AN ABSTRACT OF THE THESIS OF

Lily Zahor for the degree of Masters of Science in Environmental Science and Policy presented on May 7th 2014.

The Impact of Calcium on Transpiration in an Acid Rain Impacted Forest

Abstract approved:

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Michele L. Pruyn, Thesis Advisor

Abstract

Acid rain has continued to degrade New England forests since the early 1950s (Cogbill and Likens 1974). Pollution in the form of acid rain causes calcium to leach, or be washed out, of the soil by replacing calcium ions on soil exchange sites with hydrogen ions from the rain (DeHayes, 1999). Calcium is important to plant growth and cell regulation, and naturally occurs in the soil (Taiz and Zeiger 2010). Applying calcium fertilizers in the form of wollastonite ($CaSiO_3$) have been used in an attempt to replace the leached nutrient, which showed an in increase in health, growth, and survivorship in maple trees following application (Juice et al. 2006).

It has been shown, through a watershed-scale experiment that calcium addition can increase the forest uptake of water (Green, Bailey, and Bailey 2013). This uptake can be measured in individual trees by monitoring sap flow volumes using the Granier or thermal dissipation probe (TDP) method. The main forest type in New England is northern deciduous, which is dominated by American Beech (*Fagus grandifolia*, FAGR), Yellow Birch (*Betula alleghaniensis*,BEAL) and Sugar Maple (*Acer saccharum*, ACSA).Through studying sap flow, root conductivity and root vitality in these species and across three sites, we aimed to find out if adding calcium fertilizers will increase sapflux and root function in trees across the White Mountain National Forest. Our hypothesis was partially supported by the results, tree response seems to be dependent on site and species, and in some cases the season. Understanding how forests react to replacement of lost calcium will help land managers understand the impacts of acid rain on forest function and develop appropriate management strategies.

The Impact of Calcium on Transpiration in an Acid Rain Impacted Forest

By

Lily E. Zahor

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I understand that my thesis will become part of the permanent collection of Plymouth State University, Lamson Library. My signature below authorizes release of my thesis to any reader upon request.

Lily E. Zahor

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Introduction

Forests serve people in a multitude of ways, from wood products, wildlife habitat, and recreation, to water filters and flood control. Across the northeastern United States, forests cover the landscape with water driving many forest ecosystem processes. Water connects all aspects of a forest, moving nutrients and energy throughout a forested ecosystem (Perry, Ram, and Hart 2008). The flow and balance of nutrients is important to a healthy functioning forest within which calcium is a vital micronutrient.

Many healthy tree functions rely on calcium for processes such as: the formation of new cell walls, formation and growth of woody tissue, cell division, stoma closure, and secondary messaging for many plant responses (Taiz and Zeiger 2010; White and Broadley 2003). Herbette and Cochard (2010) found that calcium also plays a major role in the vulnerability to xylem embolism, which is air blockage in the main water conducting tissues. In addition, calcium influences the size of vessels in the xylem, which is also related to embolism (Lautner et al. 2007). Fiber cell length, important for structural support of xylem, is also affected by the amount of calcium in the tree (Lautner et al. 2007; Panshin and Zeeuw 1970).

Calcium naturally occurs in soil and is slowly introduced through bedrock weathering (Figure 1). Cations attract to negatively charged soil particles; therefore, during cation exchange calcium can be replaced by another positively charged particle and be freed into the soil solution where the nutrient can be used by the tree (Taiz and Zeiger 2010).

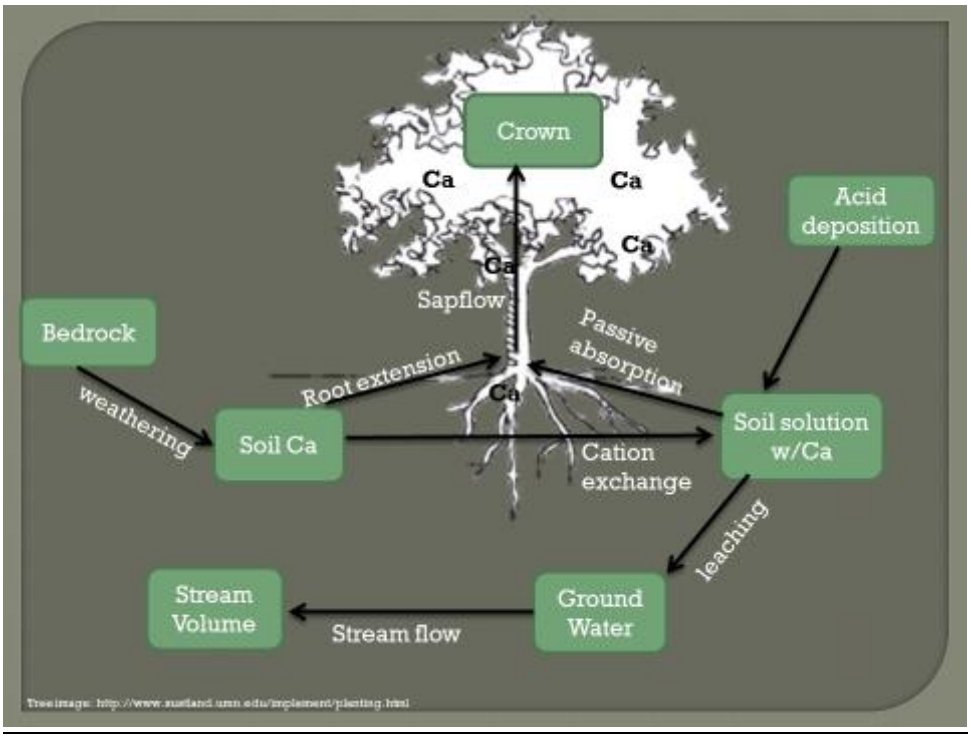

Figure 1: Calcium movement in tree and soil.

Water, calcium and other nutrients are taken up from the soil solution by the tree roots, through either passive absorption, or root extension and then flow through the xylem as tree sap. Water saturates the soil around tree roots where it can become part of the soil solution in the rhizosphere, the micro environment area immediately surrounding roots (S. A. Wilde 1958). In this zone water and nutrients are taken up by the plant. Passive absorption occurs when nutrients are taken up simultaneously during water uptake by roots, while root extension occurs when the roots grow right into the soil solution (Miller and Donahue 1990). Calcium enters the roots, becoming incorporated into root cells, or is loaded into the xylem to be distributed to other parts of the plant. The cohesive properties of water cause sap to move up the tree (Pallardy 2010), where water eventually exits the system through the leaves during transpiration. Calcium is allocated and incorporated throughout the tree to areas where it is needed such as woody tissue and leaves, eventually reentering the nutrient cycle upon leaf senescence or tree death (White and Broadley 2003; Taiz and Zeiger 2010).

Over the past 60 years the natural cycle of calcium in ecosystems has been disrupted by acid rain (Cogbill and Likens 1974). This disruption is evident in the Northeast, where the acid deposition is strongly influenced by Midwestern United States' energy production and industry. Power generation, fossil fuel burning, and industrial production emit nitrogen oxides and sulfur dioxide contributing to acid rain deposition, signified by precipitation having a pH of 5.3 or less. The Clean Air Act and amendments in the 1970s and 1990s helped reduce emissions from contributing sources. Even so, reduced emission levels remain higher than background conditions (Driscoll et al. 2001).

Given these circumstances, acid deposition has shown strong negative effects in the northern hardwood forests of the Northeast, where soil composition in many places leaves the soils vulnerable to nutrient leaching. Direct effects to the vegetation surfaces, such as the leaching of membrane associated calcium from red spruce needles, and to plant functions, like crown die back recovery, are influenced by the continuous and accumulating conditions (Driscoll et al. 2001). Acid deposition affects forest soil calcium levels because hydrogen ions, which are also positively charged, infiltrate the soil, replacing calcium ions. When the calcium is not attached to soil exchange sites , it is part of the soil solution, where it can be leached into the groundwater and flow into nearby streams (Cogbill and Likens 1974; Battles et al. 2014).

Forest trees suffer when lacking calcium, especially red spruce and sugar maple (Juice et al. 2006; Driscoll et al. 2003). Throughout the Northeastern United States and Southeastern Canada, fertilization studies have been conducted to assess how forest productivity is impacted by limiting nutrients. A meta-analysis of fertilization experiments concluded that northern hardwood forest productivity increases with the addition of nutrients, indicating that nitrogen had the largest influence (as

compared to calcium and phosphorous). However, Vadeboncoeur (2010) concluded that additional fertilization studies of calcium alone and not in combination with other nutrients were needed to find any significant productivity increase that could be related solely to calcium additions, suggesting that more research is needed in this topic area.

In an attempt to mitigate the calcium losses at the watershed-level, researchers replenished the lost nutrient with wollastonite ($CaSiO_3$) at a rate of 1.2 metric tonnes per hectare (Driscoll, Denny, and Siccama 2000).Though there are natural gradients and heterogeneity of soil calcium in the forest (A. Wilde 2013), the objective was to increase the base saturation of soil calcium from approximately 10 to 19 percent(Driscoll, Denny, and Siccama 2000) in a watershed known to have lost substantial calcium from the soil reserves due to acid rain leaching. Fertilizer addition in this form makes calcium available without adding additional micro-nutrients, as silicate is assumed to be inert in this process (Green 2013). Results of the wollastonite addition experiment showed a temporary increase in forest evapotranspiration for two and a half years following the calcium fertilizer treatment (Green, Bailey, and Bailey 2013). An increase in water uptake by the forest indicates more transpiration in plants, meaning there is more water flowing through the plants and less flowing into the streams. This increased water use could contribute to more tree growth and production(Juice et al. 2006).

The objective for our study was to measure sap flow, root conductivity, and root vitality in individual trees at wollastonite-treated and control plots at three sites within the northern hardwood forest of New Hampshire's White Mountains. We hypothesized, based on the results of the watershed experiment, that adding calcium fertilizers, in the form of wollastonite, to the soil would increase sapflux, root conductivity and vitality in treated plots.

Methods

Study Area

We conducted research at three sites, Jeffers Brook Valley (JB), Hubbard Brook Experimental Forest (HBEF), and Bartlett Experimental Forest (BEF) (Figure 2 and Table 1). These sites are located in northern New Hampshire on the White Mountain National Forest (Vadeboncoeur 2011). The area's climate consists of long, cold winters and mild to cool summers. The area receives about 1400 mm of precipitation a year, snow accounting for one third to one quarter. Snow pack usually lasts from mid-December to mid-April(Hubbard Brook Ecosystem Study). January temperatures average -9°C, and average July temperature is 18°C (Schwarz, Fahey, and McCulloch 2003).

The region's soils are derived from glacial till, formed from granite and high-grade metamorphic silicate rock parent material. This created soils that are well developed Spodosols that are coarse-loamy, moderately drained and generally acidic with a pH ranging from 3.5 to 5.5 (Schwarz, Fahey, and McCulloch 2003; Vadeboncoeur et al. 2014). Each of the sites has a different natural abundance of calcium in the soil, decreasing from west to east (A. Wilde 2013).

The forest type is comprised of northern deciduous hardwoods, dominated by American beech (*Fagus grandifolia*, FAGR), yellow birch (*Betula alleghaniensis*, BEAL) and sugar maple (*Acer saccharum*, ACSA). Most of the forest is second growth, following logging in the late 19th and early 20th centuries. Beech bark disease, which includes both insect and fungal components, has been a part of the New Hampshire landscape since the 1960s and has affected nearly all American Beech in the Northeast, though the species still remain an important part of infected forests (Shigo 1972; Taylor, McPhee, and Loo 2013; Griffin et al. 2003).

