 80:245-263.
- Driscoll, C.T., G.B. Lawrence, A.J. Bulger, T.J. Butler, C.S. Cronan, C. Eagar, K.F. Lambert, G.E. Likens, J.L. Stoddard, and K.C. Weathers. 2001. Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *BioScience* 51:180-198.
- Duchesne, L., R. Ouimet, J-D. Moore, and R. Paquin. 2005. Changes in structure and composition of maple–beech stands following sugar maple decline in Québec, Canada. *Forest Ecology and Management* 208:223-236.
- Duncan, K.A., and S.C. Huber. 2007. Sucrose synthase oligomerization and F-actin association are regulated by sucrose concentration and phosphorylation. *Plant Cell Physiology* 48:1612-1623.
- Ezaki B., K. Sasaki, H. Matsumoto, and S. Nakashima. 2005. Functions of two genes in aluminium (Al) stress resistance: repression of oxidative damage by the *AtBCB* gene and promotion of efflux of Al ions by the *NtGDII* gene. *Journal of Experimental Botany* 56:2661-2671.
- Federer, C.A., J.W. Hornbeck, L.M. Tritton, C.W. Martin, R.S. Pierce, and C.T.

- Smith.1989. Long-term depletion of calcium and other nutrients in eastern US forests. *Environmental Management* 14:593-601.
- Fenn, M.E., T.G. Huntington, S.B. McLaughlin, C. Eager, A. Gomez, and R.B. Cook. 2006. Status of soil acidification in North America. *Journal of Forest Science* 52:3-13.
- Fisk, M.C., W.R. Kessler, A. Goodale, T.J. Fahey, P.M. Groffman, and C.T. Driscoll. 2006. Landscape variation in microarthropod response to calcium addition in a northern hardwood forest ecosystem. *Pedobiologia* 50:69-78.
- Gradowski, T., and S.C. Thomas. 2006. Phosphorus limitation of sugar maple growth in central Ontario. *Forest Ecology and Management* 226:104-109.
- Hedin, L.O., L. Granat, G.E. Likens, T.A. Bulshand, J.N. Galloway, T.J. Butler, and H. Rodhe. 1994. Steep declines in atmospheric base cations in regions of Europe and North America. *Nature* 367:351-354.
- Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Science* 33:1306-1311.
- Hinesley, L. E., D. M. Pharr, L. K. Snelling, and S. K. Funderburk. 1992. Foliar raffinose and sucrose in four conifer species: Relation to seasonal temperature. *Journal of the American Society for Horticultural Science* 117:852-855.
- Hodson, M.J, and D.E. Evans. 1995. Aluminium/silicon interactions in higher plants. *Journal of Experimental Botany* 46:161-171.
- Hornbeck, J.W., S.W. Bailey, D.C. Buso, and J. B. Shanley. 1997. Streamwater

- chemistry and nutrient budgets for forested watersheds in New England: Variability and management implications. *Forest Ecology and Management* 93:73-89.
- Horsley, S.B., R.P. Long, S.W. Bailey, R.A. Hallet, and P.M. Wargo. 2002. Health of eastern North American sugar maple forests and factors affecting decline. *Northern Journal of Applied Forestry* 19:34-44.
- Hossain, A.K.M.Z., H. Koyama, and T. Hara. 2005. Sugar compositions and molecular mass distributions of hemicellulosic polysaccharides in wheat plants under aluminum stress at higher level of calcium supply. *Asian Journal of Plant Sciences* 4:11-16.
- Houde, M., and A.O. Diallo. 2008. Identification of genes and pathways associated with aluminum stress and tolerance using transcriptome profiling of wheat near-isogenic lines. *BioMed Central Genomics*. doi:10.1186/1471-2164-9-400.
- Huggett, B.A., P.G. Schaberg, G.J. Hawley, and C. Eagar. 2007. Long-term calcium addition increases growth release, wound closure, and health of sugar maple (*Acer saccharum*) trees at the Hubbard Brook Experimental Forest. *Canadian Journal of Forest Research* 37:1692-1700.
- Hultberg, H., and M. Ferm. 2004. Temporal changes and fluxes of sulphur and calcium in wet and dry deposition, internal circulation as well as in run-off and soil in a forest at Gårdsjön, Sweden. *Biogeochemistry* 68:355-363.
- Issac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue using

- a block digester. Journal of the Association of Official Analytical Chemists 59:98-100.
- Juice, J.M., T.J. Fahey, T.G. Siccama, C. T. Driscoll, E.G. Denny, C. Eagar, N.L. Cleavitt, R. Minocha, and A.D. Richardson. 2006. Response of sugar maple to calcium addition to northern hardwood forest. Ecology 87:1267-1280.
- Kinraide, T.B. 1991. Identity of the rhizotoxic aluminum species. Plant and Soil 134: 167-78.
- Kochian, L.V., M.A. Piñeros, and O.A. Hoekenga. 2005. The physiology, genetics, and molecular biology of plant aluminum resistance and toxicity. Plant and Soil 274:175-195.
- Kolb, T.E., and L.H. McCormick. 1993. Etiology of sugar maple decline in four Pennsylvania stands. Canadian Journal of Forest Research 23:2395-2402.
- König, H. 2006. *Bacillus* species in the intestines of termites and other soil invertebrates. Journal of Applied Microbiology 101:620-627.
- Lautner, S., B. Ehling, E. Windneisen, H. Rennenberg, H. Matyssek, and J. Fromm. 2007. Calcium nutrition has a significant influence on wood formation in poplar. New Phytologist 173:743-752.
- Lawrence, G.B., M.B. David, and W.C. Shortle. 1995. A new mechanism for calcium loss in forest-floor soils. Nature 378:162-165.
- Liang, Y., C. Yang, and H. Shi. 2001. Effects of silicon on growth and mineral composition of barley grown under toxic levels of aluminum. Journal of Plant Nutrition 24:229-243.

- Likens, G.E., C.T. Driscoll, D.C. Buso, M.J. Mitchell , G.M. Lovett, S.W. Bailey, T.G. Siccama, W.A. Reiners, and C. Alewell. 2002. The biogeochemistry of sulfur at Hubbard Brook. *Biogeochemistry* 60:235-316.
- Likens, G.E., C.T. Driscoll, D.C. Buso, T.G. Siccama, C.E. Johnson, G.M. Lovett, T.J. Fahey, W.A. Reiners, D.F. Ryan, C.W. Martin, and S.W. Bailey. 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* 41:89-173.
- Likens, G.E., C.T. Driscoll, and D.C. Buso 1996. Long-term effects of acid rain response and recovery of a forest ecosystem. *Science* 272:244–246.
- Long, R.P., S.B. Horsley, and P.R. Lilja. 1997. Impacts of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Canadian Journal of Forest Research* 27:1560–1573.
- Ma, J.F., P.R. Ryan, and E. Delhaize. 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends In Plant Science* 6:273-278.
- Marschner, H. 2002. *Mineral Nutrition of Higher Plants*. Academic Press, New York, NY, USA.
- Martel, J. and Y. Mauffette. 1997. Lepidopteran communities in temperate deciduous forests affected by forest decline. *Oikos* 78:48-56.
- Medvedev, S.S. 2005. Calcium signaling system in plants. *Russian Journal of Plant Physiology* 52:249-270.
- Miqyass M., H.J. van Gorkom, and C.F. Yocum. 2007. The PSII calcium site revisited. *Photosynthesis Research* 92:275-287.
- Montgomery, D.C. 2001. *Design and analysis of experiments*. John Wiley and Sons, New

York, NY, USA.

Moore, J-D., C. Camiré, and R.Ouimet. 2000. Effects of liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair watershed, Québec, Canada. *Canadian Journal of Forest Research* 30:725–732.

Moore, J-D., and R. Ouimet 2006. Ten-year effect of dolomitic lime on the nutrition, crown vigor, and growth of sugar maple. *Canadian Journal of Forest Research* 36:1834-1841.

Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Science Society of America Journal* 65:359-367.

Naik, D., E. Smith, and J.R. Cumming. 2009. Rhizosphere carbon deposition, oxidative stress and nutritional changes in two poplar species exposed to aluminum. *Tree Physiology* doi:10.1093/treephys/tpn035.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* 22:867-880.

National Atmospheric Deposition Program annual data summaries, 1997- 2007.

National Atmospheric Deposition Program (NRSP-3). 2009. NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.

Nguyen, N.T., K. Nakabayashi, J. Thompson, and K. Fujita. 2003. Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. *Tree Physiology* 23:1041-1050.

Nowak, J., and A. L. Friend 2006. Loblolly pine and slash pine responses to

- acute aluminum and acid exposures. *Tree Physiology* 26:1207-1215.
- Ouimet, R., L. Duchesne, D. Houle, and P.A. Arp. 2001. Critical loads and exceedances of acid deposition and associated forest growth in the northern hardwood and boreal coniferous forests in Québec, Canada. *Water, Air, Soil Pollution: Focus* 1:119-134.
- Ouimet, R., and C. Camiré. 1995. Foliar deficiencies of sugar maple stands associated with soil cation imbalances in the Quebec Appalachians. *Canadian Journal of Soil Science* 75:169-175.
- Ouimet, R., and J-M. Fortin. 1992. Growth and foliar nutrient status of sugar maple: Incidence of forest decline and reaction to fertilization. *Canadian Journal of Forest Research* 22:699-706.
- Pan, R-S., and R.A. Dilley. 2000. Influence of Ca^{2+} on the thylakoid lumen violaxanthin de-epoxidase activity through Ca^{2+} gating of H^+ flux at the CF_0 H^+ channel. *Photosynthesis Research* 65:141–154.
- Panda, S.K., L.B. Singha, and M.H. Khan. 2003. Does aluminum phytotoxicity induce oxidative stress in greengram (*Vigna radiata*)? *Bulgarian Journal of Plant Physiology* 29:77-86.
- Pell, E.J., J.P. Sinn, B.W. Brendley, L. Samuelson, C. Vinten-Johansen, M. Tien, and J. Skillman. 1999. Differential response of four tree species to ozone-induced acceleration of foliar senescence. *Plant, Cell Environment* 22:779-790.
- Poschenrieder, C., B. Gunsé, I. Corrales, and J. Barceló. 2008. A glance into

