

Increased sap flow in a northern hardwood forest treated with wollastonite (CaSiO_3)

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Abstract

Calcium plays a vital role in plant structure and physiology, making it an important nutrient in northern hardwood forests. Beginning in the 1950s, acidic deposition resulting from industrialization has depleted naturally-occurring soil calcium. To better understand the impact of this loss on forest ecosystem processes, researchers amended Watershed 1 at Hubbard Brook Experimental Forest with wollastonite (CaSiO_3) to restore soil Ca reserves to preindustrial levels. Watershed runoff decreased significantly following the addition—evapotranspiration was estimated to have increased over three years by 25%, 18%, and 19% , respectively (Green et. al, 2013). This study sought to clarify the findings at Watershed 1 by measuring sap flow, which can be used to estimate transpiration, in another nearby northern hardwood forest treated with wollastonite. Sap flow was measured in sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*) trees in a plot with added wollastonite and in a control plot. A two-way ANCOVA performed on the data indicates that treatment and species were main effects: trees in the wollastonite plot had significantly higher sap flow than those in the control, and sugar maple had significantly higher sap flow than yellow birch ($p < .0001$). An interaction test showed that the sap flow response to wollastonite was stronger in sugar maple than in yellow birch ($p = 0.447$). These results confirm the Watershed 1 transpiration response, suggesting soil calcium supply can substantially influence forest water cycles. This knowledge may be important in developing forest management strategies amidst continued acid deposition and other threats to calcium reserves in years ahead.

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Introduction

Calcium in Forest Ecosystems

Calcium (Ca) is unique among elements that make up living systems due to its distinctive chemical binding properties and its function as a messenger in a variety of physiological processes (Hepler and Wayne, 1985). Trees and other plants rely on calcium for structural and physiological functions including cell wall and membrane synthesis; formation and growth of woody tissue; cell division; protein synthesis; intra- and intercellular signaling; carbohydrate metabolism; and stomatal regulation (Kirkby and Pilbeam, 1984; McAinsh et al., 1996; Roberts and Harmon, 1993; Taiz and Zeiger, 2010). Calcium is the least mobile among primary plant macronutrients, making Ca supply, uptake, and distribution critical to plant growth, development, and stress response, given its diverse role in healthy plant function (McLaughlin and Wimmer, 1999).

Calcium occurs naturally in soil, introduced slowly through chemical weathering of bedrock and deposition from the atmosphere. Cation exchange allows calcium to become available in the soil solution in its cationic form, Ca^{2+} , where it can be taken up by tree roots in the rhizosphere. It may be incorporated into root cells or transported up through the xylem and allocated to other parts of the plant where needed, and reenters the nutrient cycle through leaf senescence and tree death (Miller and Donahue, 1990). Calcium is linked to biogeochemical cycles through its impact on soil aggregate formation, stabilization of soil humus, rates of litter decomposition, and acid-base status in soils and aquatic ecosystems (Driscoll et al., 1989; Marion et al., 1993; Oyonarte et al., 1994; Ulrich and Matzner, 1986). Broadly, the physiological and biogeochemical importance of calcium places limitations on forest growth and ecosystem function (McLaughlin and Wimmer, 1999).

Environmental influences that disrupt natural calcium gradients are of ecological concern. The northeastern United States has experienced increased acid deposition since the early 1950s, largely as a result of sulfur dioxide and nitrogen oxides emitted from fossil fuel combustion in Midwestern industry, energy production, and transportation. Deposition of strong acids from the atmosphere replaces calcium ions with hydrogen and aluminum ions; calcium ions leach into the groundwater and flow into nearby streams, severely impacting the calcium cycle (Cogbill and Likens, 1974). In forest ecosystems in the northeast, calcium leaching far exceeds replenishment, threatening long-term forest stability (Likens et al., 1998; Tomlinson, 2003). Controls on emissions in the United States were first implemented after passage of the 1963 Clean Air Act (CAA) and the Clean Air Act Extension of 1970, but reduced levels still remain high compared to background conditions (Driscoll et al., 2001).