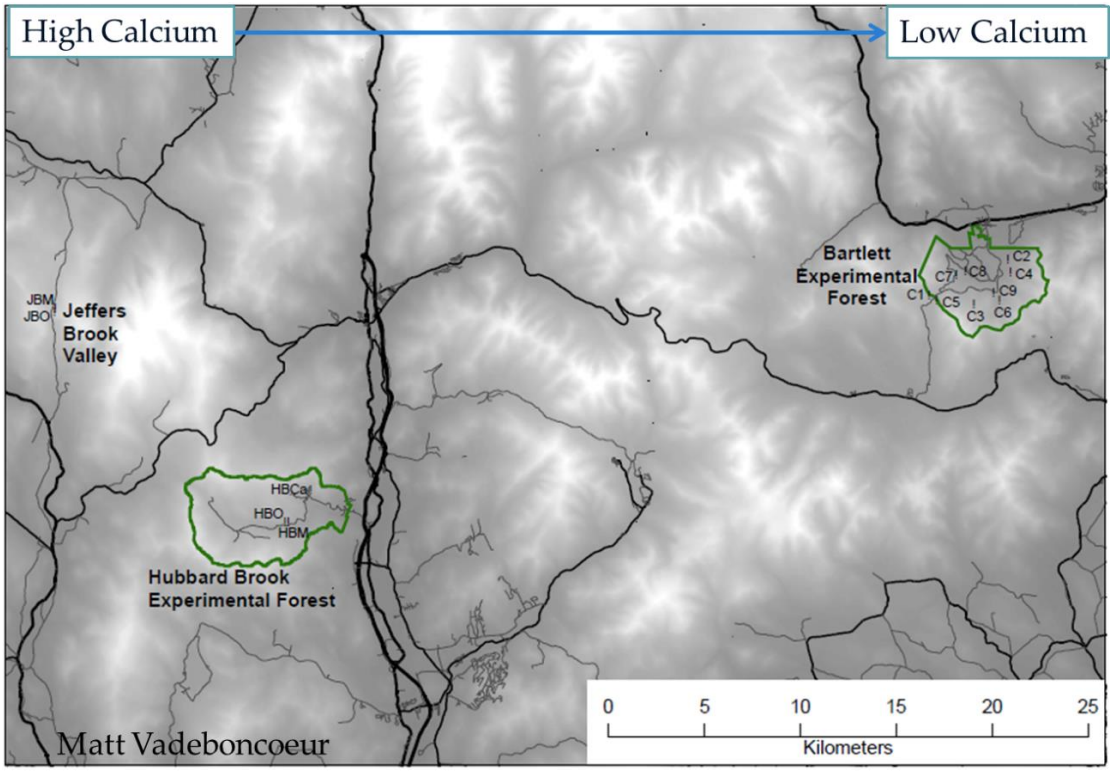

Figure 2: Site Locations and natural calcium gradient across the WMNF.

Experimental Design

Two 50 x 50 m plots (calcium-treated versus control), were selected to be representative of the forest stands at each of the three sites. Wollastonite ($CaSiO_3$) was manually applied, in October of 2011, to the calcium-treated plots at 1.2 metric tonnes per hectare with a fine powder of mineral, ground down to 10 microns (Driscoll, Denny, and Siccama 2000). Table 1 summarizes the characteristics of each site(A. Wilde 2013; Vadeboncoeur 2006).

Table 1: Site characteristics for the three calcium experiments within the White Mountain National Forest, NH.

Site	Natural Calcium	Slope	Aspect	Elevation (m)	Location (latitude and longitude)
Barttlet Experimental Forest (BEF)	Low	5-35%	NE	330	44º 03' N 71° 18' W
Hubbard Brook Experimental Forest (HBEF)	Medium	25-35%	SW	500	43º 57' N 71° 43' W
Jeffers Brook (JB)	High	30-34%	WNW	730	44º 02' N 71° 53' W

Nine relatively healthy canopy or sub-canopy trees, larger than 12.7 cm DBH, were selected for study at each plot. Ideally three trees of each of the studied species were chosen for monitoring, which was the case at both HBEF and BEF. Due to lack of availability of trees within sapflux sampling range, only two FAGR trees were measured at the calcium plot and instead four ACSA trees were sampled. Similarly, at the control plot, due to the tree availability within sapflux sampling, only one FAGR was sampled and five ACSA were sampled instead.

Sapflux: Field Operations

Tree sapflux was recorded every fifteen minutes using the Granier constant heat method, which used two probes, a CR1000 or CR800 data logger(*Campbell Scientific Inc*), a power source (two 12v marine batteries and two solar panels for every nine probes), heater boards, and a power regulator (Granier 1987). The Granier-Phillips probes, a slightly modified version of the original Granier probe, were assembled and tested in the lab prior to field deployment (Phillips, Oren, and Zimmermann 1996).

Each probe, sheathed in a 2 cm long aluminum sleeve, was inserted into 2 cm deep holes, drilled into the sapwood. As shown in Figure 3, the heated thermocouple was installed 10 cm above the reference probe and connected to the rest of the Granier sapflux system (figure adapted from Lu, Urban, and Zhao 2004). Previous years' probe locations were avoided to limit interference in sapflux measurements that could be due to scarring from the tree healing. Large defects, such as scars and areas blow dead limbs, were also avoided. Mylar sheets covered each installation site to protect from any solar heating of probes.

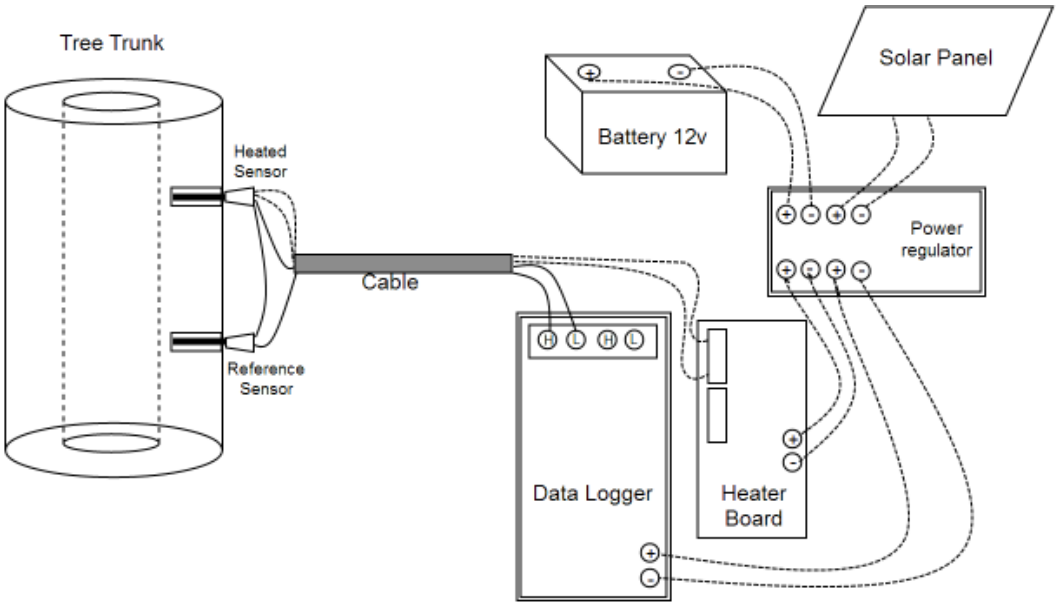

Figure 3: Diagram of Granier sapflux system set up (Adapted from Lu, Urban, and Zhao 2004).

Initial sapflux measurements were conducted in the summer of 2011 to gather pre-treatment data on the study plots. Due to limited power and equipment, only one site at BEF was set up. During this season, system requirements and limitations, such as number of batteries needed and number of sensors per data logger, were refined. Data were collected during the 2013 growing season, from early June to late September, to measure treatment and control plots. Data was collected at intervals of seven to ten days, as the system needed to be shut down periodically to let the 12v batteries recharge via the solar panels. Therefore, this system required weekly visits, to check power levels, monitor sensor and data loggers, replace broken sensors, and download data.

Root Conductivity and Vitality: Field Operations

Three replicates of coarse roots were sampled at each plot in summer and fall of 2012. Because of high variation among replicates, sample size was increased to five in 2013, and roots were sampled in spring at HBEF and at all three sites in summer. Samples were dug out of the soil, following larger roots from trees, to ensure correct species identification and plot location. Roots ranged in diameter from 18 mm to

30mm in size, with straight non branching sections about six inches long being ideal. Fine roots were also collected from each tree at HBEF for root vitality measurements. Roots, both coarse and fine, were stored in plastic bags with written labels, and stored in coolers, with ice packs, for transportation back to the laboratory.

Root Conductivity and Vitality: Laboratory Operations

Coarse and fine roots were stored in laboratory refrigerators for a maximum of 24 hours, before lab procedures were conducted. The maximum conductivity and the loss of conductivity as a result of native embolism were measured in sampled roots to calculate percent embolism. Fresh cuts were made on each end of the coarse roots while submerged in water; one end of the root xylem was then attached to tubing and perfused at a pressure of 4 kPa with filtered (0.22 um) HCl (ph 2). To measure loss of conductivity due to native embolism the hydraulic pressure head was kept low to prevent refilling of embolized tracheids. The flow rate was measured in a 2-ml graduated pipette, which was attached to the other end of the root; the temperature of the solution was also recorded for each measurement. Each sample was measured three times, for both initial (native) (ks,nat) and maximum conductivity (ks,max). Maximum conductivity was measured after samples were submerged in HCl solution and placed under vacuum (3.0 Mpa) for 34 hours (Domec and Pruyn 2008). Certain groups of samples resulted in negative embolism, which is sometimes eliminated, were kept as we suspected they might have significance because it showed up repeatedly.

The triphenyltetrazolium chloride (TTC) analysis was used to measure the amount of live tissue per mass of fine roots. Originally clear, the TTC solution was biologically reduced, by enzymes in living tissues, to formazan, a non-water-soluble, red compound. There is known to be a very strong linear relationship between spectrophotometric absorbance and tissue dry weight (Joslin and Henderson 1984). Fine roots collected during the spring of 2013 at HBEF were used for this analysis, as the start of the growing season best represents root vitality compared to other parts of the year (Cleavitt 2013).

Fine roots were sectioned into 1-2cm long pieces, dried, and split into two separate samples, one for dry weight one for triphenyltetrazolium chloride(TTC) analysis. TTC samples were placed into tubes with the TTC buffer solution (ten mL of a 0.1 M potassium phosphate buffer (pH 7.0) solution containing 0.6% TTC and 0.05% Tween 20 (Ruf and Brunner 2003) and then placed in a vacuum (3.0 Mpa) for 45 minutes to allow buffer infiltration. Vilas were then capped with parafilm and incubated in the dark for 15 hours at 30°C. After incubation samples were decanted, drained, and then double rinsed. Samples were ground with mortar and pestle using 95% ethanol and sterile sand, and then centrifuged at 70 rpm for one minute. Absorbance at 520nm was measured on a spectrophotometer for the supernatant after it was removed from the centrifuge; 95% ethanol was used as the blank. Reduction of TTC was calculated as absorbance per gram of root dry weight.

Data Analysis

Using the BaseLiner software developed by Oren and Parashkevov (2012),voltage difference was converted into sapflux, using the equation of $119*((B/X)-1^{1.23}$, where B is the baseline value, which was manually set at zero for each ordinal day, and X is the data point value. The baseline was set by visually inspecting each day's readings, then selecting the highest point of voltage difference as the zeroing out point of that day.

Daily sap flow graphs were constructed by computing the hourly sapflux medians for each individual tree. Then the medians of all tree hourly medians were computed to graph a single species over one 24 hour period to visually compare the calcium and control plots. The same was done for all trees at each plot to compare the treatment and control plot. This was also done for pretreatment data gathered during the growing season of 2011 to check that the plots had similar sapflux values before the treatment of wollastonite was applied.

Sapflux ($g/m^2/day$) for each tree on each day was totaled and designated as a Tree-day. Sapflux was only totaled for the times between 0400 and 1900 hours, as this is

when 90 to 99 percent of total sapflux occurs. Only Tree-days with 50 or more observations were used, as 60 would be a full number of observations for the studied hourly window. Any Tree-days that had a total sapflux greater than 150 g/m^2/day were eliminated, as this was found to be the maximum rate for northern hardwoods (Ewers et al. 2002). Values higher were most likely from errors that occur due to system malfunction or power fluctuations.

To understand how much of an influence the wollastonite treatment had on total daily sapflux per Tree-day, a linear model was constructed to predict values based on site and species. The influencing factors considered were potential evapotranspiration (PET), unique tree identification, DBH (cm), and treatment. The best fitting model, based on the lowest AIC, was determined for each species at each site. The predictive equations used were: Total daily sapflux (g/m^2/day) = PET+treatment+DBH+unique tree ID and, Total daily sapflux (g/m^2/day) = PET+treatment+DBH. Each of these models indicated how much of an influence the calcium treatment had on total daily sapflux.