- aluminum toxicity and resistance in plants. *Science of the Total Environment* 400:356-368.
- Quartin, V.L., H.G. Azinheira, and M.A. Nunes. 2001. Phosphorus deficiency is responsible for biomass reduction of triticale in nutrient solution with aluminum. *Journal of Plant Nutrition* 24:1901-1911.
- Rengel, Z., and D.C. Elliott. 1992. Mechanism of Al inhibition of net $^{43}\text{Ca}^{2+}$ uptake by *Amaranthus* protoplasts. *Plant Physiology* 98:632-638.
- Roy, A.K., A. Sharma, G. Talukder, and K. Talukder. 1988. Some aspects of aluminum toxicity in plants. *Botanical Review* 54:145-178.
- Saiya-Cork, K.R., R.L. Sinsabaugh, and D.R. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry* 34:1309-1315
- Salisbury, F.B., and C.W. Ross. 1992. *Plant physiology*. Wadsworth Publishing Co., Berkley, CA, USA.
- Schaberg, P.G., J.W. Tilley, G.J. Hawley, D.H. DeHayes, and S.W. Bailey. 2006. Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. *Forest Ecology and Management* 223:159-169.
- Schaberg, P.G., D.H. DeHayes, and G.J. Hawley. 2001. Anthropogenic calcium depletion: A unique threat to forest ecosystem health? *Ecosystem Health* 7:214-228.
- Schaberg, P.G., D.H. DeHayes, G.J. Hawley, G.R. Strimbeck, J.R. Cumming,

- P.F. Murakami, and C.H. Borer. 2000. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiology* 20:73-85.
- Schaedle, M., F.C. Thornton, D.J. Raynal, and H.B. Tepper. 1989. Response of tree seedlings to aluminum. *Tree Physiology* 5:337-356.
- Shamrikova, E.V., T.A. Sokolova, and I.V. Zaboieva. 2005. Acid buffer capacity of mineral horizons of podzolic and bog-podzolic soils of the Komi Republic. *Eurasian Soil Science* 38:471-479.
- Sinsabaugh, R.L., C.L. Lauber, M.N. Weintraub, B. Ahmed, S.D. Allison, C. Crenshaw, A.R. Contosta, D. Cusack, S. Frey, M.E. Gallo, T.B. Gartner, S.E. Hobbie, K. Holland, B.L. Keeler, J.S. Powers, M. Stursova, C. Takacs-Vesbach, M.P. Waldrop, M.D. Wallenstein, D.R. Zak, and L.H. Zeglin. 2008. Stoichiometry of enzyme activity at global scale. *Ecology Letters* 11:1252-1264.
- Sinsabaugh, R.L., D.R. Zak, M. Gallo, C. Lauber, and R. Amonette 2004. Nitrogen deposition and dissolved organic carbon production in northern temperate forests. *Soil Biology and Biochemistry* 36:1509-1515.
- Sinsabaugh, R.L., M.M. Carreiro, and D.A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* 60:1-24.
- Sinsabaugh, R.L., R.K. Antibus, A.E. Linkins, C.A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology* 74:1586-1593.
- Smith, I.K., T.L. Vierheller, and C.A. Thorne. 1988. Assay of glutathione

- reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid).
Analytical Biochemistry 175:408-413.
- Thornton, F.C., M. Schaedle, and D.J. Raynal. 1986a. Effect of aluminum on
sugar maple in solution culture. *Canadian Journal of Forest Research* 16:892-896.
- Thornton, F.C., M. Schaedle, and D.J. Raynal. 1986b. Effects of aluminum on
growth, development and nutrient composition of honeylocust (*Gleditsia
triacanthos* L.) seedlings. *Tree Physiology* 2:307-316.
- Tomlinson, G.H. 1993. A possible mechanism relating increased soil temperature
to forest decline. *Water, Air, Soil Pollution* 66:365-380.
- Tozawa, Y., A. Nozawa, T. Kanno, T. Narisawa, S. Masuda, K. Kasai, and H.
Nanamiya. 2008. Calcium-activated (p)ppGpp synthetase in chloroplasts of land
plants. *The Journal of Biological Chemistry* 282:35536–35545.
- Weber-Blaschke, G., and K.E. Rehfuss. 2002. Correction of Al toxicity with
European ash (*Fraxinus excelsior* L.) growing on acid soils by fertilization with
Ca and Mg carbonate and sulfate in pot experiments. *Forest Ecology and
Management* 167:173–183.
- Weisskopf, L., R-C. Le Bayon, F. Kohler, V. Page, M. Jossi, J-M. Gobat, E.
Martinoia, and M. Aragno. 2008. Spatio-temporal dynamics of bacterial
communities associated with two plant species differing in organic acid secretion:
A one-year microcosm study on lupin and wheat. *Soil Biology and Biochemistry*
40:1772-1780.
- Wilmot, T.R., D.S. Ellsworth, and M.T. Tyree. 1996. Base cation fertilization and

liming effects on nutrition and growth of Vermont sugar maple stands.

Forest Ecology and Management 84:123-134.

Yamamoto, Y., Y. Kobayashi, S. R. Devi, S. Rikiishi, and H. Matsumoto. 2002.

Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. *Plant Physiology* 128:63-72.

Zhang, J., Z. He, H. Tian, G. Zhu, and X. Peng. 2007. Identification of aluminium-

responsive genes in rice cultivars with different aluminium sensitivities. *Journal of Experimental Botany* 58:2269-2278.

Zhen, Y., J-L Qi, S-S. Wang, J. Su, G-H. Xu, M-S. Zhang L. Miao, X-X. Ping, D.

Tian, and Y-H. Yang. 2007. Comparative proteome analysis of differentially expressed proteins induced by Al toxicity in soybean. *Physiologia Plantarum* 131: 542–554.

TABLE 1. Dates and treatment applications in the long-term NuPert experiment at the Hubbard Brook Experimental Forest, New Hampshire.

Date of application:	Treatment (g/m ²)		
	Calcium	Control	Aluminum
October-95	2 ^a	-	0.9
May-96	3 ^a	-	0.9
November-96	2 ^a	-	0.9
May-97	3 ^a	-	0.9
October-99	38 ^b	-	1.8
May-01	0	-	0.9
April-02	0	-	0.9
May-04	0	-	0.9
November-05	0	-	0.9
May-08	0	-	0.9
Source:	CaCl ₂ ^a , CaSiO ₃ ^b		AlCl ₃

TABLE 2. Example ANOVA tables for the statistical analyses of tree-based and soil EEA data from the NuPert experiment at the Hubbard Brook Experimental Forest, New Hampshire. For all tree-based data and individual soil-based enzyme systems, a fully nested design was used and is depicted in its generic form (A) and with foliar APX activity (B) as examples. A mixed model was employed to test for differences among EEA means and in seasonal changes in EEA across enzyme systems. Components of this general mixed model (C) and this model applied to May EEA data (D) are depicted.

Fully nested model					
Source of variation	n	df	MS	F	P
Treatment	a	a-1	MS _A	MS _A / MS _{B(A)}	
Plot [treatment]	b	a(b-1)	MS _{B(A)}	MS _{B(A)} / MS _E	
Sample [plot {treatment}] error	c	ab(c-1)	MS _E		
Total		abc-1			

Measure: APX activity					
Source of variation	SS	n	df	MS	P
Treatment	5.27583	3	2	2.63792	0.0022
Plot [treatment]	0.81137	3	6	0.13523	0.3735
Sample/tree [plot {treatment}] error	3.86722	5	36	0.12085	
Total		45	44		

Mixed model					
Source of variation	n	df	MS	F	P
Treatment	a	a-1	MS _A	MS _A / MS _{A X B}	
Enzyme system	b	b-1	MS _B	MS _B / MS _{A X B}	
Enzyme system x treatment	c	(a-1)(b-1)	MS _{A X B}	MS _{A X B} / MS _{B(A)}	
Plot [treatment]		a(c-1)	MS _{B(A)}	MS _{B(A)} / MS _E	
Enzyme system x plot [treatment] error		(b-1){a(c-1)}	MS _E		
Total		abc-1			

Measure: May EEA across enzyme systems*						
Source of variation	SS	n	df	MS	F	P
Treatment	1.15314	3	2	0.57657	146.8677	<0.0001
Enzyme system	6.21651	5	4	1.55413	395.8777	<0.0001
Treatment x enzyme system	0.03141		8	0.00393	0.0340	>0.9990
Plot [treatment]	1.04149	4	9	0.11572	2.7431	0.0150
Enzyme system x plot [treatment] error	1.51872		36	0.04219		
Total		60	59			

*The data required a Log10 transformation for this analysis to fulfill assumptions about the homogeneity of variance.

TABLE 3. Soil addition treatment differences in foliar element concentrations for sugar maple (*Acer saccharum*) trees in summer 2006 from Ca-addition, control, and Al-addition plots in the NuPert experiment at the Hubbard Brook Experimental Forest, New Hampshire.

Element ($\mu\text{g/g}$)	Treatment			df	P
	Calcium	Control	Aluminum		
Al	14.8 \pm 0.7	17.3 \pm 1.1	21.6 \pm 1.9	2, 6	0.2770
Ca	8400.0 ^a \pm 600.0	4700.0 ^b \pm 200.0	4600.0 ^b \pm 400.0	2, 6	0.0182
Fe	42.9 \pm 1.8	40.1 \pm 1.1	39.5 \pm 1.7	2, 6	0.5528
K	9100.0 \pm 400.0	8700.0 \pm 400.0	8800.0 \pm 300.0	2, 6	0.8264
Mg	1100.0 \pm 100.0	1000.0 \pm 100.0	800.0 \pm 100.0	2, 6	0.3929
Mn	1211.7 \pm 122.8	1124.7 \pm 117.4	1030.1 \pm 80.0	2, 6	0.6713
P	1390.0 ^a \pm 50.0	1210.0 ^{ab} \pm 30.0	1140.0 ^b \pm 30.0	2, 6	0.0411

Notes: Element concentration values are foliar means \pm SE. $P < 0.05$ represent significant differences attributable to treatment using ANOVA. Contrasting superscript letters indicate significant ($P < 0.05$) differences among treatments using Tukey's HSD post hoc test.

TABLE 4. Foliar carbohydrate concentrations for sugar maple (*Acer saccharum*) trees in 2006 from soil Ca-addition, control, and Al-addition plots at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire.

Foliar carbohydrates (mg/cm ³)	Treatment			df	P
	Calcium	Control	Aluminum		
Total sugars	0.110 ± 0.010	0.118 ± 0.014	0.118 ± 0.013	2, 6	0.8743
Sucrose	0.092 ± 0.008	0.101 ± 0.013	0.094 ± 0.010	2, 6	0.7829
Glucose	0.009 ± 0.001	0.009 ± 0.001	0.010 ± 0.001	2, 6	0.0878
Fructose	0.010 ± 0.002	0.009 ± 0.001	0.012 ± 0.002	2, 6	0.2669

Notes: Values are carbohydrate concentration means ± SE. ANOVA was used to test for treatment differences.