HBEF Wollastonite Addition Experiment

In the fall of 1999, a whole-watershed wollastonite (CaSiO_3) addition was implemented at Watershed 1 (W1) in Hubbard Brook Experimental Forest (HBEF) to investigate the role of calcium supply in the structure and function of base-poor forest and aquatic ecosystems. Researchers replenished calcium at the watershed-level with wollastonite (CaSiO_3) at a rate of 1.2 metric tons Ca per hectare, with the objective of increasing the soil base saturation from approximately 10 to 19% (Driscoll, 2000). W1 had previously lost substantial calcium from the soil reserves due to acid deposition, and a base saturation of 19% was believed to be the level at HBEF 65 years ago, before significant deposition began.

The effort was a major endeavor and the first of its kind. Wollastonite was pelletized into 1.5 to 4 mm diameter pellets with a lignin sulfonate binder (~2% wet weight) and dropped onto

W1 by helicopter (Peters et al., 2004). A great variety of measurements have assessed the response of major ecosystem processes, and these continue today (Battles, 2015).

Unexpectedly, the addition significantly impacted the water balance of Watershed 1. Compared to mean annual streamflow before the addition and compared to a reference watershed, discharge decreased significantly five months after the addition and remained low for three years. Watershed scale evapotranspiration (ET) was estimated by subtracting discharge from precipitation, assuming negligible groundwater exchange and interannual water storage. It was estimated that ET increased by 25%, 18%, and 19% each year following the addition before returning to base levels (Green et al., 2013). The effect on streamflow was so severe that for many years researchers believed that there was an error in the stream discharge measurements (J. Blum, personal communication). However, discharge measurement accuracy was confirmed and it became apparent that the calcium addition stimulated transpiration through fine root growth, uptake of calcium, and primary production. It was suggested that the transience of the response could be due to more efficient water use from improved stomatal functioning, or a secondary limitation effect (Green et al., 2013). The scale of this response was of similar magnitude to the effects of clear-cutting experiments conducted at Hubbard Brook, indicating previously unrecognized implications of soil Ca concentrations for the water cycle (Martin et al., 2000). Still, the transpiration response remains puzzling, and the mechanisms behind it are unclear.

There are two caveats to consider with this experiment. First, the wollastonite treatment added silicon (Si) as well as calcium to Watershed 1 (Driscoll, 2000). Silicon is ubiquitous in nature and is not considered an essential nutrient, but an effect of Si or a combined effect with Ca on tree growth and evapotranspiration cannot be ruled out (Currie and Perry, 2007). Second, pH in the surface soil horizons increased following the wollastonite addition (Nezat et al., 2010).

Soil pH impacts the solubility of nutrients and minerals, making them more or less available for uptake by plants, and can also influence plant growth by affecting the activity of beneficial microorganisms (Miransari, 2013). An effect of overall change in soil pH also cannot be ruled out.

In 2011, additional wollastonite addition plots were established by the Multiple Element Limitation in Northern Hardwood Ecosystems project (MELNHE) with the aim of clarifying underlying mechanisms of the HBEF whole-watershed transpiration response. Wollastonite was applied at Bartlett Experimental Forest, Hubbard Brook Experimental Forest and Jeffers Brook Forest in sites of varying stand age at the same rate used in HBEF W1 in 1999. Studies of the effects on ecosystem processes in these plots are ongoing.

Sap Flow

Xylem sap flow measurements can be used to estimate transpiration, and diurnal variation in sap flow can reveal mechanisms for efficient water use (Granier, 1987). Thus, studying sap flow measurements in the MELNHE plots amended with wollastonite could give insight into the role of calcium in forest water use and clarify the W1 transpiration response.