Coarse conductivity (ks, kg $m^{-1}\cdot MPA^{-1}\cdot s^{-1}$) was calculated for samples according to Darcy's law. Root conductivity was calculated as the mass flow rate of the HCl solution divided by the pressure gradient across the root segment, and standardized by the xylem cross-sectional area. To account for changes in water properties due to temperature fluctuations, conductivity calculations were corrected to 20 ° C. Native embolism was determined by comparing the initial (or native) conductivity (ks,nat) of root segments to the maximum conductivity (ks,max) Percent embolism of coarse root conductivity was calculated as (ks,max - -ks,nat/ks,max)*100 (Domec and Pruyn 2008). Roots from the calcium-treated and control plots were compared using the Wilcoxon T-test, a non-parametric test, to measure any significant difference in percent embolism between the two treatments. The test was also used for each of the three species groups and three sites to test for a treatment effect.

The results from TTC analysis of the fine roots from the calcium- treated and control plot were compared using the Wilcoxon T-test, a non-parametric test, to test for any significant difference in absorbance per mass between the two treatments. The test was also used for each of the three species groups to test for a treatment effect.

Results

Calculating total sapflux for Tree-days by site, species and treatment.

Combining the sapflux data into one 24 hour period allows visualization of the overall cumulative trend for each site and species. Pretreatment data from the 2011 growing season at the BEF site are graphed (figure 4); displaying sapflux ($g/m^2/sec$) for each species and all trees at the calcium (dashed line) and control (solid line) plots are shown. BEAL trees at the control plot were missing from this data set, though the calcium trees follow the same trend as the other measured species, peaking around 1000 hours and having a maximum flow close to 20 $g/m^2/sec$. FAGR shows some difference having higher sapflux at the control plot when compared to the calcium; with median total daily sapflux for calcium at 572,283 $g/m^2/day$ and for control at 441,589 $g/m^2/day$. ACSA suggests little to no difference between daily sapflux comparing the two treatments, with median total daily sapflux for calcium at 412,704 $g/m^2/day$ and for control at 516,456 $g/m^2/day$. This trend, of little to no difference, is also shown for all species comparing trees at the two plots; with median total daily sapflux for calcium at 499,945 $g/m^2/day$ and for control at 514,881 $g/m^2/day$ (Christ 2011).

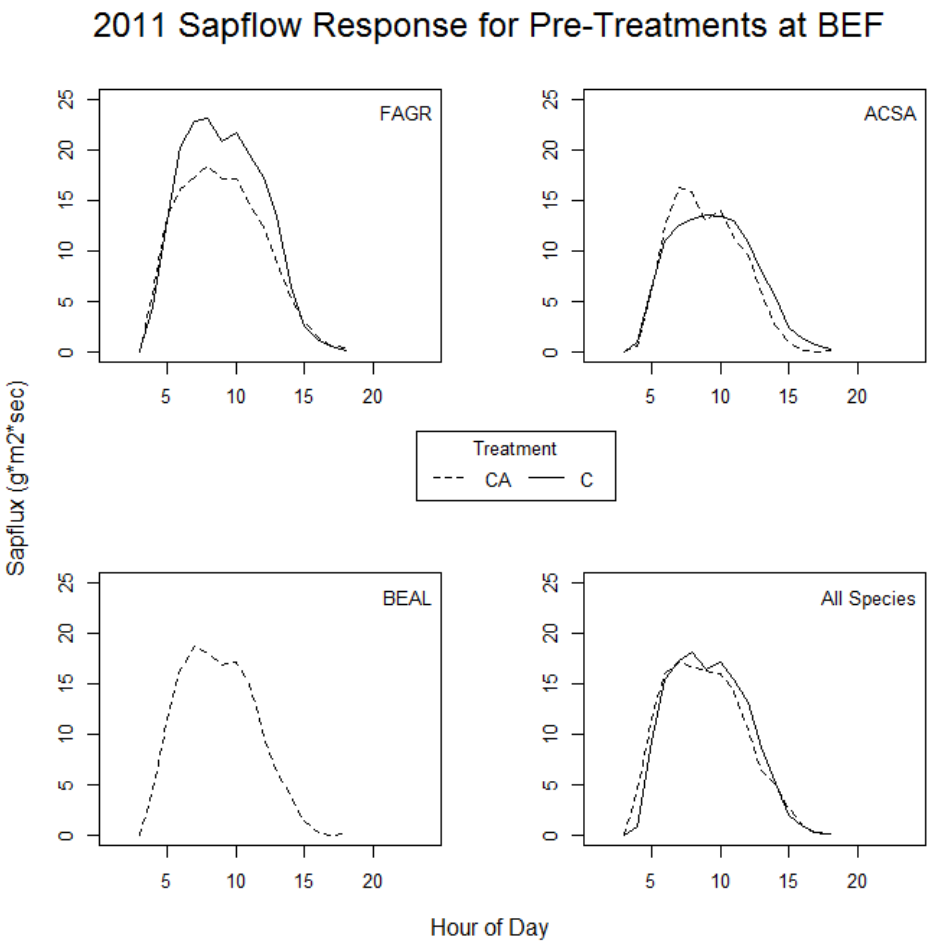

Figure 4: 2011 Hourly medians for all days and trees at BEF.

Figure 5 showed sapflux (g/m^2/sec) graphs for each species and all trees for the calcium (dashed line) and control (solid line) at the BEF site. FAGR trees' sapflux tended to be slightly higher at the calcium plot during the middle part of the day compared to control, with median total daily sapflux for calcium at 434,115 g/m^2/day and control at 272,655 g/m^2/day. BEAL trees also tended to follow the same trend of higher sapflux at the calcium plot compared to the control during the middle part of the day; with median total daily sapflux for calcium at 636.129 g/m2/day and control at 407,610 g/m^2/day. In contrast, ACSA control trees showed a trend of very high sapflux compared to the calcium plot during the majority of the day, with median total daily sapflux for calcium at 285,772 g/m^2/day and control at 224,145 g/m^2/day. Comparing all tree species for calcium versus control at BEF, there seems to be little

to no difference in the trends between the calcium treated plot and the control plot; with median total daily sapflux for calcium at 444,654 g/m^2/day and control at 324,144 g/m^2/day. This might be caused by a cancelling out effect because of the contrasting trends among the species.

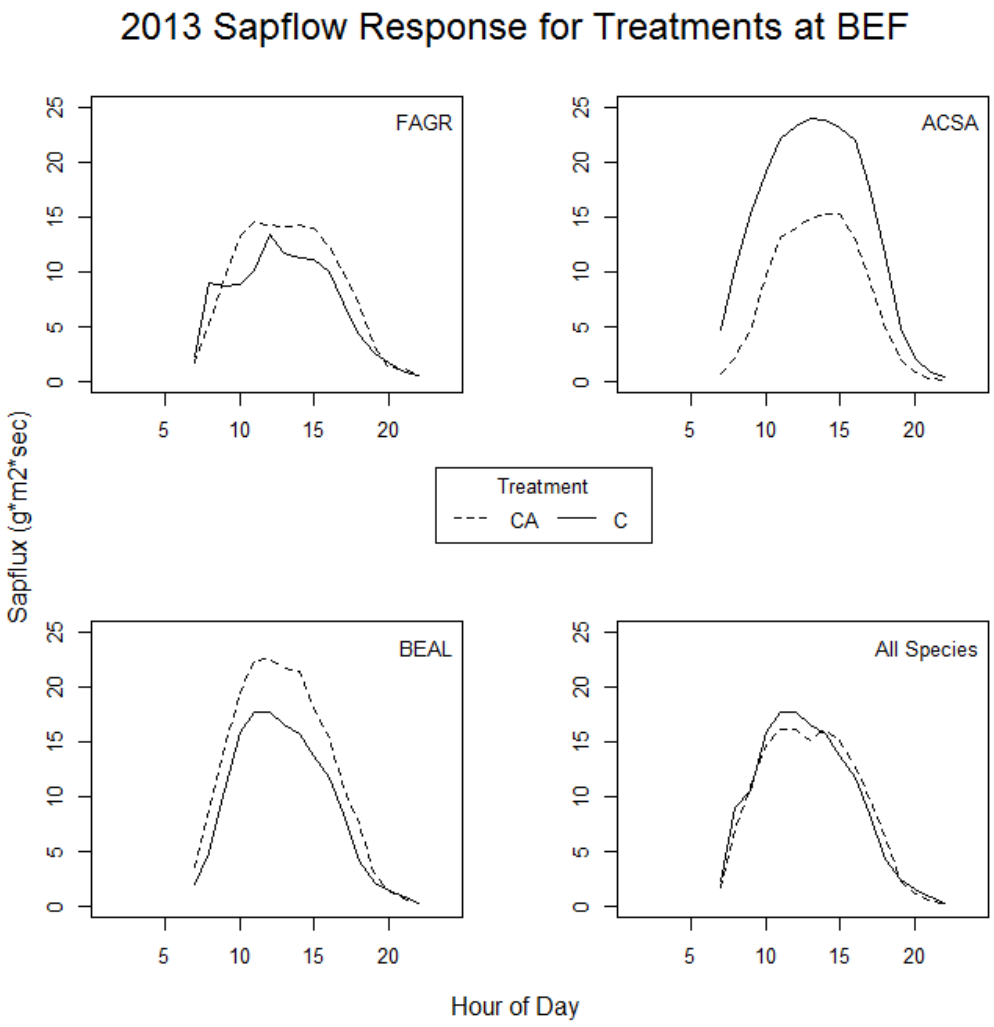

Figure 5: 2013 Hourly medians for all Tree-days at BEF.

HBEF sapflux (g/m^2/sec) plotted as all Tree-days for each species and all trees for the calcium (dashed line) versus control (solid lined) did not show contrasting trends, like from those at BEF (Figure 5). FAGR trees showed little to no difference at the control vs. calcium plots, with median total daily sapflux for calcium at 500,629 g/m^2/day and

for control at 621,495 g/m^2/day. Both BEF ACSA and BEAL trees tended to have higher sapflux for the majority of the day for trees at the control plot compared to trees at the calcium treated plot. This trend was more noticeable for ACSA with median total daily sapflux for calcium at 193,284 g/m^2/day and for control at 562,554 g/m^2/day. BEAL had median total daily sapflux, reflecting the opposite trend, for calcium at 447,534 g/m^2/day and for control at 392,017 g/m^2/day. Combining the medians for all three species supported the ACSA and BEAL trend of potentially higher sapflux at the control versus calcium plots, with median total daily sapflux for calcium at 405,837 g/m2/day and for control at 551.943 g/m2/day.