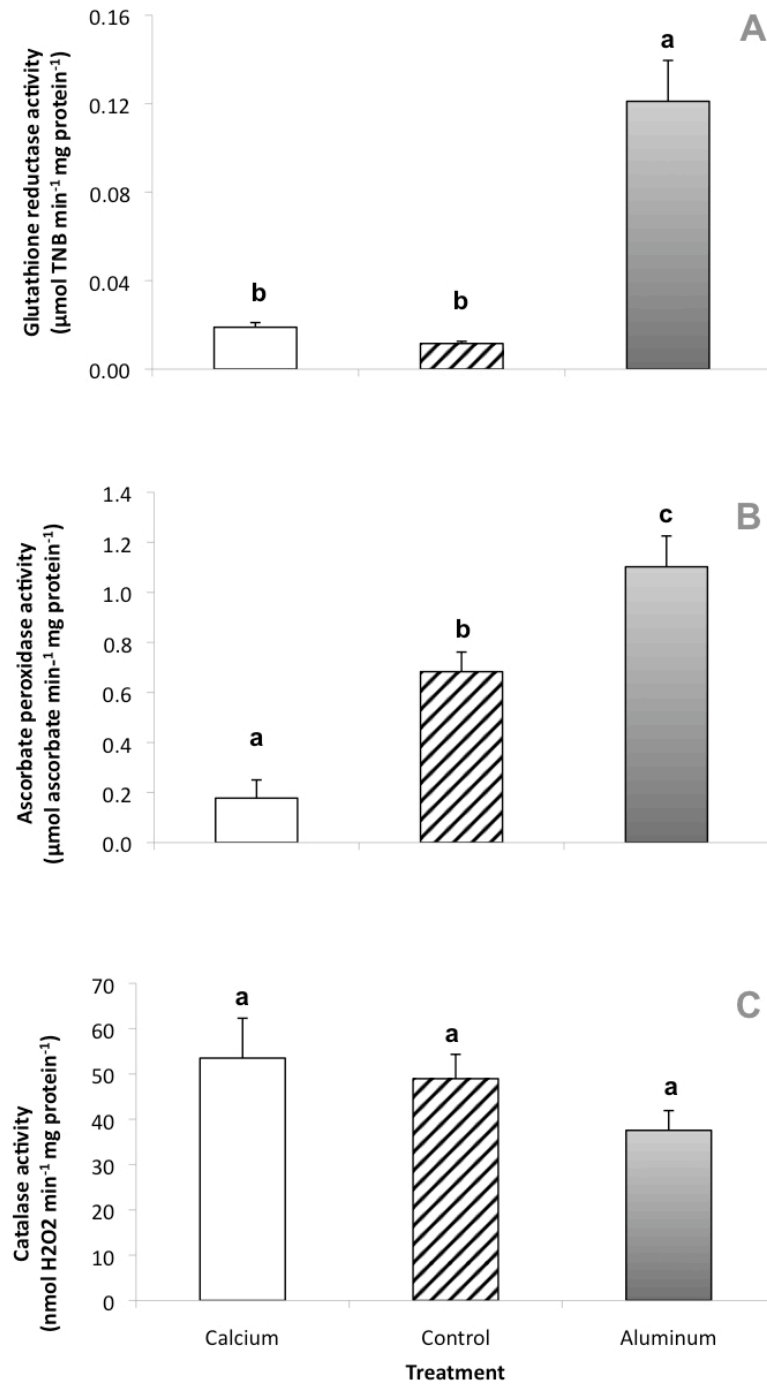


FIG. 1. Foliar glutathione reductase (A), ascorbate peroxidase (B), and catalase (C) antioxidant enzyme activity in summer 2006 for sugar maple (*Acer saccharum*) trees from soil Ca-addition, control, and Al-addition plots at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire. Activity level values are means \pm SE. Contrasting letters above bars denote statistically significant ($P < 0.05$, Tukey's HSD post hoc) differences among treatments.

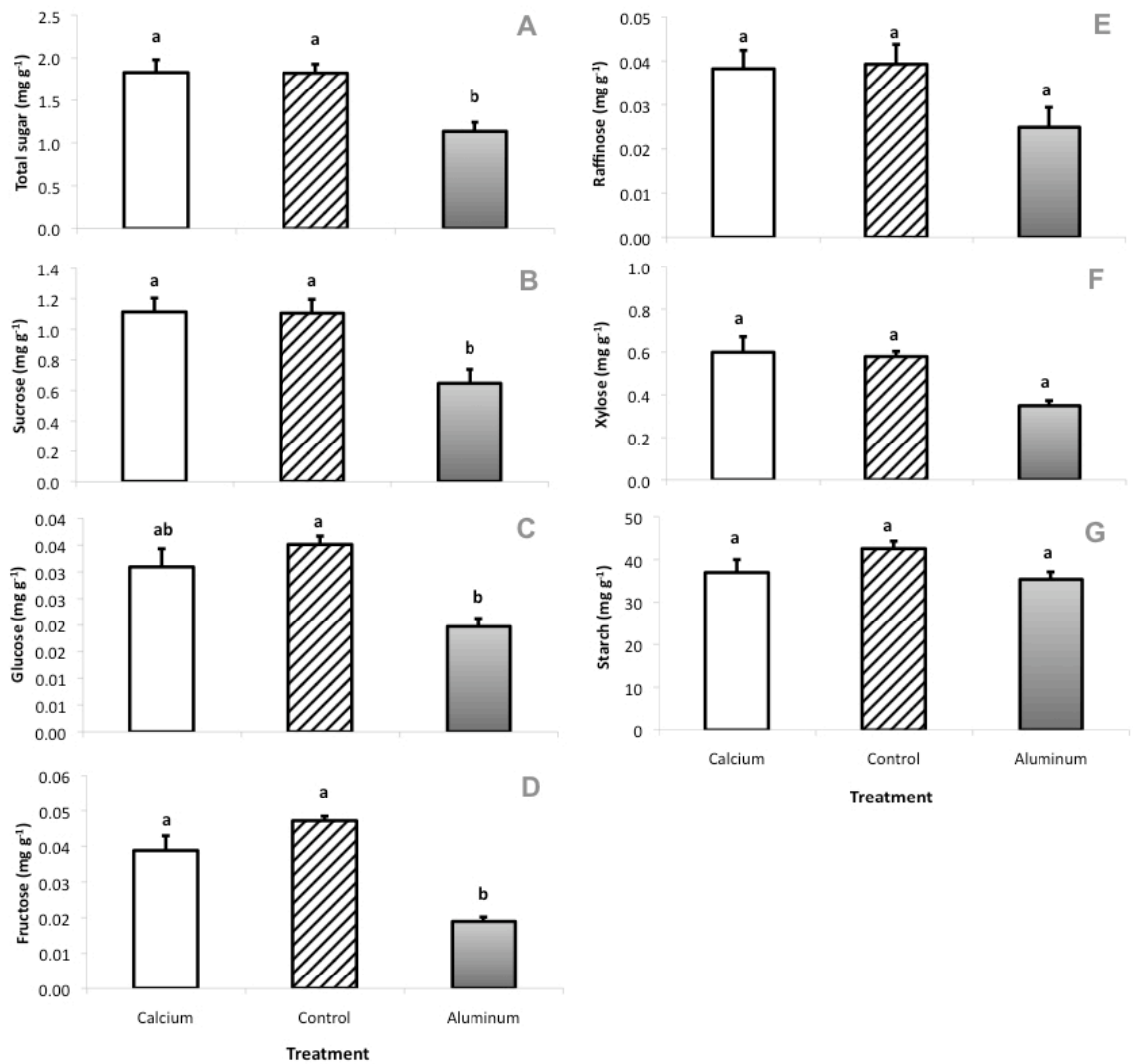


FIG. 2. Woody shoot carbohydrate concentrations of total sugars (A), sucrose (B), glucose (C), fructose (D), raffinose (E), xylose (F), and starch (G) in summer 2006 for sugar maple (*Acer saccharum*) trees from soil Ca-addition, control, and Al-addition plots at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire. Values are means \pm SE. Contrasting letters above bars indicate statistically significant ($P < 0.05$) differences among treatments using Tukey's HSD post hoc test.