While phloem sap contains photosynthesized sucrose and other stored carbohydrates, xylem sap is water, minerals, and nutrients transported up the sapwood, from roots to aerial parts of the tree in response to tension, pressure and osmotic gradients. The xylem vasculature pathway has hydraulic continuity, so variation in tension or pressure is immediately transferred (Joly and Dixon, 1894). Transpiration induces sap flow: evaporative water loss when stomata are open during photosynthesis creates negative pressure in the xylem, which pulls water from the soil through the stem and branches up to the leaves. This is the cohesion-tension theory; the

negative pressure creates a tension pull, and the cohesion property of water enables it to move upward (Joly and Dixon, 1894). In the absence of photosynthesis, sap may rise up through the xylem due to root pressure, when a gradient exists between the soil water and roots due to high mineral and organic compound concentrations. The movement of sap is thus mediated by a number of factors, including soil water availability, humidity, and stomatal control (Meinzer et al., 2004).

On an individual tree scale, most variation in sap flow is caused by changes in weather. On sunny days, sap flow measurements for an individual tree will show a characteristic diurnal pattern: sap flow peaks in the early afternoon, when light intensity and photosynthetic activity is highest, then drops to a minimum in the early morning. Generally, on warmer, sunnier days, sap flow is higher, and rain events disrupt sap flow. Within species, sap flow variation is highly dependent on tree crown class (dominant, codominant, intermediate, suppressed) (Granier, 1987; Horna et al., 2011).

The objective of this study was to measure sap flow in individual trees at wollastonite-treated and control plots at a site within a northern hardwood forest of New Hampshire's White Mountains. We hypothesized that adding calcium to the soil in the form of wollastonite would increase sap flow based on the results of the HBEF Watershed 1 experiment..

Methods

Site Description

Sap flow was studied in Jeffers Brook Forest (JB) in the White Mountain National Forest of northeastern New Hampshire (Table 1). The site is a deciduous hardwood forest dominated by

sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*). The White Mountains region experiences a relatively short growing season, with mild, cool summers and long, cold winters. Average January temperature is around -9°C with long periods of low temperatures from -12°C to -18°C , while average July temperature is around 19°C. Mean annual precipitation is 1200- 1400 mm which is well distributed throughout the year, with about one third falling as snow (Schwarz et al., 2003; Smith and Martin, 2001). Soils in the region are well developed Spodosols derived from glacial till of metamorphic and igneous origin; they are moderately-drained, acidic ($3.5 \leq \text{pH} \leq 5.5$) and relatively infertile, with sandy loam textures (Hubbard Brook Ecosystem Study; Schwarz et al., 2003). The forest consists of a mature stand, presumed to be last cut around 1900 (+/- 20 years), and a young stand mechanically clear-cut in 1985 (Vadeboncoeur, unpublished data). Sap flow was measured in the mature stand only. Other characteristics of Jeffers Brook Forest including latitude, longitude, elevation, slope and aspect are given in Table 1.

	Cut	Latitude	Longitude	Elevation	Slope	Aspect	Dates Sampled	Trees Sampled
Jeffers Brook	~1900	44° 03' N	71° 88' W	730 m	30-40%	WNW	7/21/2014- 8/7/2014	10 sugar maple and 8 yellow birch

Table 1. Site characteristics and sampling dates for Jeffers Brook Forest (Vadeboncoeur unpublished data).