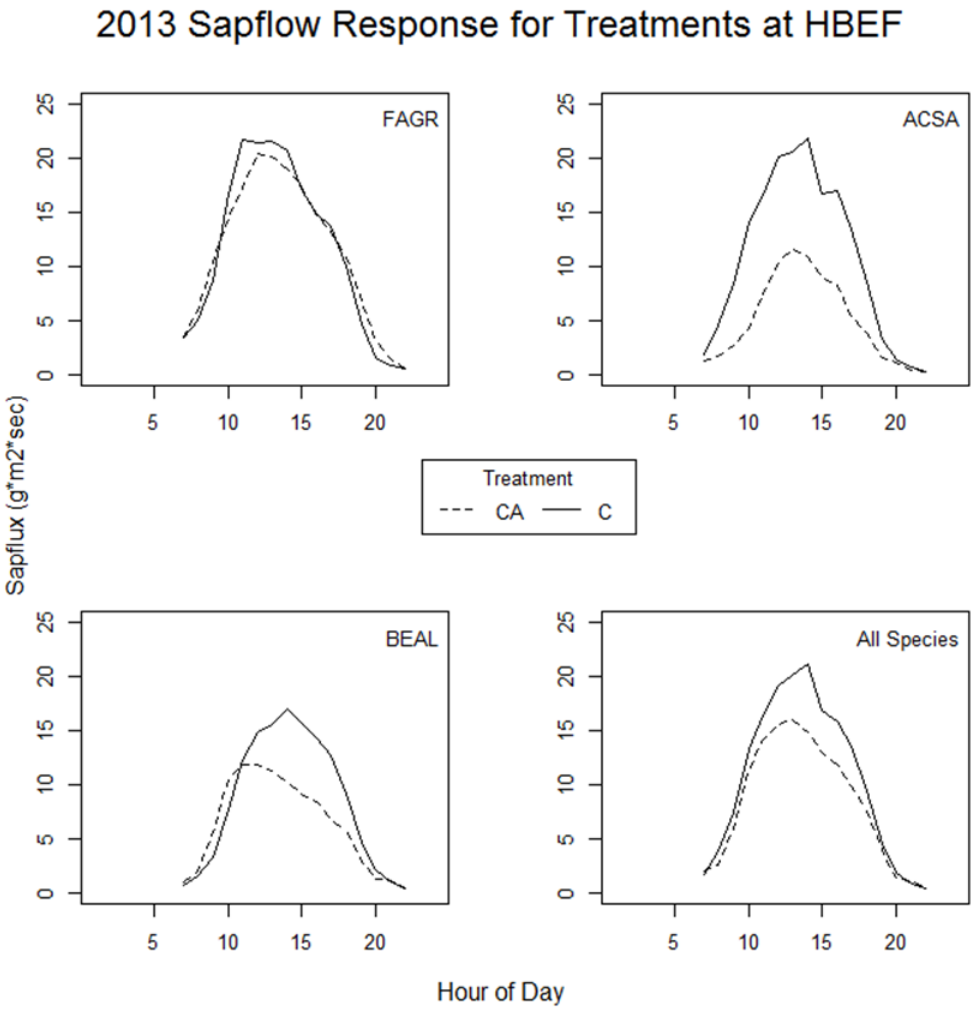

Figure 6: 2013 Hourly medians for all days and trees at HBEF.

Sapflux ($g/m^2/sec$) graphs for each species and all trees for the calcium (dashed line) and control (solid line) at the JB site can be seen in figure 6. Similar to the pattern at BEF, FAGR trees showed higher sapflux at the calcium plot compared to the control during the middle part of the day, with median total daily sapflux for calcium at 523,264 $g/m^2/day$ and for control at 284,445 $g/m^2/day$. ACSA trees showed higher sapflux at the control compared to the calcium, following the same trend seen for this species at HBEF, with median total daily sapflux for calcium at 303,012 $g/m^2/day$ and for control at 336,888 $g/m^2/day$. Unlike HBEF, BEAL trees showed higher daily sapflux for the calcium versus control plots especially during peak sapflux hours, with median total daily sapflux for calcium at 760,729 $g/m^2/day$ and for control at 326,241 $g/m^2/day$. Overall trend for all species combined at JB showed a trend of higher sapflux at the calcium vs. control plots with median total daily sapflux for calcium at 484,276 $g/m^2/day$ and for control at 326,241 $g/m^2/day$.

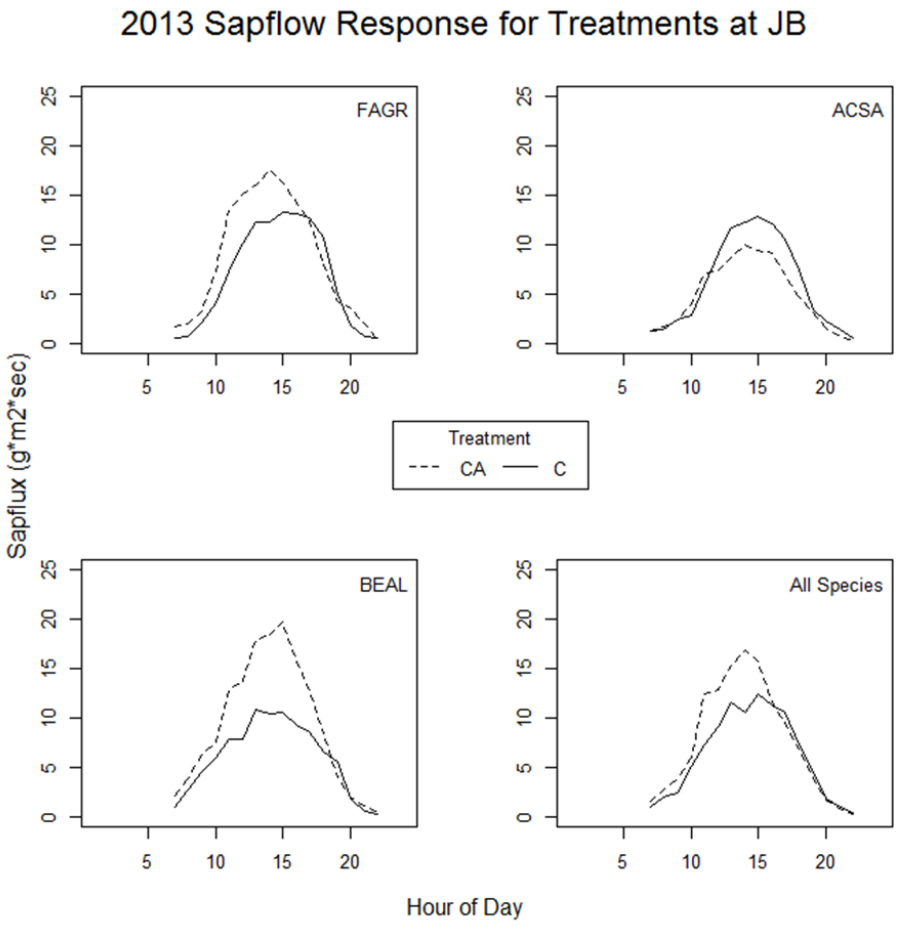

Figure 7: 2013 Hourly medians for all days and trees at JB.

Predictive Linear Model for total daily sapflux per Tree-day

Each site and species combination used one of the two linear models to predict total daily sapflux that was to account for potential evapotranspiration (PET), treatment, diameter at breast height (DBH), and variance between individual trees (unique ID). The two models were: Total daily sapflux ($g/m^2/day$) = PET+treatment+DBH+unique tree ID, and Total daily sapflux ($g/m^2/day$) = PET+treatment+DBH. The best fit between the two linear models was determined for each species and site, based on the lowest AIC value (Table2). The influence of the calcium treatment as a driver of total daily sapflux in comparison to the control is also reported along with the associated p-value.

All three tree species at the BEF site had the best fitting linear model when taking into account PET, treatment, DBH, and unique ID, with the strongest fitting for ACSA with an AIC of 588.10. For BEF ACSA and FAGR, with an AIC of 846.00, the calcium treatment negatively impacted total daily sapflux as compared to the control. This negative influence was significant only for ACSA at BEF, with a p-value of 0.03. In contrast, the BEF BEAL trees treated with calcium, which were also significant with a p-value of 2.2E-05, positively correlated with total daily sapflux, modelled with an AIC of 995.10. At HBEF, FAGR and ACSA were modeled best by accounting for PET, treatment, DBH and Tree ID. In contrast, the BEAL model did not include Tree ID, but did include the three other parameters. Although not significant, these models also predicted a negative relationship between calcium treatment and total daily sapflux. As with HBEF, JB FAGR and ACSA were best modeled when accounting for PET, treatment, DBH, and unique ID. Still matching HBEF, JB BEAL was best modeled when excluding Tree ID and using the other three parameters for daily sapflux. Unlike HBEF, all three JB models showed calcium had a negative influence on total daily sapflux, though they were not significant.

Table 2: Fit of linear model for total daily sapflux for site and species. (Significant values p<0.05, in bold italics)

Site	Species	Total daily sap flow Linear Model	R2	AIC	CA influence (g/m2/day)	p-value
BEF	FAGR	Tsap= PET + treatment + DBH + unique ID	0.74	846.00	-1171612.00	0.10
BEF	ACSA	Tsap=PET+ treatment + DBH + unique ID	0.93	588.10	-133632.00	***0.03***
BEF	BEAL	Tsap= PET + treatment + DBH + unique ID	0.69	995.10	940419.00	***2.2E-05***
HBEF	FAGR	Tsap= PET + treatment + DBH + unique ID	0.99	112.50	-242515.00	0.10
HBEF	ACSA	Tsap= PET + treatment + DBH + unique ID	0.69	429.50	-233185.00	0.20
HBEF	BEAL	Tsap= PET + treatment + DBH	0.96	156.10	-36484.00	0.88
JB	FAGR	Tsap= PET + treatment + DBH + unique ID	0.99	112.50	-242515.00	0.10
JB	ACSA	Tsap=PET+ treatment + DBH + unique ID	0.69	429.50	-233185.00	0.20
JB	BEAL	Tsap= PET + treatment +DBH	0.96	156.10	-36484.00	0.88

Coarse root conductivity and fine root vitality

Studying coarse root conductivity resulted in significant differences (Wilcox test (R Core Team 2013); α of 0.05) in percent embolism between calcium versus control paired plots at BEF and HBEF (Table 3). The results show each sample season broken down by treatment, then site and species. The W and p-values are reported along with the median of each treatment for the tested pair. Four significant pairs of calcium vs control, three at BEF and over all sites, showed higher percent embolism for the calcium plot. With all trees combined, calcium plots showed significantly higher embolism at BEF for all three sampling seasons.

Table 3: Results of Wilcox T-test for treatment effect on percent embolism in coarse roots. (Significant values,p<0.05 in bold italics)

Is there a significant difference in % Embolism between CA vs C plots (Wilcox T-test)			All trees	Sites			Species		
				BEF	HBEF	JB	ACSA	BEAL	FAGR
Sample Season	Summer 2012	W	308.50	22.00	32.00	39.50	27.00	32.00	37.00
		p-value	0.52	***0.03***	0.74	0.20	0.33	1.00	0.55
	Fall 2012	W	353.00	16.00	96.00	24.00	40.00	53.00	35.00
		p-value	***0.01***	***0.01***	0.96	0.17	0.12	0.13	1.00
	Spring 2013	W	74.00	(samples only taken at HBEF)			9.00	9.00	6.00
		p-value	***0.00***				0.10	0.10	0.70
	Summer 2013	W	744.00	39.00	178.00	26.00	71.00	95.00	71.50
		p-value	0.39	***0.01***	***0.00***	0.20	0.78	0.71	0.26

Median %E CA vs C plots			All trees	Sites			Species		
				BEF	HBEF	JB	ACSA	BEAL	FAGR
Sample Season	Summer 2012	CA	87.08	***89.80***	73.32	47.87	89.03	47.87	90.56
		C	54.11	***50.37***	49.09	90.18	82.56	-9.32	52.67
	Fall 2012	CA	***88.54***	***99.45***	74.82	71.75	91.35	89.88	78.78
		C	***53.63***	***46.53***	62.06	33.27	57.44	-4.48	92.93
	Spring 2013	CA	***-26.52***	(samples only taken at HBEF)			-26.52	-45.74	-8.28
		C	***55.03***				57.74	71.96	41.71
	Summer 2013	CA	67.06	***86.69***	***19.12***	78.72	57.27	74.56	52.03
		C	69.83	***53.96***	***96.41***	20.54	86.98	60.20	72.90

During all three sample seasons BEF showed a significant difference in percent embolism for all tree species, with 86.69 to 99.45 percent at the calcium plots and

46.53 to 53.37 percent at the control plots. During summer 2013 at the HBEF site there was a significant difference in percent embolism with the control plot being higher, with 96.41 percent, than the calcium, which had 19.12 percent.

In contrast two other significant pairs, at HBEF, showed a higher percent embolism for the control plot over the calcium treated plot. In Spring of 2013 HBEF, which was the only site measured for that sample season, showed the same trend with the control plot having a significantly higher percent embolism, at 55.03 percent compared to the -26.52 percent at the calcium plot. These results of negative embolism found are addressed in the discussion.

The results of the Wilcox T-test show that there are no significant differences in root vitality, though there was a trend of higher absorbency at the calcium plot, as indicated by TTC between calcium verses control pairs (Table 4). The sample season was broken down by treatment, then by species. The W and p-values are reported along with the median of each treatment for the tested pair.

Table 4: Results for Wilcox T-test for treatment effect on TTC response in fine roots.