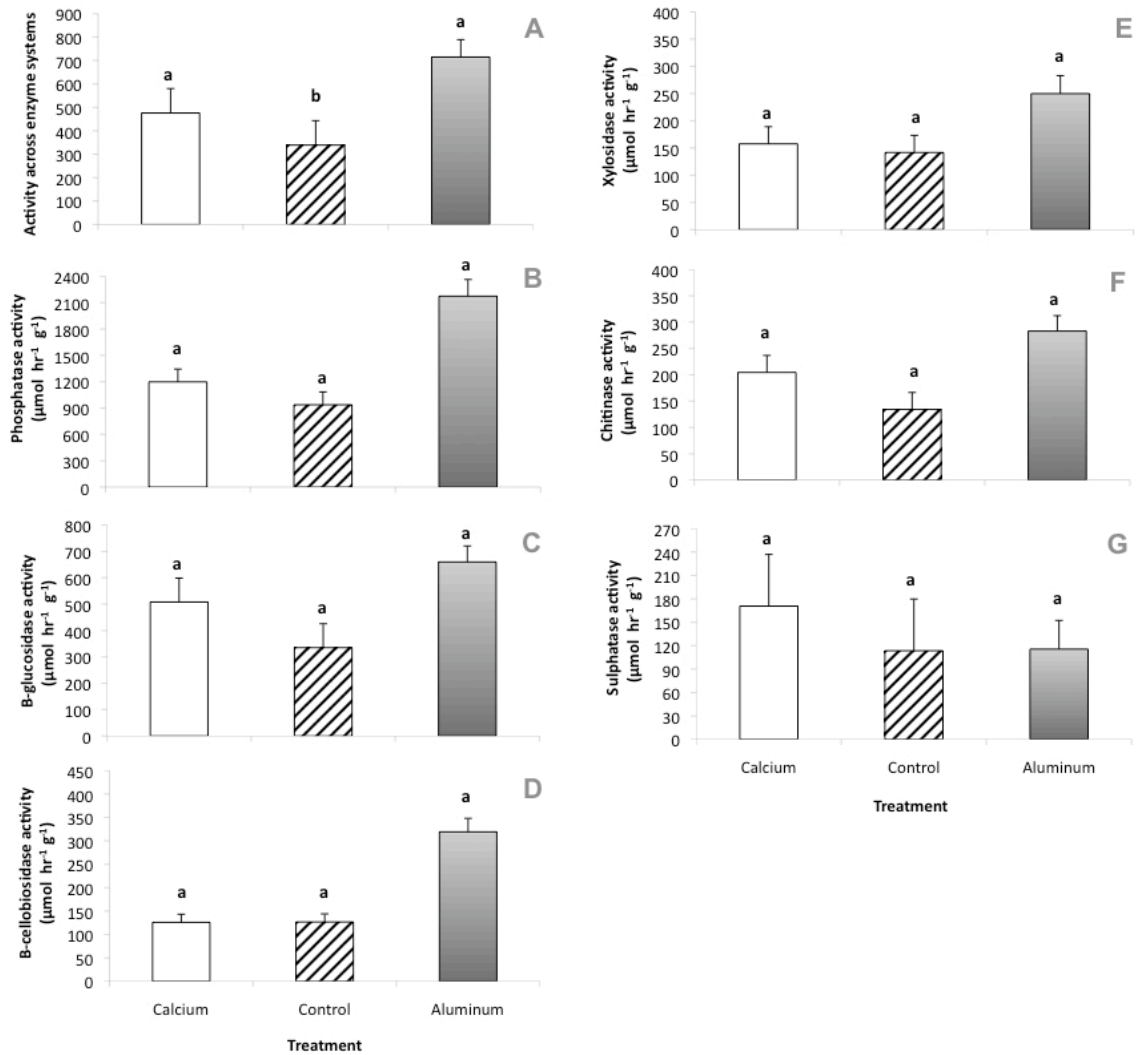


FIG. 3. September 2007 extracellular enzyme activity (EEA) assessed across enzyme systems (A) in soil treatments of Ca-addition, control, and Al-addition at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire. Individual EEA assays in fall are of phosphatase (B), β -glucosidase (C), β -cellobiosidase (D), xylosidase (E), chitinase (F), and sulphatase (G). Activity level values are means \pm SE. Contrasting letters above bars indicate statistically significant ($P < 0.05$) differences among treatments using Tukey's HSD post hoc test.

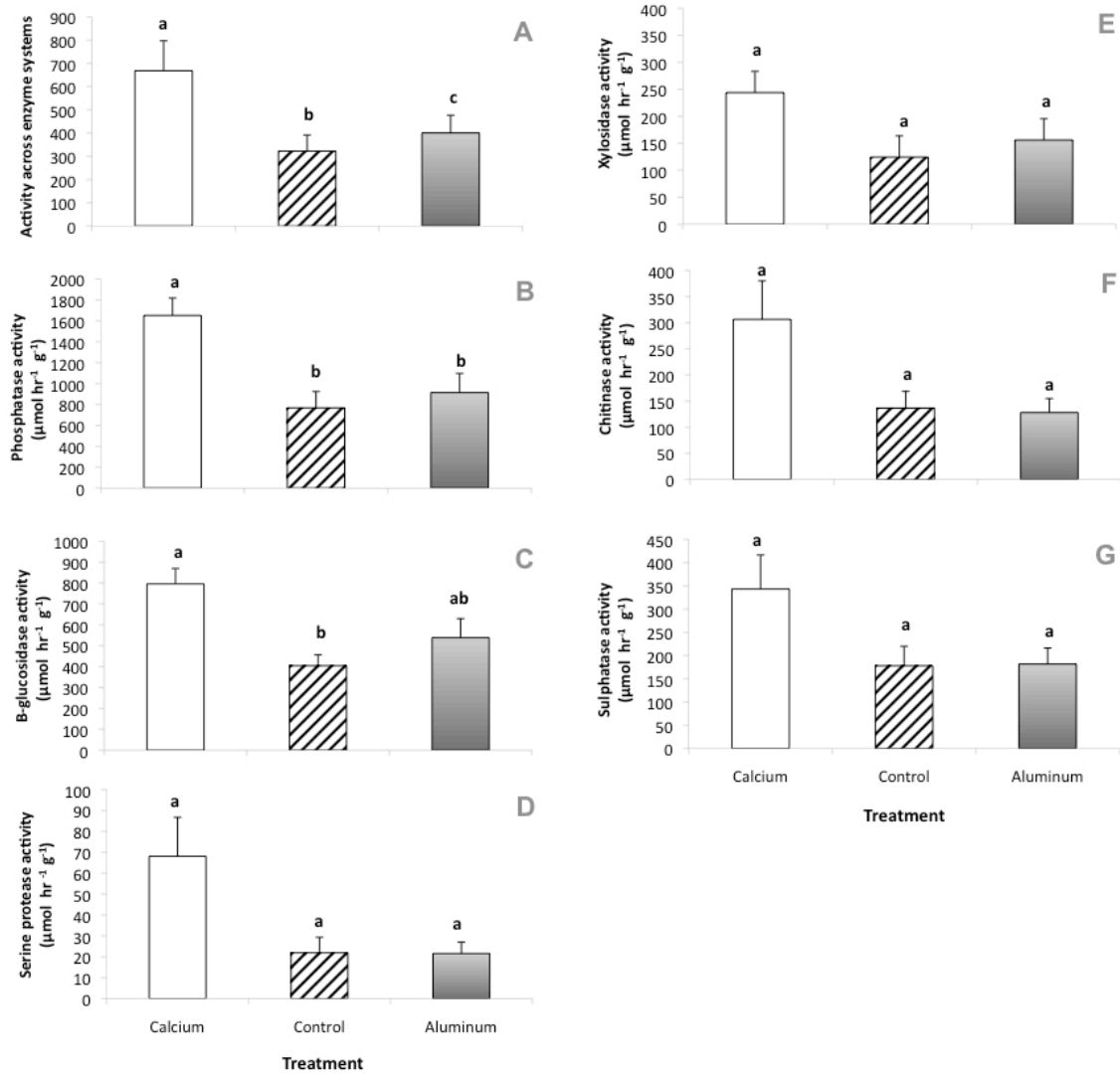


FIG. 4. May 2008 extracellular enzyme activity (EEA) assessed across enzyme systems (A) in soil treatments of Ca-addition, control, and Al-addition at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire. Individual EEA assays in spring are of phosphatase (B), β -glucosidase (C), serine protease (D), xylosidase (E), chitinase (F), and sulphatase (G). Activity level values are means \pm SE. Contrasting letters above bars indicate statistically significant ($P < 0.05$) differences among treatments using Tukey's HSD post hoc test.

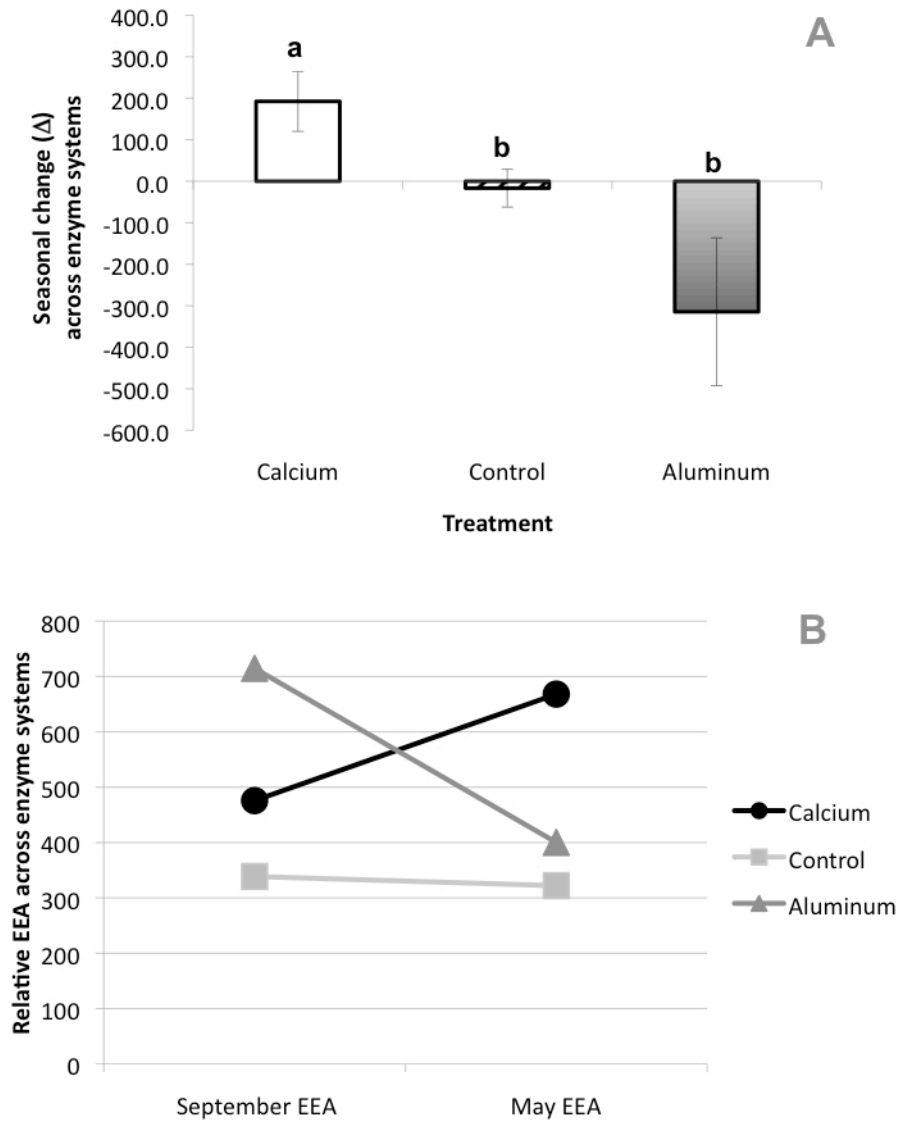


FIG. 5. Seasonal change (Δ = May activity – September activity) in soil extracellular enzyme activities (EEA) across enzyme systems (A), and means of EEA across enzyme systems over time (B) in Ca-addition, control, and Al-addition treatments between September 2007 and May 2008 at the NuPert experiment, Hubbard Brook Experimental Forest, New Hampshire. Seasonal differences in enzyme activities across enzyme systems are means \pm SE in A, whereas only mean values are plotted in B. Contrasting letters above bars indicate statistically significant ($P < 0.05$) differences among treatments using Tukey's HSD post hoc test.

Comprehensive Bibliography

- Abbasi, F., H. Onodera, S. Toki, H. Tanaka, and S. Komatsu. 2004. OsCDPK13, a calcium-dependent protein kinase gene from rice, is induced by cold and gibberellin in rice leaf sheath. *Plant Molecular Biology* 55:541-552.
- Aber, J.D., C.L. Goodale, S.V. Ollinger, M-L. Smith, A.H. Magill, M.E. Martin, and R.A. Hallet, J.L. Stoddard. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? *BioScience* 53:375-389.
- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems. *BioScience* 48:921-934.
- Acosta-Martínez, V., and M.A. Tabatabai. 2000. Enzyme activities in a limed agricultural soil. *Biology and Fertility in Soils* 31:85-91.
- Aebi, H. 1984. Catalase *in vitro*. Pages 121-126 in S.P. Colowick and N.O. Kaplan, editors. *Methods of enzymology*. Academic Press, New York, NY, USA.
- Ahn, S.J., M. Sivaguru, G.C. Chung, Z. Rengel, and H. Matsumoto. 2001. Aluminum-induced growth inhibition is associated with impaired efflux and influx of H⁺ across the plasma membrane in root apices of squash (*Curcubita pepo*). *Journal of Experimental Botany* 53:1959-1966.
- Allen, G.J., S.R. Muir, and D. Sanders. 1995. Release of Ca²⁺ from individual plant vacuoles by both Insp(3) and cyclic ADP-ribose. *Science* 268: 735-737.
- Bailey, S.W., S.B. Horsley, and R.P. Long. 2005. Thirty years of change in forest

- soils of the Allegheny Plateau, Pennsylvania. Soil Science Society of America Journal 69:681-690.
- Balsberg Pålsson, A-M. 1990. Influence of aluminium on biomass, nutrients, soluble carbohydrates and phenols in beech (*Fagus sylvatica*). Physiologia Plantarum 78:79-84.
- Bangerth, F. 1979. Calcium-related physiological disorders of plants. Annual Review of Phytopathology 17:97-122.
- Barwe, S., M. Sathiyabama, and C. Jayabaskaran. 2001. Induction of chitinase activity by exogenous cytokinins in excised darkgrown cucumber cotyledons: involvement of Ca²⁺ and staurosporine sensitive protein kinase(s) in cytokinin signaling. Journal of Plant Physiology 158:1-7.
- Becana, M. 2007. Oxidative stress as a response to salinity and aluminum. Consortium LOTASSA workshop. October 25-26. Porto Alegre, Brazil.
- Bélangier, C., B. Desrosiers, and K. Lee. 1997. Microbial extracellular enzyme activity in marine sediments: extreme pH to terminate reaction and sample storage. Aquatic Microbial Ecology 13:187-196.
- Bernard, B.K., S. Tsubuku, and S. Shioya. 1998. Acute toxicity and genotoxicity studies of a microbial transglutaminase. International Journal of Toxicology 17:703-721.
- Berridge, M.J., P. Lipp, and M.D. Bootman. 2000. The versatility and universality of calcium signaling. Nature Reviews Molecular Cell Biology 1:11-21.
- Berteau, O., A. Guillot, A. Benjdia, and S. Rabot. 2006. A new type of bacterial

- sulfatase reveals a novel maturation pathway in Prokaryotes. *The Journal of Biological Chemistry* 281:22464-22470.
- Bhuja, P., K. McLachlan, J. Stephens, and G. Taylor. 2004. Accumulation of 1,3- β -D-glucans, in response to aluminum and cytosolic calcium in *Triticum aestivum*. *Plant Cell Physiology* 45:543-549.
- Boscolo, P.R.S., M. Menossi, and R.A. Jorge. 2003. Aluminum-induced oxidative stress in maize. *Phytochemistry* 62:181-189.
- Bowman, W.D., C.C. Cleveland, L. Halada, J. Hresko, and J.S. Baron. 2008. Negative impact of nitrogen deposition on soil buffering capacity. *Naturegeoscience* 1:767-770.
- Brady, N.C., and R.R. Weil. 2002. *The nature and properties of soil*. Prentice-Hall, Inc., Upper Saddle River, New Jersey, USA.
- Bruce, R.C., L.A. Warrell, D.G. Edwards, and L.C. Bell. 1988. Effects of aluminum and calcium in the soil solution of acid soils on root elongation of *Glycine max* cv. Forrest. *Australian Journal of Agricultural Research* 39:319-338.
- Bush, D.S. 1995. Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology. Plant Molecular Biology* 46:95-122.
- Carreiro, M.M., R.L. Sinsabaugh, D.A. Repert, and D.F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359-2365.
- Chadwick, O.A., and J. Chorover. 2001. The chemistry of pedogenic thresholds. *Geoderma* 100:321-353.

- Chagnon, M., D. Paré, C. Hébert, and C. Camiré. Effects of experimental liming on collembolan communities and soil microbial biomass in a southern Quebec sugar maple (*Acer saccharum* Marsh.) stand. *Applied Soil Ecology* 17:81-90.
- Chen, C., M. Gao, J. Liu, and H. Zhu. 2007. Fungal symbiosis in rice requires an ortholog of a legume common symbiosis gene encoding a Ca²⁺/calmodulin-dependent protein kinase. *Plant Physiology* 145:1619-1628.
- Chen, L-S. 2006. Physiological responses and tolerance of plant shoot to aluminum toxicity. *Journal of Plant Physiology and Molecular Biology* 32:143-155.
- Chen L-S., Y-P. Qi, B.R. Smith, and X-H. Liu. 2005a. Aluminum-induced decrease in CO₂ assimilation in citrus seedlings is unaccompanied by decreased activities of key enzymes involved in CO₂ assimilation. *Tree Physiology* 25:317-324.
- Chen, L-S., Y-P. Qi, and X-H. Liu. 2005b. Effects of aluminum on light energy utilization and photoprotective systems in citrus leaves. *Annals of Botany* 96:35-41.
- Cheong, Y.H., K-N. Kim, G. K. Pandey, R. Gupta, J. J. Grant, and S. Luan. 2003. CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in *Arabidopsis*. *The Plant Cell* 15:1833-1845.
- Coleman, D.C. 2008. From peds to paradoxes: Linkages between soil biota and their influences on ecological processes. *Soil Biology and Biochemistry* 40: 271-289.
- Coleman, D.C., D.A. Crossley, and P.F. Hendrix. 2004. Fundamentals of soil

ecology. Elsevier Academic Press, Burlington, MA, USA.

- Courchesne, F., B. Côte', J. W. Fyles, W. H. Hendershot, P. M. Biron, A. G. Roy, and M-C. Turmel. 2005. Recent changes in soil chemistry in a forested ecosystem of southern Québec, Canada. *Soil Science Society of America Journal* 69:1298–1313.
- Cronan, C.S., and D.F. Grigal. 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality* 24:209-226.
- Dai, Z., Y. Liu, X. Wang, and D. Zhao. 1998. Changes in pH, CEC and exchangeable acidity of some forest soils in southern China during the last 32-35 years. *Water, Air, and Soil Pollution* 108:377-390.
- Darveau, M., J. Martel, J-L DesGranges, and Y. Mauffette. 1997. Associations between forest decline and bird and insect communities in northern hardwoods. *Canadian Journal of Forest Research* 27:876-882.
- Davies, H.W., and L.S. Monk-Talbot. 1990. Permeability characteristics and membrane lipid composition of potato tuber cultivars in relation to Ca^{2+} deficiency. *Phytochemistry* 29:2833–2825.
- DeForest, J.L., D.R. Zak, K.S. Pregitzer, and A.J. Burton. 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. *Soil Science Society of America Journal* 68:132-138.
- DeHayes, D.H., P.G. Schaberg, G.J. Hawley, and G.R. Strimbeck. 1999. Alteration of membrane-associated calcium leads to membrane destabilization and foliar injury in red spruce. *Bioscience* 49:789–800.

- DeHayes, D.H., P.G. Schaberg, G.J. Hawley, C.H. Borer, J.R. Cumming, and G.R. Strimbeck. 1997. Physiological implications of seasonal variation in membrane-associated calcium in red spruce mesophyll cells. *Tree Physiology* 17:687-695.
- Delhaize, E., and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiology* 107:315-321.
- de la Fuente, J.M., V. Ramirez-Rodriguez, J.L. Cabrera-Ponce, and L. Herrera-Estrella. 1997. Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science* 276:1566-1568.
- de Vries, W., J.J.M. van Grinsven, N. van Breemen, E.E.J.M. Leeters, and P.C. Jansen. 1995. Impacts of acid deposition on concentrations and fluxes of solutes in acid sandy forest soils in the Netherlands. *Geoderma* 67:17-43.
- Dijkstra, F.A. 2003. Calcium mineralization in the forest floor and surface beneath different tree species in the northeastern US. *Forest Ecology and Management* 175:185-194.
- Dijkstra, F.A., and M.M. Smits. 2002. Tree species effects on calcium cycling: The role of calcium uptake in deep soils. *Ecosystems* 5:385-398.
- Dilley, R.A. 2004. On why thylakoids energize ATP formation using either delocalized or localized proton gradients – a Ca^{2+} mediated role in thylakoid stress responses. *Photosynthesis Research* 80:245-263.
- Driscoll, C.T., G.B. Lawrence, A.J. Bulger, T.J. Butler, C.S. Cronan, C. Eagar,

- K.F. Lambert, G.E. Likens, J.L. Stoddard, and K.C. Weathers. 2001. Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *BioScience* 51:180-198.
- Duchesne, L., R. Ouimet, J-D. Moore, and R. Paquin. 2005. Changes in structure and composition of maple–beech stands following sugar maple decline in Québec, Canada. *Forest Ecology and Management* 208:223-236.
- Duncan, K.A., and S.C. Huber. 2007. Sucrose synthase oligomerization and F-actin association are regulated by sucrose concentration and phosphorylation. *Plant Cell Physiology* 48:1612-1623.
- Egli, M. 1998. Estimates of current and historical fluxes of N, Ca, and K in the forest ecosystem Möhlin. *GeoJournal* 44:73-89.
- Ezaki B., K. Sasaki, H. Matsumoto, and S. Nakashima. 2005. Functions of two genes in aluminium (Al) stress resistance: Repression of oxidative damage by the *AtBCB* gene and promotion of efflux of Al ions by the *NtGDII* gene. *Journal of Experimental Botany* 56:2661-2671.
- Federer, C.A., J.W. Hornbeck, L.M. Tritton, C.W. Martin, R.S. Pierce, and C.T. Smith. 1989. Long-term depletion of calcium and other nutrients in eastern US forests. *Environmental Management* 14:593-601.
- Fenn, M.E., T.G. Huntington, S.B. McLaughlin, C. Eager, A. Gomez, and R.B. Cook. 2006. Status of soil acidification in North America. *Journal of Forest Science* 52:3-13.
- Fernandez, I.J., L.E. Rustad, S.A. Norton, J.S. Kahl, and B.J. Cosby. 2003.