Is there a significant difference in TTC between CA vs C plots at HBEF (Wilcoxon T-test)		All trees	Species		
			ACSA	BEAL	FAGR
Spring 2013	W	25.00	2.00	3.00	3.00
	p-value	0.32	0.40	1.00	0.70
Median TTC CA vs C at HBEF (absorbency per gram)		All trees	Species		
			ACSA	BEAL	FAGR
Spring 2013	CA	18.82	20.00	25.86	17.65
	C	17.35	17.35	19.89	15.15

Discussion

Our hypothesis, that the calcium fertilization treatments of wollastonite would increase sapflux, root conductivity, and root vitality, was partially supported. Tree response seems to be dependent on site and species, and in some cases the season. A range of different trends was found, which could be explained by a variety of physiological mechanisms.

The results from the total daily sapflux linear models for BEAL trees at BEF site support our hypothesis of increased sapflux in response to wollastonite addition (Table 2). The results showed that the calcium treatment has a significant positive influence on total daily sapflux. This was supported by the trends shown in sapflux graphs displaying hourly medians over one 24 hour period. The other significant linear model (Table 2) was also at BEF, ACSA, though in this species the calcium treatment had a negative influence on total daily sapflux, not supporting the hypothesis. This trend too was supported by the 24 hour sapflux graphs, which showed higher sapflux at the control plot vs calcium.

In the case of BEAL, calcium could be allowing the trees to take up more water and move it up the xylem at higher volumes than the control trees. Lautner et al. (2007) showed a decrease in lignin and fiber length in the xylem, which are important components of plant cell wall structure, in calcium deficient aspen compared to optimally supplied trees (Panshin and Zeeuw 1970). Since calcium is important in this wood wall cell formation, the positive influence could be due to stronger more stable

conductive tissues being formed with the added nutrient (Fromm 2010). This was supported by the 24 hour sapflux graphs at BEF and JB, which both showed higher sapflux in BEAL. Different tree species have varying life history strategies. ACSA trees, which showed a decrease in sapflux with the calcium addition, may be using the added calcium in different ways than the BEAL, and could be a factor behind the contrasting trend between the two species.

The higher sapflux rate of ACSA control trees vs calcium are supported by 24 sapflux graphs for all three sites. Lower total daily sapflux in calcium treated sites could result from better stomata regulation in the leaves, which control transpiration, as calcium is vital part of this function (Taiz and Zeiger 2010). If the wollastonite treated trees have better control of their stoma closure, a possible effect would be reduced sapflux. An examination of stomata conductance between the treatments would be one way to further investigate decreased sapflux. Since the total daily sapflux linear models had treatment as a significant influence only at BEF, which was the naturally poorest calcium site compared to JB and HBEF, evidenced by lower levels of calcium in the foliage (A. Wilde 2013). Natural calcium soil variation might also influence how trees react to wollastonite.

Measurements of coarse root conductivity also showed a significant difference between percent embolisms in calcium treated and control trees at BEF (Table 3). For the three seasons sampled (summer 2012, fall 2012, and summer 2013) there was higher percent embolism at the calcium treated plot compared to the control for all the tree species. This suggested that the native conductivity of the xylem in the roots was less than the maximum conductivity. This was most likely from air bubbles blocking the xylem, preventing the coarse roots from moving water at full capacity. Though the successful removal of air blockages in the roots indicated that embolisms are not permanent and the conductive xylem is still functional.

A study on calcium's role in wood formation in aspen seedlings (*Populus tremula* × *Populus tremuloides* clones) showed a decrease in both vessel size and wood increment growth in calcium starved trees, compared to optimally supplied trees. It was also shown that the calcium starved aspen had less cell formation in radial direction for cambial and xylem growth (Lautner et al. 2007). The researchers also concluded that vessel size is the main controlling factor in water movement since transpiration increased with vessel size, but this larger size also left the xylem more vulnerable to cavitation (Lautner et al. 2007). The more efficient water moving calcium treated roots, with their higher chance of embolism, could have caused the negative influence that calcium treatment has on conductivity at BEF (all sample seasons) and HBEF (spring 2013). These significant differences occurred at both BEF and HBEF, which are poorest and second poorest in natural soil calcium levels, again suggesting that this landscape characteristic might have an influence on how trees respond to wollastonite.

Studying the percent embolisms of the individual species at BEF gives more insight to how root mechanisms might vary among them. Herbette and Cochard (2010) found that 70 percent of variation between species, when measuring 50 percent conductivity loss, is due to the presence of calcium in the cell wall. During all three sample seasons FAGR had higher embolism at the calcium plot compared to the control. This trend was also true for ACSA during the summer and fall of 2012, with 88.06 and 98.15 percent embolism each (Table 5). The high percent embolisms in these species at the wollastonite addition plots support the negative influence calcium treatment had on the model for predicting total daily sapflux (Table 2).

For both summer and fall 2012 BEAL had negative embolism at the control plots, meaning that native conductivity of the xylem in the roots was greater than the maximum conductivity. A possible reason for the negative embolism found in BEAL is the larger, more vulnerable root xylem collapsed when exposed to the vacuum force,

rather than pulling out any existing air bubbles. Root conductivity at the control plot had a relatively high percent embolism during the two sample seasons (summer and fall 2012), 73.10 and 100 percent (Table 5). It is possible that BEAL at the calcium plot had higher native conductivity than the control, due to the increased calcium allowing for formation of longer fibers in the xylem. Even though there might have been higher native conductivity for calcium BEAL, the vessels are more vulnerable to cavitation compared to the control BEAL roots and could have collapsed when put under vacuum (Lautner et al. 2007). This would support the positive influence calcium had the other model for total daily sapflux for BEAL at BEF.

During the summer of 2013 at BEF the trends of the previous year were reversed compared to summer 2012 for both ACSA and BEAL. ACSA trees had higher embolism at the control plot compared to the calcium, with -100.98 and 81.40 percent respectively during summer 2013; negative embolism meaning that the vacuum could not remove the existing embolism or some sort of damage occurred. BEAL trees also had higher embolism at the calcium plot compared to the control, with 82.78 and 60.23 percent each during summer 2013 (Table 5). This change from the year before could be due to another factor that was not measured, such as winter damage to the roots(Cox and Zhu 2003).

Table 5: Median percent embolism by species at BEF.

median % embolism BEF		ACSA	BEAL	FAGR
Summer 2012	CA	88.06	73.09	90.84
	C	51.55	-87.11	52.67
Fall 2012	CA	98.15	100.00	99.49
	C	69.52	-10.91	71.94
Summer 2013	CA	81.40	82.78	91.37
	C	-100.89	60.20	55.60

During spring 2013 at HBEF there was greater embolism, actually negative, at the wollastonite treated plot compared to the control plot. Though there were no significant differences between calcium and control plots at HBEF regarding the influence of treatment on the total daily sapflux model, there was a significant

difference in root embolism conductivity during the spring and summer of 2013. During HBEF summer 2013 there was a reverse trend compared to early that spring, with greater embolism at the control compared to the calcium plot. (Table 3). These trends, especially the negative embolism, could be pointing to changing mechanisms with in the trees.

The negative embolism observed under the maximum conductivity measurements from roots collected at the calcium plot during spring 2013, a time for fast new growth, could be explained by the larger and less structurally-sound xylem subsequently collapsing while under vacuum pressure of the maximum conductivity test. This would support the negative correlation between calcium treatment and total daily sapflux at HBEF (even though not significant) (Table2). The switch in percent embolism trends, from negative embolism (spring 2013) to very low embolism (summer 2013) at HBEF calcium treated plot, in the later growing season could be due to the xylem being repaired in the roots of the calcium trees after the high embolism that occurred in the spring. Wollastonite treated plots could provide more calcium for this repair to take place, and might be the cause for such low embolism at the treated plot for the HBEF site later in the season (Lautner et al. 2007).

The study of fine roots to measure root vitality yielded no significant differences comparing the calcium and control plots at HBEF. This was true at the whole plot level and the species level. Even so, there was an overall trend of higher absorbency per mass at the wollastonite treated plot, across all three species (Table 4). As calcium is important in forming new cell walls, the treated plot would have more of the nutrient available to grow better functioning fine roots (Cox and Zhu 2003). A study of sugar maple fine roots showed the least amount of damage for calcium treated trees, compared to a control and aluminum addition (Halman et al. 2013). With larger sample replication, the trend of higher vitality could reveal significant

trends for the calcium treatment, which would support the hypothesis for calcium improving fine root vitality.

Another reason higher conductivity and higher sapflux were not always seen paired together could be explained by the finding of Herbette and Cochard (2010), who found that removing calcium from cell walls did increase chances of cavitation but had no effect on xylem transportation of water. Even though all three mechanisms, of sapflux, root conductivity and root vitality, have roles in the transportation of water and other nutrients, these three are not always very closely linked when responding to calcium levels(Herbette and Cochard 2010).

Conclusion

The results from our study did not universally support our hypothesis that calcium fertilizers of wollastonite would lead to increased sapflux, root conductivity and vitality. Transpiration mechanisms are complicated and tree physiological response to increased levels of calcium occurs in a multitude of ways. Tree physiology has many drivers happening below, above and within the stem, all of which involve calcium. Ecological processes below the whole tree level could have an influence on calcium acquisition and use. Mycorrhizal fungi, which form a mutualistic relationship with plant roots and vary by species, can enhance water and nutrient uptake for their host. In particular the fungi can produce an acid that combines with calcium to form calcium oxalate, which can help prevent leaching of this nutrient (Perry, Ram, and Hart 2008). Wollastonite fertilization increased the percentage of mycorrhizal colonization in ACSA seedlings compared to control seedlings (Juice et al. 2006). At the landscape scale tree harvesting practices can have an influence on soil nutrient levels. If the nutrients taken or washed away as a result of timber harvesting are not naturally or artificially replenished before the next harvest cycle, the forest soil nutrients could suffer long term damage. A study showed that under several different scenarios, calcium would be one of the soil nutrients to be depleted due to harvesting practices (Vadeboncoeur et al. 2014)

Coarse root conductivity may be more sensitive to calcium levels than sapflux, though it may have many mechanisms driving the response to the fertilization. Fine root vitality could be a valuable metric toward monitoring the effects of wollastonite additions, though this study suggested a larger sample size was needed. This research can help forest managers understand the impacts acid rain has on soil calcium and forest function, along with the possible effects of wollastonite mitigation to the forest system. Further study of tree responses to calcium addition, such as root and stem anatomy can help explain the mechanisms behind the varied outcomes.

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Appendixes

Appendix A : Table of total daily sapflux for each individual Tree-day

PET	Ordinal day	ID	Unique ID	DBH (cm)	species	site	treatment	Total sap ($g/m^2/day$)
1.420	200	AB1	AB1bc	24.5	FAGR	BEF	C	427590
0.983	206	AB1	AB1bc	24.5	FAGR	BEF	C	249462
1.690	208	AB1	AB1bc	24.5	FAGR	BEF	C	328536
	`	AB1	AB1bc	24.5	FAGR	BEF	C	294426
1.018	212	AB1	AB1bc	24.5	FAGR	BEF	C	273177
0.946	214	AB1	AB1bc	24.5	FAGR	BEF	C	237735
	215	AB1	AB1bc	24.5	FAGR	BEF	C	272655
0.636	216	AB1	AB1bc	24.5	FAGR	BEF	C	243036
1.056	217	AB1	AB1bc	24.5	FAGR	BEF	C	257004
0.964	219	AB1	AB1bc	24.5	FAGR	BEF	C	251496
0.409	220	AB1	AB1bc	24.5	FAGR	BEF	C	211770
1.118	223	AB1	AB1bc	24.5	FAGR	BEF	C	266769
1.148	224	AB1	AB1bc	24.5	FAGR	BEF	C	232533
1.754	199	AB2	AB2bc	30	FAGR	BEF	C	797013
1.420	200	AB2	AB2bc	30	FAGR	BEF	C	655623
0.947	201	AB2	AB2bc	30	FAGR	BEF	C	370719
1.241	202	AB2	AB2bc	30	FAGR	BEF	C	325881
0.946	214	AB2	AB2bc	30	FAGR	BEF	C	842409
0.636	216	AB2	AB2bc	30	FAGR	BEF	C	113796
1.690	208	SM2	SM2bc	20.9	ACSA	BEF	C	273204
	211	SM2	SM2bc	20.9	ACSA	BEF	C	215550
1.018	212	SM2	SM2bc	20.9	ACSA	BEF	C	232740
0.636	216	SM2	SM2bc	20.9	ACSA	BEF	C	123012
1.056	217	SM2	SM2bc	20.9	ACSA	BEF	C	142533
0.800	218	SM2	SM2bc	20.9	ACSA	BEF	C	141822
1.118	223	SM2	SM2bc	20.9	ACSA	BEF	C	144999
0.947	201	SM3	SM3bc	42.2	ACSA	BEF	C	778770
1.241	202	SM3	SM3bc	42.2	ACSA	BEF	C	727830
1.056	217	SM3	SM3bc	42.2	ACSA	BEF	C	723231
1.690	208	YB1	YB1bc	41.6	BEAL	BEF	C	256158
	211	YB1	YB1bc	41.6	BEAL	BEF	C	207144
1.018	212	YB1	YB1bc	41.6	BEAL	BEF	C	203382
0.946	214	YB1	YB1bc	41.6	BEAL	BEF	C	314667
0.636	216	YB1	YB1bc	41.6	BEAL	BEF	C	243648
1.056	217	YB1	YB1bc	41.6	BEAL	BEF	C	246357
0.800	218	YB1	YB1bc	41.6	BEAL	BEF	C	93177
1.176	231	YB1	YB1bc	41.6	BEAL	BEF	C	177003
1.252	232	YB1	YB1bc	41.6	BEAL	BEF	C	184482

0.341	243	YB1	YB1bc	41.6	BEAL	BEF	C	203130
0.719	244	YB1	YB1bc	41.6	BEAL	BEF	C	282780
0.503	246	YB1	YB1bc	41.6	BEAL	BEF	C	485505
0.983	206	YB2	YB2bc	45.8	BEAL	BEF	C	599877
1.690	208	YB2	YB2bc	45.8	BEAL	BEF	C	667125
	211	YB2	YB2bc	45.8	BEAL	BEF	C	643419
1.018	212	YB2	YB2bc	45.8	BEAL	BEF	C	623358
1.056	217	YB2	YB2bc	45.8	BEAL	BEF	C	611271
1.118	223	YB2	YB2bc	45.8	BEAL	BEF	C	581724
0.713	226	YB2	YB2bc	45.8	BEAL	BEF	C	508059
0.800	229	YB2	YB2bc	45.8	BEAL	BEF	C	407610
1.176	231	YB2	YB2bc	45.8	BEAL	BEF	C	374391
1.252	232	YB2	YB2bc	45.8	BEAL	BEF	C	382968
0.959	236	YB2	YB2bc	45.8	BEAL	BEF	C	395919
1.156	237	YB2	YB2bc	45.8	BEAL	BEF	C	453636
1.093	240	YB2	YB2bc	45.8	BEAL	BEF	C	439641
	242	YB2	YB2bc	45.8	BEAL	BEF	C	520983
0.719	244	YB2	YB2bc	45.8	BEAL	BEF	C	617634
0.581	207	YB3	YB3bc	44.1	BEAL	BEF	C	322407
0.946	214	YB3	YB3bc	44.1	BEAL	BEF	C	460809
1.056	217	YB3	YB3bc	44.1	BEAL	BEF	C	601785
1.176	231	YB3	YB3bc	44.1	BEAL	BEF	C	584838
	242	YB3	YB3bc	44.1	BEAL	BEF	C	524493
0.341	243	YB3	YB3bc	44.1	BEAL	BEF	C	361782
0.983	206	AB1	AB1bca	25.1	FAGR	BEF	CA	616968
0.832	209	AB1	AB1bca	25.1	FAGR	BEF	CA	622746
0.636	216	AB1	AB1bca	25.1	FAGR	BEF	CA	652401
1.056	217	AB1	AB1bca	25.1	FAGR	BEF	CA	830781
0.800	218	AB1	AB1bca	25.1	FAGR	BEF	CA	647919
0.964	219	AB1	AB1bca	25.1	FAGR	BEF	CA	699516
0.409	220	AB1	AB1bca	25.1	FAGR	BEF	CA	600714
	211	AB2	AB2bca	40.7	FAGR	BEF	CA	483201
0.636	216	AB2	AB2bca	40.7	FAGR	BEF	CA	398394
1.056	217	AB2	AB2bca	40.7	FAGR	BEF	CA	434115
	215	AB3	AB3bca	38.6	FAGR	BEF	CA	266391
0.636	216	AB3	AB3bca	38.6	FAGR	BEF	CA	233298
1.056	217	AB3	AB3bca	38.6	FAGR	BEF	CA	385722
0.800	218	AB3	AB3bca	38.6	FAGR	BEF	CA	176958
0.964	219	AB3	AB3bca	38.6	FAGR	BEF	CA	244026
0.409	220	AB3	AB3bca	38.6	FAGR	BEF	CA	136377
1.079	222	AB3	AB3bca	38.6	FAGR	BEF	CA	228915

1.018	212	SM1	SM1bac	55.4	ACSA	BEF	CA	132327
0.946	214	SM1	SM1bac	55.4	ACSA	BEF	CA	75267
	215	SM1	SM1bac	55.4	ACSA	BEF	CA	127404
0.636	216	SM1	SM1bac	55.4	ACSA	BEF	CA	89550
1.056	217	SM1	SM1bac	55.4	ACSA	BEF	CA	145656
0.964	219	SM1	SM1bac	55.4	ACSA	BEF	CA	66231
0.409	220	SM1	SM1bac	55.4	ACSA	BEF	CA	55449
	210	SM2	SM2bca	54	ACSA	BEF	CA	265005
0.946	214	SM2	SM2bca	54	ACSA	BEF	CA	304902
1.056	217	SM2	SM2bca	54	ACSA	BEF	CA	311823
0.800	218	SM2	SM2bca	54	ACSA	BEF	CA	420228
	262	SM2	SM2bca	54	ACSA	BEF	CA	537759
1.690	208	SM3	SM3bca	43.3	ACSA	BEF	CA	774045
	211	SM3	SM3bca	43.3	ACSA	BEF	CA	727191
1.018	212	SM3	SM3bca	43.3	ACSA	BEF	CA	693027
0.636	216	SM3	SM3bca	43.3	ACSA	BEF	CA	446859
1.056	217	SM3	SM3bca	43.3	ACSA	BEF	CA	539820
0.409	220	SM3	SM3bca	43.3	ACSA	BEF	CA	266643
0.353	257	YB1	YB1bca	37.8	BEAL	BEF	CA	504675
0.704	261	YB1	YB1bca	37.8	BEAL	BEF	CA	636129
	262	YB1	YB1bca	37.8	BEAL	BEF	CA	603810
1.690	208	YB2	YB2bca	42.8	BEAL	BEF	CA	772335
	215	YB2	YB2bca	42.8	BEAL	BEF	CA	668682
1.056	217	YB2	YB2bca	42.8	BEAL	BEF	CA	692784
1.690	208	YB3	YB3bca	34.5	BEAL	BEF	CA	661779
0.832	209	YB3	YB3bca	34.5	BEAL	BEF	CA	442449
	211	YB3	YB3bca	34.5	BEAL	BEF	CA	663012
0.946	214	YB3	YB3bca	34.5	BEAL	BEF	CA	514476
1.056	217	YB3	YB3bca	34.5	BEAL	BEF	CA	668763
0.704	261	YB3	YB3bca	34.5	BEAL	BEF	CA	419310
	262	YB3	YB3bca	34.5	BEAL	BEF	CA	389520
0.115	179	AB1	AB1hc	15.1	FAGR	HBEF	C	367929
0.401	180	AB1	AB1hc	15.1	FAGR	HBEF	C	249417
1.154	181	AB1	AB1hc	15.1	FAGR	HBEF	C	503613
	187	AB1	AB1hc	15.1	FAGR	HBEF	C	681381
	188	AB1	AB1hc	15.1	FAGR	HBEF	C	562347
0.946	214	AB1	AB1hc	15.1	FAGR	HBEF	C	672129
1.056	217	AB1	AB1hc	15.1	FAGR	HBEF	C	803682
0.125	221	AB1	AB1hc	15.1	FAGR	HBEF	C	155115
1.079	222	AB1	AB1hc	15.1	FAGR	HBEF	C	749592
1.118	223	AB1	AB1hc	15.1	FAGR	HBEF	C	831807

1.148	224	AB1	AB1hc	15.1	FAGR	HBEF	C	707760
0.713	226	AB1	AB1hc	15.1	FAGR	HBEF	C	367902
0.669	228	AB1	AB1hc	15.1	FAGR	HBEF	C	673695
0.800	229	AB1	AB1hc	15.1	FAGR	HBEF	C	705573
1.176	231	AB1	AB1hc	15.1	FAGR	HBEF	C	683388
1.252	232	AB1	AB1hc	15.1	FAGR	HBEF	C	769608
1.515	233	AB1	AB1hc	15.1	FAGR	HBEF	C	694035
	234	AB1	AB1hc	15.1	FAGR	HBEF	C	736713
0.996	235	AB1	AB1hc	15.1	FAGR	HBEF	C	596043
0.959	236	AB1	AB1hc	15.1	FAGR	HBEF	C	515106
1.156	237	AB1	AB1hc	15.1	FAGR	HBEF	C	575181
0.295	238	AB1	AB1hc	15.1	FAGR	HBEF	C	453627
0.824	239	AB1	AB1hc	15.1	FAGR	HBEF	C	562140
	242	AB1	AB1hc	15.1	FAGR	HBEF	C	524988
1.056	217	AB2	AB2hc	16.8	FAGR	HBEF	C	723015
0.713	226	AB2	AB2hc	16.8	FAGR	HBEF	C	406899
1.252	232	AB2	AB2hc	16.8	FAGR	HBEF	C	538047
	234	AB2	AB2hc	16.8	FAGR	HBEF	C	543816
1.156	237	AB2	AB2hc	16.8	FAGR	HBEF	C	437778
0.295	238	AB2	AB2hc	16.8	FAGR	HBEF	C	197307
0.824	239	AB2	AB2hc	16.8	FAGR	HBEF	C	359082
	215	AB3	AB3hc	32.4	FAGR	HBEF	C	1077408
0.636	216	AB3	AB3hc	32.4	FAGR	HBEF	C	530505
1.056	217	AB3	AB3hc	32.4	FAGR	HBEF	C	1187280
0.800	218	AB3	AB3hc	32.4	FAGR	HBEF	C	951525
0.964	219	AB3	AB3hc	32.4	FAGR	HBEF	C	945765
0.409	220	AB3	AB3hc	32.4	FAGR	HBEF	C	179883
1.118	223	AB3	AB3hc	32.4	FAGR	HBEF	C	892377
1.148	224	AB3	AB3hc	32.4	FAGR	HBEF	C	838944
0.864	227	AB3	AB3hc	32.4	FAGR	HBEF	C	646947
0.800	229	AB3	AB3hc	32.4	FAGR	HBEF	C	933273
1.176	231	AB3	AB3hc	32.4	FAGR	HBEF	C	478098
1.252	232	AB3	AB3hc	32.4	FAGR	HBEF	C	714528
1.515	233	AB3	AB3hc	32.4	FAGR	HBEF	C	551943
	234	AB3	AB3hc	32.4	FAGR	HBEF	C	1011771
0.996	235	AB3	AB3hc	32.4	FAGR	HBEF	C	788355
1.156	237	AB3	AB3hc	32.4	FAGR	HBEF	C	657360
0.824	239	AB3	AB3hc	32.4	FAGR	HBEF	C	500796
	242	AB3	AB3hc	32.4	FAGR	HBEF	C	481536
0.719	244	AB3	AB3hc	32.4	FAGR	HBEF	C	574074
	215	SM1	SM1hc	34.4	ACSA	HBEF	C	502128