- Experimental acidification causes soil base-cation depletion at the Bear Brook Watershed in Maine. *Soil Science Society of America Journal* 67:1909-1919.
- Fink, S. 1991. Unusual patterns in the distribution of calcium oxalate in spruce needles and their possible relationships to the impacts of pollutants. *New Phytologist* 119:41-51.
- Fisk, M.C., W.R. Kessler, A. Goodale, T.J. Fahey, P.M. Groffman, and C.T. Driscoll. 2006. Landscape variation in microarthropod response to calcium addition in a northern hardwood forest ecosystem. *Pedobiologia* 50:69-78.
- Foyer, C.H., M. Lelandais, and K.J. Kunert. 1994. Photooxidative stress in plants. *Physiologia Planta* 92:696-717.
- Fromm, J., and S. Lautner. 2007. Electrical signals and their physiological significance in plants. *Plant, Cell, and Environment* 30:249-257.
- Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Cosby. 2003. The nitrogen cascade. *BioScience* 53:341- 356.
- Gaulin, E., N. Dramé, C. Lafitte, T. Torto-Alalibo, Y. Martinez, C. Ameline-Torregrosa, M. Khatib, H. Mazarguil, F. Villalba-Mateos, S. Kamoun, C. Mazars, B. Dumas, A. Bottin, M-T. Esquerré-Tugayé and M. Rickauera. 2006. Cellulose binding domains of a *Phytophthora* cell wall protein are novel pathogen-associated molecular patterns. *The Plant Cell* 18:1766-1777.
- Giannakoula, A., M. Moustakas, P. Mylona, I. Papadakis, and T. Yupsanis. 2008.

- Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal of Plant Physiology* 165:385-396.
- Gbondo-Tugbawa, S.S., and C.T. Driscoll. 2003. Factors controlling long-term changes in soil pools of exchangeable basic cations and stream acid neutralizing capacity in a northern hardwood forest ecosystem. *Biogeochemistry* 63:161-185.
- Gradowski, T., and S.C. Thomas. 2006. Phosphorus limitation of sugar maple growth in central Ontario. *Forest Ecology and Management* 226:104-109.
- Guo, P., G. Bai, B. Carver, R. Li, A. Bernardo, and M. Baum. 2007. Transcriptional analysis between two wheat near-isogenic lines contrasting in aluminum tolerance under aluminum stress. *Molecular Genetics and Genomics* 277:1-12.
- Hardin, S.C., H. Winter, and S.C. Huber. 2004. Phosphorylation of the amino terminus of maize sucrose synthase in relation to membrane association and enzyme activity. *Plant Physiology* 134:1427-1438.
- Hartwell, B.L., and F.R. Pember. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *Soil Science* 6:259-281.
- Hebe, G., A. Hager, and P. Salzer. 1999. Initial signaling processes induced by elicitors of ectomycorrhizal-forming fungi in spruce cells can also be triggered by G-protein-activating mastoparan and protein phosphatase inhibiting cantharidin. *Planta* 207:418-425.

- Hedin, L.O., L. Granat, G.E. Likens, T.A. Bulshand, J.N. Galloway, T.J. Butler, and H. Rodhe. 1994. Steep declines in atmospheric base cations in regions of Europe and North America. *Nature* 367:351-354.
- Heim, A., I. Brunner, E. Frossard, and J. Luster. 2003. Aluminum effects on *Picea abies* at low solution concentrations. *Soil Science Society of America Journal* 67:895-898.
- Hendrix, D.L. 1993. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Science* 33:1306-1311.
- Hinesley, L. E., D. M. Pharr, L. K. Snelling, and S. K. Funderburk. 1992. Foliar raffinose and sucrose in four conifer species: Relation to seasonal temperature. *Journal of the American Society for Horticultural Science* 117:852-855.
- Hodge, A., I.J. Alexander, and G.W. Gooday. 1995. Chitinolytic activities of *Eucalyptus pilularis* and *Pinus sylvestris* root systems challenged with mycorrhizal and pathogenic fungi. *New Phytologist* 131:255-261.
- Hodson, M.J, and D.E. Evans. 1995. Aluminium/silicon interactions in higher plants. *Journal of Experimental Botany* 46:161-171.
- Hornbeck, J.W., S.W. Bailey, D.C. Buso, and J. B. Shanley. 1997. Streamwater chemistry and nutrient budgets for forested watersheds in New England: Variability and management implications. *Forest Ecology and Management* 93:73-89.
- Horsley, S.B., R.P. Long, S.W. Bailey, R.A. Hallet, and P.M. Wargo. 2002. Health of

- eastern North American sugar maple forests and factors affecting decline. Northern Journal of Applied Forestry 19:34-44.
- Hossain, A.K.M.Z., H. Koyama, and T. Hara. 2005. Sugar compositions and molecular mass distributions of hemicellulosic polysaccharides in wheat plants under aluminum stress at higher level of calcium supply. Asian Journal of Plant Sciences 4:11-16.
- Houde, M., and A.O. Diallo. 2008. Identification of genes and pathways associated with aluminum stress and tolerance using transcriptome profiling of wheat near-isogenic lines. BioMed Central Genomics. doi:10.1186/1471-2164-9-400.
- Huggett, B.A., P.G. Schaberg, G.J. Hawley, and C. Eagar. 2007. Long-term calcium addition increases growth release, wound closure, and health of sugar maple (*Acer saccharum*) trees at the Hubbard Brook Experimental Forest. Canadian Journal of Forest Research 37:1692-1700.
- Hultberg, H., and M. Ferm. 2004. Temporal changes and fluxes of sulphur and calcium in wet and dry deposition, internal circulation as well as in run-off and soil in a forest at Gårdsjön, Sweden. Biogeochemistry 68:355-363.
- Huntington, T.G., R.P. Hooper, C.E. Johnson, B.T. Aulenbach, R. Cappellato, and A.E. Blum. 2000. Calcium depletion in a southeastern United States forest ecosystem. Soil Science Society of America Journal 64:1845-1858.
- Igawa M., T. Kase, K. Satake, and H. Okochi. 2002. Severe leaching of calcium ions from fir needles caused by acid fog. Environmental Pollution 119:375-82.
- Issac, R.A., and W.C. Johnson. 1976. Determination of total nitrogen in plant tissue using

- a block digester. *Journal of the Association of Official Analytical Chemists* 59:98-100.
- Jandl, R., C. Alewell, and J. Prietzel. 2004. Calcium loss in central European soils. *Soil Science Society of America Journal* 68:588-595.
- Jegerschöld, C., A.W. Rutherford, and T.A. Mattioli. 2000. Calcium binding to the photosystem II subunit CP29. *The Journal of Biological Chemistry* 275:12781–12788.
- Jiang, Y., and B. Huang. 2001. Effect of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *Journal of Experimental Botany* 52:341-349.
- Juice, J.M., T.J. Fahey, T.G. Siccama, C.T. Driscoll, E.G. Denny, C. Eagar, N.L. Cleavitt, R. Minocha, and A.D. Richardson. 2006. Response of sugar maple to calcium addition to northern hardwood forest. *Ecology* 87:1267-1280.
- Kinraide, T.B. 1991. Identity of the rhizotoxic aluminum species. *Plant and Soil* 134: 167-78.
- Kinraide, T.B., and D.R. Parker. 1990. Apparent phytotoxicity of mononuclear hydroxyl aluminum to four dicotyledonous species. *Plant Physiology* 79:283-288.
- Knight, H. 2000. Calcium signaling during abiotic stress in plants. *International Review of Cytology* 195:269-324.
- Kochian, L.V., M.A. Piñeros, and O.A. Hoekenga. 2005. The physiology, genetics, and molecular biology of plant aluminum resistance and toxicity. *Plant and Soil* 274:175-195.

- Kolb, T.E., and L.H. McCormick. 1993. Etiology of sugar maple decline in four Pennsylvania stands. *Canadian Journal of Forest Research* 23:2395-2402.
- König, H. 2006. *Bacillus* species in the intestines of termites and other soil invertebrates. *Journal of Applied Microbiology* 101:620-627.
- Konno, H., T. Nakato, and K. Katoh. 2002. Characteristics, hydrolysis of cell wall polymers, and response to calcium deficiency of a cell wall-associated β -galactosidase from carrot cells. *Journal of Plant Physiology* 159:1-8.
- Larsen, T., E. Lydersen, D. Tang, Y. He, J. Gao, H. Liu, L. Duan, H. Seip, R. Vogt, J. Mulder, M. Shao, Y. Wang, H. Shang, X. Zhang, S. Solberg, W. Aas, T. Økland, O. Eilertsen, V. Angell, Q. Lu, D. Zhao, R. Xiang, J. Xiao, and J. Luo. 2006. Acid rain in China. *Environmental Science and Technology* 40:418-425.
- Lautner, S., B. Ehlting, E. Windneisen, H. Rennenberg, H. Matyssek, and J. Fromm. 2007. Calcium nutrition has a significant influence on wood formation in poplar. *New Phytologist* 173:743-752.
- Lautner, S., T.E.E. Grams, R. Matyssek, and J. Fromm. 2005. Characteristics of electrical signals in poplar and responses in photosynthesis. *Plant Physiology* 138:2200-2209.
- Lawrence, G.B., M.B. David, and W.C. Shortle. 1995. A new mechanism for calcium loss in forest-floor soils. *Nature* 378:162-165.
- Legge, R.L., J.E. Thompson, J.E. Baker, and M. Liberman. 1982. The effect of calcium on the fluidity and phase properties of microsomal membranes isolated from Golden Delicious apples *Malus*. *Plant Cell Physiology* 23:161-169.