0.636	216	SM1	SM1hc	34.4	ACSA	HBEF	C	307548
1.056	217	SM1	SM1hc	34.4	ACSA	HBEF	C	592038
0.800	218	SM1	SM1hc	34.4	ACSA	HBEF	C	473589
0.964	219	SM1	SM1hc	34.4	ACSA	HBEF	C	535905
0.409	220	SM1	SM1hc	34.4	ACSA	HBEF	C	224496
1.118	223	SM1	SM1hc	34.4	ACSA	HBEF	C	604575
1.148	224	SM1	SM1hc	34.4	ACSA	HBEF	C	472959
0.800	229	SM1	SM1hc	34.4	ACSA	HBEF	C	613953
0.664	230	SM1	SM1hc	34.4	ACSA	HBEF	C	527751
1.176	231	SM1	SM1hc	34.4	ACSA	HBEF	C	661977
1.252	232	SM1	SM1hc	34.4	ACSA	HBEF	C	668151
1.515	233	SM1	SM1hc	34.4	ACSA	HBEF	C	681534
	234	SM1	SM1hc	34.4	ACSA	HBEF	C	722214
0.996	235	SM1	SM1hc	34.4	ACSA	HBEF	C	603018
0.959	236	SM1	SM1hc	34.4	ACSA	HBEF	C	701019
1.156	237	SM1	SM1hc	34.4	ACSA	HBEF	C	562005
0.824	239	SM1	SM1hc	34.4	ACSA	HBEF	C	389970
0.319	177	SM4	SM4hc	18.3	ACSA	HBEF	C	190539
1.118	223	SM4	SM4hc	18.3	ACSA	HBEF	C	499545
1.148	224	SM4	SM4hc	18.3	ACSA	HBEF	C	427158
0.800	229	SM4	SM4hc	18.3	ACSA	HBEF	C	492201
1.176	231	SM4	SM4hc	18.3	ACSA	HBEF	C	440424
1.252	232	SM4	SM4hc	18.3	ACSA	HBEF	C	518724
1.515	233	SM4	SM4hc	18.3	ACSA	HBEF	C	491256
	234	SM4	SM4hc	18.3	ACSA	HBEF	C	562554
0.996	235	SM4	SM4hc	18.3	ACSA	HBEF	C	470403
1.156	237	SM4	SM4hc	18.3	ACSA	HBEF	C	644418
0.295	238	SM4	SM4hc	18.3	ACSA	HBEF	C	421461
0.824	239	SM4	SM4hc	18.3	ACSA	HBEF	C	472095
0.325	241	SM4	SM4hc	18.3	ACSA	HBEF	C	97506
	242	SM4	SM4hc	18.3	ACSA	HBEF	C	565083
0.719	244	SM4	SM4hc	18.3	ACSA	HBEF	C	697176
0.436	178	SM3	SM3hc	36.5	ACSA	HBEF	C	148878
0.946	214	SM3	SM3hc	36.5	ACSA	HBEF	C	622431
0.636	216	SM3	SM3hc	36.5	ACSA	HBEF	C	514584
1.056	217	SM3	SM3hc	36.5	ACSA	HBEF	C	741798
0.800	218	SM3	SM3hc	36.5	ACSA	HBEF	C	597069
0.964	219	SM3	SM3hc	36.5	ACSA	HBEF	C	852345
0.409	220	SM3	SM3hc	36.5	ACSA	HBEF	C	355878
1.118	223	SM3	SM3hc	36.5	ACSA	HBEF	C	892035
1.148	224	SM3	SM3hc	36.5	ACSA	HBEF	C	712881

1.252	232	SM3	SM3hc	36.5	ACSA	HBEF	C	782307
1.515	233	SM3	SM3hc	36.5	ACSA	HBEF	C	781227
	234	SM3	SM3hc	36.5	ACSA	HBEF	C	910107
0.996	235	SM3	SM3hc	36.5	ACSA	HBEF	C	947970
0.959	236	SM3	SM3hc	36.5	ACSA	HBEF	C	808281
	242	SM3	SM3hc	36.5	ACSA	HBEF	C	708507
0.719	244	SM3	SM3hc	36.5	ACSA	HBEF	C	556614
	187	YB1	YB1hc	46.1	BEAL	HBEF	C	484218
	188	YB1	YB1hc	46.1	BEAL	HBEF	C	363843
0.946	214	YB1	YB1hc	46.1	BEAL	HBEF	C	589347
0.636	216	YB1	YB1hc	46.1	BEAL	HBEF	C	276381
0.800	218	YB1	YB1hc	46.1	BEAL	HBEF	C	297927
0.964	219	YB1	YB1hc	46.1	BEAL	HBEF	C	484290
0.409	220	YB1	YB1hc	46.1	BEAL	HBEF	C	141444
1.148	224	YB1	YB1hc	46.1	BEAL	HBEF	C	352854
0.800	229	YB1	YB1hc	46.1	BEAL	HBEF	C	288837
1.176	231	YB1	YB1hc	46.1	BEAL	HBEF	C	225963
1.252	232	YB1	YB1hc	46.1	BEAL	HBEF	C	303939
	234	YB1	YB1hc	46.1	BEAL	HBEF	C	376749
0.996	235	YB1	YB1hc	46.1	BEAL	HBEF	C	327222
1.156	237	YB1	YB1hc	46.1	BEAL	HBEF	C	248976
1.655	176	YB4	YB4hc	28.7	BEAL	HBEF	C	411408
1.154	181	YB4	YB4hc	28.7	BEAL	HBEF	C	218772
	187	YB4	YB4hc	28.7	BEAL	HBEF	C	301023
	188	YB4	YB4hc	28.7	BEAL	HBEF	C	195480
0.946	214	YB4	YB4hc	28.7	BEAL	HBEF	C	431784
1.056	217	YB4	YB4hc	28.7	BEAL	HBEF	C	561654
0.800	218	YB4	YB4hc	28.7	BEAL	HBEF	C	525204
0.964	219	YB4	YB4hc	28.7	BEAL	HBEF	C	598113
1.148	224	YB4	YB4hc	28.7	BEAL	HBEF	C	569547
1.176	231	YB4	YB4hc	28.7	BEAL	HBEF	C	611559
1.252	232	YB4	YB4hc	28.7	BEAL	HBEF	C	620100
	234	YB4	YB4hc	28.7	BEAL	HBEF	C	670185
0.996	235	YB4	YB4hc	28.7	BEAL	HBEF	C	475866
1.156	237	YB4	YB4hc	28.7	BEAL	HBEF	C	507051
0.824	239	YB4	YB4hc	28.7	BEAL	HBEF	C	407286
	242	YB4	YB4hc	28.7	BEAL	HBEF	C	355617
	187	AB1	AB1hca	41.2	FAGR	HBEF	CA	535743
	188	AB1	AB1hca	41.2	FAGR	HBEF	CA	592794
1.470	193	AB1	AB1hca	41.2	FAGR	HBEF	CA	446733
0.631	195	AB1	AB1hca	41.2	FAGR	HBEF	CA	599238

0.581	207	AB1	AB1hca	41.2	FAGR	HBEF	CA	415953
1.690	208	AB1	AB1hca	41.2	FAGR	HBEF	CA	463509
1.018	212	AB1	AB1hca	41.2	FAGR	HBEF	CA	394317
0.636	216	AB1	AB1hca	41.2	FAGR	HBEF	CA	271692
1.056	217	AB1	AB1hca	41.2	FAGR	HBEF	CA	465516
0.800	218	AB1	AB1hca	41.2	FAGR	HBEF	CA	314460
	210	AB2	AB2hca	25.2	FAGR	HBEF	CA	376083
1.056	217	AB2	AB2hca	25.2	FAGR	HBEF	CA	796464
0.581	207	AB3	AB3hca	43.4	FAGR	HBEF	CA	944433
	210	AB3	AB3hca	43.4	FAGR	HBEF	CA	858123
0.946	214	AB3	AB3hca	43.4	FAGR	HBEF	CA	944226
0.636	216	AB3	AB3hca	43.4	FAGR	HBEF	CA	1367748
	187	SM1	SM1hca	14.5	ACSA	HBEF	CA	249192
1.690	208	SM1	SM1hca	14.5	ACSA	HBEF	CA	304767
0.832	209	SM1	SM1hca	14.5	ACSA	HBEF	CA	193284
1.056	217	SM1	SM1hca	14.5	ACSA	HBEF	CA	405837
0.319	177	SM2	SM2hca	35	ACSA	HBEF	CA	77346
0.436	178	SM2	SM2hca	35	ACSA	HBEF	CA	95004
0.508	189	SM2	SM2hca	35	ACSA	HBEF	CA	116316
0.523	190	SM2	SM2hca	35	ACSA	HBEF	CA	145062
	210	SM2	SM2hca	35	ACSA	HBEF	CA	207882
	211	SM2	SM2hca	35	ACSA	HBEF	CA	614772
0.946	214	SM2	SM2hca	35	ACSA	HBEF	CA	355968
1.056	217	SM2	SM2hca	35	ACSA	HBEF	CA	538884
	188	SM3	SM3hca	16.8	ACSA	HBEF	CA	164538
0.738	192	SM3	SM3hca	16.8	ACSA	HBEF	CA	27360
1.056	217	SM3	SM3hca	16.8	ACSA	HBEF	CA	184536
0.436	178	YB1	YB1hca	28.4	BEAL	HBEF	CA	240939
0.115	179	YB1	YB1hca	28.4	BEAL	HBEF	CA	371205
0.401	180	YB1	YB1hca	28.4	BEAL	HBEF	CA	294012
0.832	209	YB1	YB1hca	28.4	BEAL	HBEF	CA	699264
	210	YB1	YB1hca	28.4	BEAL	HBEF	CA	269586
1.018	212	YB1	YB1hca	28.4	BEAL	HBEF	CA	613602
	215	YB1	YB1hca	28.4	BEAL	HBEF	CA	476064
0.636	216	YB1	YB1hca	28.4	BEAL	HBEF	CA	460998
1.056	217	YB1	YB1hca	28.4	BEAL	HBEF	CA	434070
0.800	218	YB1	YB1hca	28.4	BEAL	HBEF	CA	687051
0.996	235	AB1	AB1jc	13.7	FAGR	JB	C	284445
0.115	179	SM1	SM1jc	19.1	ACSA	JB	C	202599
0.996	235	SM1	SM1jc	19.1	ACSA	JB	C	187119
1.515	233	SM2	SM2jc	24.6	ACSA	JB	C	223227

1.156	237	SM2	SM2jc	24.6	ACSA	JB	C	188946
	172	SM3	SM3jc	35.6	ACSA	JB	C	655632
0.996	235	SM3	SM3jc	35.6	ACSA	JB	C	247950
0.959	236	SM3	SM3jc	35.6	ACSA	JB	C	336888
1.156	237	SM3	SM3jc	35.6	ACSA	JB	C	348885
0.917	157	SM6	SM6jc	28.5	ACSA	JB	C	638361
0.436	178	SM6	SM6jc	28.5	ACSA	JB	C	136773
	188	SM6	SM6jc	28.5	ACSA	JB	C	341514
	203	SM6	SM6jc	28.5	ACSA	JB	C	311841
0.996	235	SM6	SM6jc	28.5	ACSA	JB	C	399762
0.959	236	SM6	SM6jc	28.5	ACSA	JB	C	567108
1.156	237	SM6	SM6jc	28.5	ACSA	JB	C	418248
0.295	238	YB1	YB1jc	36.6	BEAL	JB	C	111204
1.420	200	YB2	YB2jc	28.2	BEAL	JB	C	397368
1.420	200	YB3	YB3jc	21.1	BEAL	JB	C	326241
1.515	233	AB1	AB1jca	21.3	FAGR	JB	CA	448614
	172	AB2	AB2jca	29.4	FAGR	JB	CA	812880
	188	AB2	AB2jca	29.4	FAGR	JB	CA	519939
1.420	200	AB2	AB2jca	29.4	FAGR	JB	CA	611595
1.156	237	AB2	AB2jca	29.4	FAGR	JB	CA	526590
0.295	238	AB2	AB2jca	29.4	FAGR	JB	CA	165159
0.508	189	SM2	SM2jca	34.1	ACSA	JB	CA	110043
	234	SM2	SM2jca	34.1	ACSA	JB	CA	235683
1.787	186	SM8	SM8jca	37.8	ACSA	JB	CA	714123
	188	SM8	SM8jca	37.8	ACSA	JB	CA	370341
0.508	189	SM8	SM8jca	37.8	ACSA	JB	CA	142452
1.156	237	SM8	SM8jca	37.8	ACSA	JB	CA	370467
1.787	186	YB3	YB3jca	36.8	BEAL	JB	CA	806256
0.523	190	YB4	YB4jca	49.1	BEAL	JB	CA	305595
	234	YB4	YB4jca	49.1	BEAL	JB	CA	818082
1.156	237	YB4	YB4jca	49.1	BEAL	JB	CA	715203

Appendix B: Table of median percent embolism for each coarse root measurement

Sample Season	Site	Treatment	Species	percent embolism median
FL12	BEF	CA	FAGR	96.18
FL12	BEF	CA	FAGR	98.97
FL12	BEF	CA	FAGR	100.00
FL12	BEF	CA	FAGR	100.00
FL12	BEF	CA	ACSA	97.38
FL12	BEF	CA	ACSA	98.15
FL12	BEF	CA	ACSA	99.93
FL12	BEF	CA	BEAL	98.10
FL12	BEF	CA	BEAL	100.00
FL12	BEF	CA	BEAL	100.00
FL12	BEF	C	FAGR	35.61
FL12	BEF	C	FAGR	71.94
FL12	BEF	C	FAGR	100.00
FL12	BEF	C	ACSA	-8.18
FL12	BEF	C	ACSA	57.44
FL12	BEF	C	ACSA	81.59
FL12	BEF	C	ACSA	100.00
FL12	BEF	C	BEAL	-86.86
FL12	BEF	C	BEAL	-10.91
FL12	BEF	C	BEAL	22.78
FL12	JB	CA	FAGR	69.55
FL12	JB	CA	FAGR	73.95
FL12	JB	CA	ACSA	-262.04
FL12	JB	CA	ACSA	43.87
FL12	JB	CA	ACSA	52.93
FL12	JB	CA	ACSA	85.31
FL12	JB	CA	ACSA	100.00
FL12	JB	CA	BEAL	88.54
FL12	JB	C	FAGR	97.90
FL12	JB	C	ACSA	10.00
FL12	JB	C	ACSA	23.63
FL12	JB	C	ACSA	42.91
FL12	JB	C	ACSA	53.63
FL12	JB	C	ACSA	61.35
FL12	JB	C	ACSA	77.24
FL12	JB	C	BEAL	-61.30
FL12	JB	C	BEAL	-29.64

FL12	JB	C	BEAL	-4.48
FL12	HBEF	CA	FAGR	52.03
FL12	HBEF	CA	FAGR	57.53
FL12	HBEF	CA	FAGR	70.76
FL12	HBEF	CA	FAGR	83.60
FL12	HBEF	CA	ACSA	62.92
FL12	HBEF	CA	ACSA	74.82
FL12	HBEF	CA	ACSA	98.65
FL12	HBEF	CA	ACSA	100.00
FL12	HBEF	CA	BEAL	-197.45
FL12	HBEF	CA	BEAL	-142.10
FL12	HBEF	CA	BEAL	60.92
FL12	HBEF	CA	BEAL	84.59
FL12	HBEF	CA	BEAL	89.88
FL12	HBEF	CA	BEAL	100.00
FL12	HBEF	CA	BEAL	100.00
FL12	HBEF	C	FAGR	49.94
FL12	HBEF	C	FAGR	92.93
FL12	HBEF	C	FAGR	100.00
FL12	HBEF	C	ACSA	96.38
FL12	HBEF	C	BEAL	-121.46
FL12	HBEF	C	BEAL	-80.66
FL12	HBEF	C	BEAL	-20.63
FL12	HBEF	C	BEAL	39.05
FL12	HBEF	C	BEAL	61.42
FL12	HBEF	C	BEAL	62.06
FL12	HBEF	C	BEAL	100.00
FL12	HBEF	C	BEAL	100.00
FL12	HBEF	C	BEAL	100.00
SM12	BEF	CA	ACSA	87.08
SM12	BEF	CA	ACSA	89.03
SM12	BEF	CA	BEAL	48.46
SM12	BEF	CA	BEAL	97.73
SM12	BEF	CA	FAGR	87.12
SM12	BEF	CA	FAGR	90.56
SM12	BEF	CA	FAGR	91.12
SM12	BEF	CA	FAGR	98.77
SM12	BEF	C	ACSA	-0.35
SM12	BEF	C	ACSA	40.05
SM12	BEF	C	ACSA	50.37
SM12	BEF	C	ACSA	52.75

SM12	BEF	C	ACSA	85.44
SM12	BEF	C	ACSA	93.56
SM12	BEF	C	BEAL	-128.36
SM12	BEF	C	BEAL	-87.11
SM12	BEF	C	BEAL	90.91
SM12	BEF	C	FAGR	45.51
SM12	BEF	C	FAGR	49.86
SM12	BEF	C	FAGR	55.48
SM12	BEF	C	FAGR	95.15
SM12	JB	CA	ACSA	-100.47
SM12	JB	CA	ACSA	91.13
SM12	JB	CA	BEAL	-205.36
SM12	JB	CA	BEAL	47.87
SM12	JB	CA	BEAL	76.82
SM12	JB	CA	FAGR	32.04
SM12	JB	CA	FAGR	100.00
SM12	JB	C	ACSA	82.56
SM12	JB	C	ACSA	93.23
SM12	JB	C	BEAL	-16.63
SM12	JB	C	BEAL	87.14
SM12	JB	C	BEAL	100.00
SM12	JB	C	FAGR	33.68
SM12	JB	C	FAGR	95.26
SM12	JB	C	FAGR	96.29
SM12	HBEF	CA	ACSA	80.73
SM12	HBEF	CA	ACSA	94.64
SM12	HBEF	CA	ACSA	95.11
SM12	HBEF	CA	BEAL	-37.27
SM12	HBEF	CA	BEAL	-7.77
SM12	HBEF	CA	FAGR	35.28
SM12	HBEF	CA	FAGR	65.91
SM12	HBEF	CA	FAGR	94.00
SM12	HBEF	C	ACSA	11.28
SM12	HBEF	C	ACSA	88.22
SM12	HBEF	C	ACSA	94.40
SM12	HBEF	C	BEAL	-78.76
SM12	HBEF	C	BEAL	-9.32
SM12	HBEF	C	BEAL	86.39
SM12	HBEF	C	FAGR	26.47
SM12	HBEF	C	FAGR	49.09
SM12	HBEF	C	FAGR	96.42

SM13	BEF	CA	FAGR	55.68
SM13	BEF	CA	FAGR	91.37
SM13	BEF	CA	FAGR	100.00
SM13	BEF	CA	ACSA	57.08
SM13	BEF	CA	ACSA	71.05
SM13	BEF	CA	ACSA	91.76
SM13	BEF	CA	ACSA	100.00
SM13	BEF	CA	BEAL	25.82
SM13	BEF	CA	BEAL	52.46
SM13	BEF	CA	BEAL	78.87
SM13	BEF	CA	BEAL	86.69
SM13	BEF	CA	BEAL	100.00
SM13	BEF	CA	BEAL	100.00
SM13	BEF	C	FAGR	51.42
SM13	BEF	C	FAGR	52.46
SM13	BEF	C	FAGR	55.60
SM13	BEF	C	FAGR	69.83
SM13	BEF	C	FAGR	100.00
SM13	BEF	C	ACSA	-252.38
SM13	BEF	C	ACSA	-110.06
SM13	BEF	C	ACSA	-91.73
SM13	BEF	C	ACSA	55.47
SM13	BEF	C	BEAL	-4.92
SM13	BEF	C	BEAL	40.52
SM13	BEF	C	BEAL	60.20
SM13	BEF	C	BEAL	65.74
SM13	BEF	C	BEAL	93.39
SM13	JB	CA	FAGR	89.75
SM13	JB	CA	FAGR	94.29
SM13	JB	CA	ACSA	-166.79
SM13	JB	CA	ACSA	-119.98
SM13	JB	CA	ACSA	-18.82
SM13	JB	CA	ACSA	88.58
SM13	JB	CA	ACSA	100.00
SM13	JB	CA	BEAL	63.08
SM13	JB	CA	BEAL	73.21
SM13	JB	CA	BEAL	78.28
SM13	JB	CA	BEAL	79.17
SM13	JB	CA	BEAL	93.09
SM13	JB	C	FAGR	-1806.42
SM13	JB	C	ACSA	-155.25

SM13	JB	C	ACSA	90.94
SM13	JB	C	BEAL	17.79
SM13	JB	C	BEAL	20.54
SM13	JB	C	BEAL	57.48
SM13	JB	C	BEAL	86.09
SM13	HBEF	CA	FAGR	-5200.78
SM13	HBEF	CA	FAGR	-819.02
SM13	HBEF	CA	FAGR	-546.82
SM13	HBEF	CA	FAGR	-493.94
SM13	HBEF	CA	FAGR	48.39
SM13	HBEF	CA	ACSA	-240.48
SM13	HBEF	CA	ACSA	19.12
SM13	HBEF	CA	ACSA	57.47
SM13	HBEF	CA	BEAL	15.92
SM13	HBEF	CA	BEAL	26.22
SM13	HBEF	CA	BEAL	40.07
SM13	HBEF	CA	BEAL	71.06
SM13	HBEF	CA	BEAL	75.91
SM13	HBEF	C	FAGR	72.90
SM13	HBEF	C	FAGR	90.88
SM13	HBEF	C	FAGR	96.23
SM13	HBEF	C	FAGR	96.58
SM13	HBEF	C	FAGR	99.18
SM13	HBEF	C	ACSA	86.98
SM13	HBEF	C	ACSA	95.71
SM13	HBEF	C	ACSA	97.51
SM13	HBEF	C	ACSA	98.15
SM13	HBEF	C	ACSA	100.00
SM13	HBEF	C	BEAL	53.63
SM13	HBEF	C	BEAL	95.98
SM13	HBEF	C	BEAL	98.95
SM13	HBEF	C	BEAL	99.02
SP13	HBEF	CA	ACSA	-94.17
SP13	HBEF	CA	ACSA	-26.52
SP13	HBEF	CA	ACSA	24.39
SP13	HBEF	CA	BEAL	-56.05
SP13	HBEF	CA	BEAL	-45.74
SP13	HBEF	CA	BEAL	48.48
SP13	HBEF	CA	FAGR	-32.73
SP13	HBEF	CA	FAGR	-8.28
SP13	HBEF	CA	FAGR	52.05

SP13	HBEF	C	ACSA	44.76
SP13	HBEF	C	ACSA	57.74
SP13	HBEF	C	ACSA	95.22
SP13	HBEF	C	BEAL	55.03
SP13	HBEF	C	BEAL	71.96
SP13	HBEF	C	BEAL	73.28
SP13	HBEF	C	FAGR	36.98
SP13	HBEF	C	FAGR	41.71
SP13	HBEF	C	FAGR	50.39

Appendix C: TTC results for each fine root measured

Date	Treatment	Site	ID	Spp	abs/dry weight (g)
Spring 2014	C	HBEF	AB1	FAGR	11.36
Spring 2014	C	HBEF	AB2	FAGR	15.15
Spring 2014	C	HBEF	AB3	FAGR	19.73
Spring 2014	C	HBEF	SM1	ACSA	18.60
Spring 2014	C	HBEF	SM2	ACSA	10.22
Spring 2014	C	HBEF	SM3	ACSA	17.35
Spring 2014	C	HBEF	YB1	BEAL	19.89
Spring 2014	C	HBEF	YB2	BEAL	22.06
Spring 2014	C	HBEF	YB3	BEAL	17.09
Spring 2014	CA	HBEF	AB1	FAGR	17.65
Spring 2014	CA	HBEF	AB2	FAGR	13.76
Spring 2014	CA	HBEF	AB3	FAGR	55.56
Spring 2014	CA	HBEF	SM1	ACSA	20.00
Spring 2014	CA	HBEF	SM2	ACSA	15.00
Spring 2014	CA	HBEF	SM3	ACSA	38.00
Spring 2014	CA	HBEF	YB1	BEAL	35.71
Spring 2014	CA	HBEF	YB2	BEAL	16.00