- Liang, Y., C. Yang, and H. Shi. 2001. Effects of silicon on growth and mineral composition of barley grown under toxic levels of aluminum. *Journal of Plant Nutrition* 24:229-243.
- Likens, G.E., C.T. Driscoll, D.C. Buso, M.J. Mitchell, G.M. Lovett, S.W. Bailey, T.G. Siccama, W.A. Reiners, and C. Alewell. 2002. The biogeochemistry of sulfur at Hubbard Brook. *Biogeochemistry* 60:235-316.
- Likens, G.E., C.T. Driscoll, D.C. Buso, T.G. Siccama, C.E. Johnson, G.M. Lovett, T.J. Fahey, W.A. Reiners, D.F. Ryan, C.W. Martin, and S.W. Bailey. 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* 41:89-173.
- Likens, G.E., C.T. Driscoll, and D.C. Buso. 1996. Long-term effects of acid rain response and recovery of a forest ecosystem. *Science* 272:244-246.
- Liu, H-T., D-Y. Sun, and R-G. Zhou. 2005. Ca²⁺ and AtCaM3 are involved in the expression of heat shock protein gene in *Arabidopsis*. *Plant, Cell, and Environment* 28:1276-1284.
- Long, R.P., S.B. Horsley, and P.R. Lilja. 1997. Impacts of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Canadian Journal of Forest Research* 27:1560-1573.
- Ludwig, A.A., T. Romeis, and J.D.G. Jones. 2004. CDPK-mediated signaling pathways: Specificity and cross-talk. *Journal of Experimental Botany* 55:181-188.
- Ma, J.F., P.R. Ryan, and E. Delhaize. 2001. Aluminium tolerance in plants and the complexing role of organic acids. *Trends In Plant Science* 6:273-278.
- Macció, D., A. Fabra, and S. Castro. 2001. Acidity and calcium interaction affect

- the growth of *Bradyrhizobium* sp. and the attachment to peanut roots. *Soil Biology and Biochemistry* 34:201-208.
- Marschner, H. 2002. *Mineral Nutrition of Higher Plants*. Academic Press, New York, NY, USA.
- Martel, J. and Y. Mauffette. 1997. Lepidopteran communities in temperate deciduous forests affected by forest decline. *Oikos* 78:48-56.
- McLaughlin, S.B., and R. Wimmer. 1999. Tansley Review No. 104. Calcium physiology and terrestrial ecosystem processes. *New Phytologist* 142:373-417.
- McLaughlin, S.B. 1985. Effects of air pollution on forests - a critical review. *Journal of the Air Pollution Control Association* 35:512-534.
- Medvedev, S.S. 2005. Calcium signaling system in plants. *Russian Journal of Plant Physiology* 52:249-270.
- Merino, A., F. Macías, and E. García-Rodeja. 2000. Element fluxes and buffer reactions in acidified soils from a humid-temperate region of southern Europe. *Water, Air, and Soil Pollution* 120:217-228.
- Miqyass M., H.J. van Gorkom, and C.F. Yocum. 2007. The PSII calcium site revisited. *Photosynthesis Research* 92:275-287.
- Monroy, A.F., F. Sarhan, and R.S. Dhindsa. 1993. Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression: Evidence for the role of calcium. *Plant Physiology* 102:1227-1235.
- Montgomery, D.C. 2001. *Design and analysis of experiments*. John Wiley and Sons, New York, NY, USA.

- Moore, J-D., C. Camiré, and R.Ouimet. 2000. Effects of liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair watershed, Québec, Canada. *Canadian Journal of Forest Research* 30:725–732.
- Moore, J-D., and R. Ouimet 2006. Ten-year effect of dolomitic lime on the nutrition, crown vigor, and growth of sugar maple. *Canadian Journal of Forest Research* 36:1834-1841.
- Myers, R.T., D.R. Zak, D.C. White, and A. Peacock. 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Science Society of America Journal* 65:359-367.
- Naik, D., E. Smith, and J.R. Cumming. 2009. Rhizosphere carbon deposition, oxidative stress and nutritional changes in two poplar species exposed to aluminum. *Tree Physiology* doi:10.1093/treephys/tpn035.
- Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* 22:867-880.
- National Atmospheric Deposition Program annual data summaries, 1997- 2007.
- National Atmospheric Deposition Program (NRSP-3). 2009. NADP Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.
- Nguyen, N.T., N.D. Heip, and K. Fujita. 2005. Iron enhances aluminum-induced leaf necrosis and plant growth inhibition in *Eucalyptus camaldulensis*. *Plant and Soil* 277:139-152.
- Nguyen, N.T., K. Nakabayashi, J. Thompson, and K. Fujita. 2003. Role of

- exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. *Tree Physiology* 23:1041-1050.
- Nowak, J., and A. L. Friend 2006. Loblolly pine and slash pine responses to acute aluminum and acid exposures. *Tree Physiology* 26:1207-1215.
- Nykvist, N. 2000. Tropical forests can suffer from a serious deficiency of calcium after logging. *Ambio* 29:310-313.
- Ouimet, R., L. Duchesne, D. Houle, and P.A. Arp. 2001. Critical loads and exceedances of acid deposition and associated forest growth in the northern hardwood and boreal coniferous forests in Québec, Canada. *Water, Air, Soil Pollution: Focus* 1:119-134.
- Ouimet, R., and C. Camiré. 1995. Foliar deficiencies of sugar maple stands associated with soil cation imbalances in the Quebec Appalachians. *Canadian Journal of Soil Science* 75:169-175.
- Ouimet, R., and J-M. Fortin. 1992. Growth and foliar nutrient status of sugar maple: Incidence of forest decline and reaction to fertilization. *Canadian Journal of Forest Research* 22:699-706.
- Palta, J.P., and P.H. Li. 1978. Cell membrane properties in relation to freezing injury. *In* P.H. Li and A. Sakai, editors. *Plant cold hardiness and freezing stress*. Academic Press, London, UK.
- Pan, R-S., and R.A. Dilley. 2000. Influence of Ca^{2+} on the thylakoid lumen violaxanthin de-epoxidase activity through Ca^{2+} gating of H^+ flux at the CF_0 H^+ channel. *Photosynthesis Research* 65:141–154.

- Panda, S.K., L.B. Singha, and M.H. Khan. 2003. Does aluminum phytotoxicity induce oxidative stress in greengram (*Vigna radiata*)? *Bulgarian Journal of Plant Physiology* 29:77-86.
- Pandey, G.K., J.J. Grant, Y.H. Cheong, B-G. Kim, L.G. Li, and S. Luan. 2008. Calcineurin-B-like protein CBL9 interacts with target kinase CIPK3 in the regulation of ABA response in seed germination. *Molecular Plant* 1:238-248.
- Pandey, G.K., Y.H. Cheong, K-N. Kim, J. J. Grant, L. Li, W. Hung, C. D'Angelo, S. Weinl, J. Kudla, and S. Luan. 2004. The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in *Arabidopsis*. *The Plant Cell* 16:1912-1924.
- Pandey, S., S.B. Tiwari, K.C. Upadhyaya, and S.K. Sopory. 2000. Calcium signaling: Linking environmental signals to cellular functions. *Critical Review in Plant Science* 19:291-318.
- Paul, E. 2007. *Soil microbiology, ecology, and biochemistry*. Academic Press. Burlington, MA.
- Pell, E.J., J.P. Sinn, B.W. Brendley, L. Samuelson, C. Vinten-Johansen, M. Tien, and J. Skillman. 1999. Differential response of four tree species to ozone-induced acceleration of foliar senescence. *Plant, Cell Environment* 22:779-790.
- Polle, A., and H. Rennenberg. 1992. Field studies on Norway spruce trees at high altitudes: II. Defense systems against oxidative stress in needles. *New Phytologist* 121:635-642.
- Polle, A., and H. Rennenberg. 1994. Protection from oxidative stress in transgenic plants.

- Biochemical Society Transcripts 22:936-940.
- Poschenrieder, C., B. Gunsé, I. Corrales, and J. Barceló. 2008. A glance into aluminum toxicity and resistance in plants. *Science of the Total Environment* 400:356-368.
- Prietzl, J. 2001. Arylsulfatase activities in soils of the Black Forest/Germany- seasonal variation and effect of $(\text{NH}_4)_2\text{SO}_4$ fertilization. *Soil Biology and Biochemistry* 33:1317-1328.
- Quartin, V.L., H.G. Azinheira, and M.A. Nunes. 2001. Phosphorus deficiency is responsible for biomass reduction of triticale in nutrient solution with aluminum. *Journal of Plant Nutrition* 24:1901-1911.
- Rampazzo, N., and W.E.H. Blum. 1992. Changes in chemistry and mineralogy of forest soils by acid rain. *Water, Air, and Soil Pollution* 61:209-220.
- Raven, P.H., R.F. Evert, and S.E. Eichorn. 1999. *Biology of plants*. W.H. Freeman and Company/Worth Publishers, New York, New York, USA.
- Rengel, Z., and D.C. Elliott. 1992. Mechanism of Al inhibition of net $^{43}\text{Ca}^{2+}$ uptake by *Amaranthus* protoplasts. *Plant Physiology* 98:632-638.
- Reggiani, R., and A. Bertani 2003. Anaerobic amino acid metabolism. *Russian Journal of Plant Physiology* 50: 821-824.
- Richards, K.D., E.J. Schott, Y.K. Sharma, K.R. Davis, and R.C. Gardner. 1998. Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. *Plant Physiology* 116:409-418.
- Rizzuto, R., and T. Pozzan. 2006. Microdomains of intracellular Ca^{2+} : Molecular determinants and functional consequences. *Physiological Reviews* 86: 369-408.

- Rodríguez, M., E. Canales, C. J. Borroto, E. Carmona, J. López, M. Pujol, and O. Borrás-Hidalgo. 2006. Identification of genes induced upon water-deficit stress in a drought-tolerant rice cultivar. *Journal of Plant Physiology* 163:577-584.
- Roos, W. 2000. Ion mapping in plant cells-methods and applications in signal transduction research. *Planta* 210:347-370.
- Roy, A.K., A. Sharma, G. Talukder, and K. Talukder. 1988. Some aspects of aluminum toxicity in plants. *Botanical Review* 54:145-178.
- Rudnick, R.L., and S. Gao. 2003. Composition of the continental crust. *in* R. L. Rudnick, editor. The crust. volume 3, Pages 1-64 *in* H.D. Holland and K.K. Turekian, editors. *Treatise on geochemistry*. Elsevier-Pergamon, Oxford, UK.
- Saiya-Cork, K.R., R.L. Sinsabaugh, and D.R. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry* 34:1309-1315
- Salisbury, F.B., and C.W. Ross. 1992. *Plant physiology*. Wadsworth Publishing Co., Berkley, CA, USA.
- Sanders, D., C., Brownlee, and J.F. Harper. 1999. Communicating with calcium. *Plant Cell* 11:691-706.
- Sangawan, V., I. Foulds, J. Singh, and R.S. Dhindsa. 2001. Cold-activation of *Brassica napus BN115* promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca²⁺ influx. *The Plant Journal* 27:1-12.
- Sauter, M., and A. Hager. 1989. The mycorrhizal fungus *Amanita muscaria*

- induces chitinase activity in roots and in suspension-cultured cells of its host *Picea abies*. *Planta* 179:61–66.
- Schaberg, P.G., J.W. Tilley, G.J. Hawley, D.H. DeHayes, and S.W. Bailey. 2006. Associations of calcium and aluminum with the growth and health of sugar maple trees in Vermont. *Forest Ecology and Management* 223:159-169.
- Schaberg, P.G., G.R. Strimbeck, S.G. McNulty, D.H. DeHayes, G.J. Hawley, and P.F. Murakami. 2002. Effects of chronic N fertilization on foliar membranes, cold tolerance, and carbon storage in montane red spruce. *Canadian Journal of Forest Research* 32:1351-1359.
- Schaberg, P.G., D.H. DeHayes, and G.J. Hawley. 2001. Anthropogenic calcium depletion: A unique threat to forest ecosystem health? *Ecosystem Health* 7:214-228.
- Schaberg, P.G., and D.H. DeHayes. 2000. Physiological and environmental causes of freezing injury in red spruce. Chapter 6 *in* Responses of northern U.S. forests to environmental change.
- Schaberg, P.G., D.H. DeHayes, G.J. Hawley, G.R. Strimbeck, J.R. Cumming, P.F. Murakami, and C.H. Borer. 2000. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiology* 20:73-85.
- Schaedle, M., F.C. Thornton, D.J. Raynal, and H.B. Tepper. 1989. Response of tree seedlings to aluminum. *Tree Physiology* 5:337-356.
- Shamrikova, E.V., T.A. Sokolova, and I.V. Zaboeva. 2005. Acid buffer capacity of

- mineral horizons of podzolic and bog-podzolic soils of the Komi Republic. Eurasian Soil Science 38:471-479.
- Schmitz-Eiberger, M., R. Haefs, and G. Noga. 2002. Calcium deficiency – influence on the antioxidative defense system in tomato plants. Journal of Plant Physiology 159:733-742.
- Semin, B.K., L.N. Davletshina, A.A Bulychev, I.I. Ivanov, M. Seibert, and A.B. Rubin 2007. Effect of calcium chelators on the formation and oxidation of the slowly relaxing reduced plastoquinone pool in calcium depleted PSII membranes. Investigation of the F_0 Yield. Biochemistry (Moscow) 72:1205-1215.
- Sheen, J. 1996. Ca^{2+} -dependent protein kinases and stress signal transduction in plants. Science 274:1900-1902.
- Shigihara, A., K. Matsumoto, N. Sakurai, and M. Igawa. 2008. Growth and physiological responses of beech seedlings to long-term exposure of acid fog. Science of the Total Environment 391:124-131.
- Sieciechowicz, K.A., K.W. Joy, and R.J. Ireland. 1988. Diurnal changes in asparaginase activity in pea leaves. I. Journal of Experimental Botany 203:695-706.
- Sinsabaugh, R.L., C.L. Lauber, M.N. Weintraub, B. Ahmed, S.D. Allison, C. Crenshaw, A.R. Contosta, D. Cusack, S. Frey, M.E. Gallo, T.B. Gartner, S.E. Hobbie, K. Holland, B.L. Keeler, J.S. Powers, M. Stursova, C. Takacs-Vesbach, M.P. Waldrop, M.D. Wallenstein, D.R. Zak, and L.H. Zeglin. 2008. Stoichiometry of enzyme activity at global scale. Ecology Letters 11:1252-1264.

- Sinsabaugh, R.L., M.E. Gallo, C. Lauber, M.P. Waldrop, and D.R. Zak. 2005.
Extracellular enzyme activities and soil organic matter dynamics for northern
hardwood forests receiving simulated nitrogen deposition. *Biogeochemistry*
75:201-215.
- Sinsabaugh, R.L., D.R. Zak, M. Gallo, C. Lauber, and R. Amonette 2004.
Nitrogen deposition and dissolved organic carbon production in northern
temperate forests. *Soil Biology and Biochemistry* 36:1509-1515.
- Sinsabaugh, R.L., M.M. Carreiro, and D.A. Repert. 2002. Allocation of
extracellular enzymatic activity in relation to litter composition, N deposition, and
mass loss. *Biogeochemistry* 60:1-24.
- Sinsabaugh, R.L., R.K. Antibus, A.E. Linkins, C.A. McClaugherty, L. Rayburn, D.
Repert, and T. Weiland. 1993. Wood decomposition: nitrogen and phosphorus
dynamics in relation to extracellular enzyme activity. *Ecology* 74:1586-1593.
- Smith, I.K., T.L. Vierheller, and C.A. Thorne. 1988. Assay of glutathione
reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid).
Analytical Biochemistry 175:408-413.
- Song, X., J. Hong, Y. Shuquan, and Z. Guomo. 2008. Detection of acid rain
stress effect on plant using hyperspectral data in Three Gorges region, China.
Chinese Geographical Science 18:249-254.
- Sverdrup, H., G. Thelin, M. Robles, I. Stjernquist, and J. Sørensen. 2006.

- Assessing nutrient sustainability of forest production for different tree species considering Ca, Mg, K, N and P at Björnstorps Estate, Sweden. *Biogeochemistry* 81:219-238.
- St. Clair, S.B., J.E. Carlson, and J.P. Lynch. 2005. Evidence for oxidative stress in sugar maple stands growing on acidic, nutrient imbalanced forest soils. *Oecologia* 145:258-269.
- Tabuchi, A., S. Kikuia, and H. Matsumoto. 2004. Differential effects of aluminum on osmotic potential and sugar accumulation in the root cells of Al-resistant and Al-sensitive wheat. *Physiologia Plantarum* 120:106-112.
- Takezawa, D., Z.H. Liu, G. An, and B.W. Poovaiah. 1995. Calmodulin gene family in potato: Developmental and touch-induced expression of the mRNA encoding a novel isoform. *Plant Molecular Biology* 27:693-703.
- Tang, M., R-Q. Zhang, H. Chen, H-H. Zhang, and Z-Q Tian. 2008. Induced hydrolytic enzymes of ectomycorrhizal fungi against pathogen *Rhizoctonia solani*. *Biotechnology Letters* 30:1777-1782.
- Tao, F., Y. Hayashi, and E. Lin. 2002. Soil vulnerability and sensitivity to acid deposition in China. *Water, Air, and Soil Pollution* 140:247-260.
- Tesfaye, M., S.J. Temple, D.L. Allan, C.P. Vance, and D.A. Samac. 2001. Overexpression of malate dehydrogenase in transgenic alfalfa enhances organic acid synthesis and confers tolerance to aluminum. *Plant Physiology* 127:1836-1844.
- Thornton, F.C., M. Schaedle, and D.J. Raynal. 1986a. Effect of aluminum on

- sugar maple in solution culture. *Canadian Journal of Forest Research* 16:892-896.
- Thornton, F.C., M. Schaedle, and D.J. Raynal. 1986b. Effects of aluminum on growth, development and nutrient composition of honeylocust (*Gleditsia triacanthos* L.) seedlings. *Tree Physiology* 2:307-316.
- Tomlinson, G.H. 1993. A possible mechanism relating increased soil temperature to forest decline. *Water, Air, Soil Pollution* 66:365-380.
- Tozawa, Y., A. Nozawa, T. Kanno, T. Narisawa, S. Masuda, K. Kasai, and H. Nanamiya. 2008. Calcium-activated (p)ppGpp synthetase in chloroplasts of land plants. *The Journal of Biological Chemistry* 282:35536–35545.
- Vahisalu, T., H. Kollist, Y-F. Wang, N. Nishimura, W-Y. Chan, G. Valerio, A. Lamminmäki, M. Brosché, H. Moldau, R. Desikan, J.I. Schroeder, and J. Kangasjärvi. 2008. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signaling. *Nature* doi:10.1038/nature06608.
- van Breemen, N., J. Mulder, and C.T. Driscoll,. 1983. Acidification and alkalization of soils. *Plant and Soil* 75:283-308.
- von Stedingk, E., P.F. Pavlov, V.A. Grinkevich, and E. Glaser. 1999. The precursor of the F₁ β subunit of the ATP synthase is covalently modified upon binding to plant mitochondria. *Plant Molecular Biology* 41:505-515.
- von Uexkull, H.R., and E. Mutert. 1995. Global extent, development and economic impact of acid soils. *Plant and Soil* 171:1-15.
- Watmough, S.A. 2002. A dendrochemical survey of sugar maple (*Acer*

- saccharum* Marsh) in south-central Ontario, Canada. *Water, Air, and Soil Pollution* 136:165-187.
- Wagatsuma, T., and M. Kaneko. 1987. High toxicity of hydroxy-aluminum polymer ions to plant roots. *Soil Science and Plant Nutrition* 33:57-67.
- Weber-Blaschke, G., and K.E. Rehfues. 2002. Correction of Al toxicity with European ash (*Fraxinus excelsior* L.) growing on acid soils by fertilization with Ca and Mg carbonate and sulfate in pot experiments. *Forest Ecology and Management* 167:173–183.
- Wei, H., and J.L. Wang. 2005. Characteristics of acid rain in Jinyun Mountain, Chongqing, China. *Applied Ecology and Environmental Research* 3:29-37.
- Weisskopf, L., R-C. Le Bayon, F. Kohler, V. Page, M. Jossi, J-M. Gobat, E. Martinoia, and M. Aragno. 2008. Spatio-temporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: A one-year microcosm study on lupin and wheat. *Soil Biology and Biochemistry* 40:1772-1780.
- Whittaker, R.H. 1953. A consideration of climax theory: The climax as a population and pattern. *Ecological Monographs* 23:41-78.
- Wilmot, T.R., D.S. Ellsworth, and M.T. Tyree. 1996. Base cation fertilization and liming effects on nutrition and growth of Vermont sugar maple stands. *Forest Ecology and Management* 84:123-134.
- Wise, R.R., and A.W. Naylor. 1987. Chilling-enhanced photooxidation: The

peroxidative destruction of lipids during chilling injury to photosynthesis and ultrastructure. *Plant Physiology* 83:272-277.

Yamamoto, Y., Y. Kobayashi, S. R. Devi, S. Rikiishi, and H. Matsumoto. 2002.

Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. *Plant Physiology* 128:63-72.

Yamamoto, Y., Y. Kobayashi, and H. Matsumoto. 2001. Lipid peroxidation is an

early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiology* 125:199-208.

Yang, T., S. Chaudhuri, L. Yang, Y. Chen, and B.W. Pooviah. 2004.

Calcium/calmodulin up-regulates a cytoplasmic receptor-like kinase in plants. *The Journal of Biological Chemistry* 279:42552-42559.

Young, J.J., S. Mehta, M. Israelsson, J. Godoski, E. Grill, and J.I. Schroeder.

2006. CO₂ signaling in guard cells: Calcium sensitivity response modulation, a Ca²⁺-independent phase, and CO₂ insensitivity of the *gca2* mutant. *Proceedings of the National Academy of Sciences* 103:7506-7511.

Zhang, J., Z. He, H. Tian, G. Zhu, and X. Peng. 2007. Identification of aluminium-

responsive genes in rice cultivars with different aluminium sensitivities. *Journal of Experimental Botany* 58:2269-2278.

Zhen, Y., J-L Qi, S-S. Wang, J. Su, G-H. Xu, M-S. Zhang L. Miao, X-X. Ping, D.

Tian, and Y-H. Yang. 2007. Comparative proteome analysis of differentially expressed proteins induced by Al toxicity in soybean. *Physiologia Plantarum* 131: 542–554.