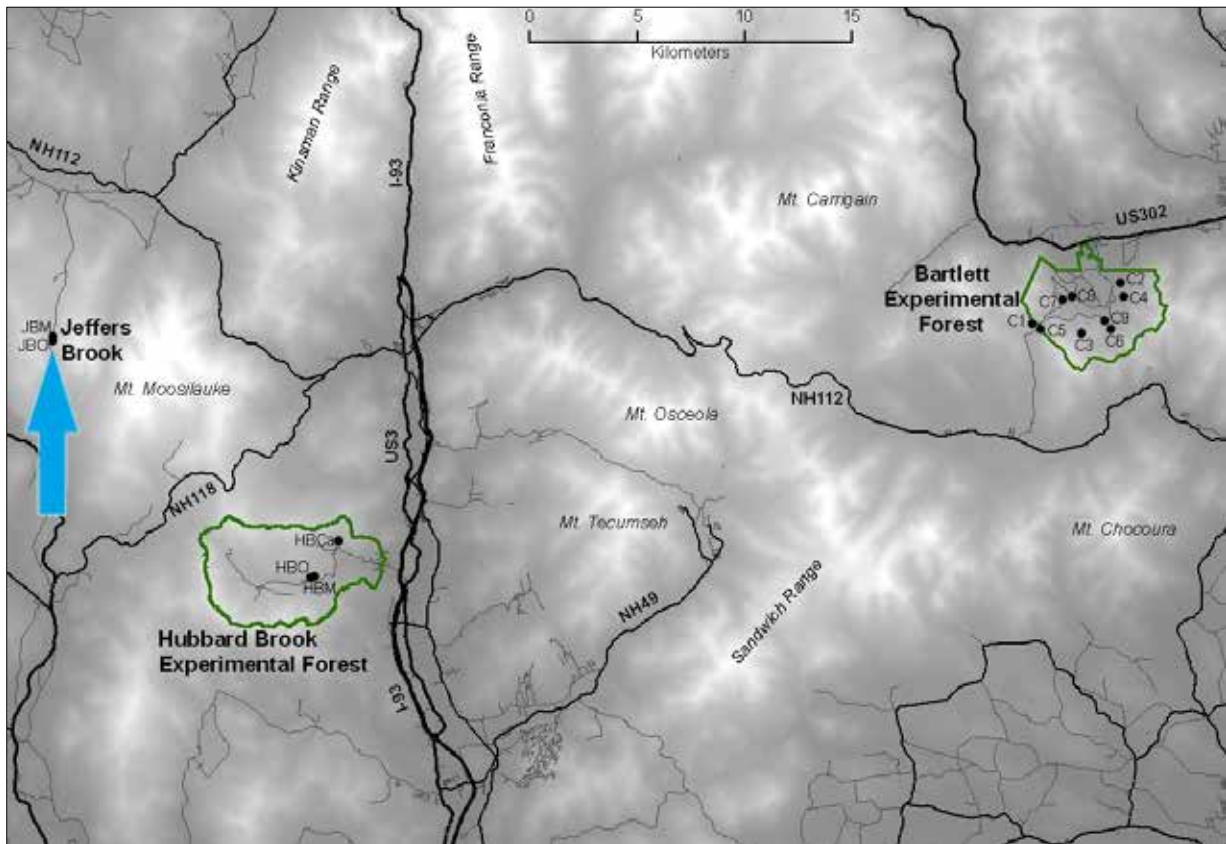


Figure 1. Site location in White Mountain National Forest (Vadeboncoeur 2011)

Experimental Design

A wollastonite (CaSiO_3) treatment plot and a control plot were selected for measuring sap flow (two plots total). The plots are 30 x 30 m (0.25 ha) with an additional 10 m buffer zone around the perimeter. Wollastonite was applied in 2011 by the Multiple Element Limitation in Northern Hardwood Ecosystems project using the same application rate as the 1999 wollastonite addition experiment at HBEF Watershed 1, 1.2 metric tons Ca per hectare (Peters et al., 2004). Healthy canopy trees presumed to be of uniform age were selected for measurement. All trees are located inside the plot (not in the buffer zone). Sap flow was measured in five sugar maple

trees in both plots, three yellow birch in the control plot, and five yellow birch in the wollastonite plot. Tree class and distance from the data logger station were the limiting factors in tree selection, and there were fewer yellow birch in the control plot that met these criteria. Measurements were collected in both plots simultaneously for 1-2 weeks in late summer (Table 1).

Sap Flow Measurements

Sap flow was measured using the Granier method, or thermal dissipation probe (TDP) method, an accurate and inexpensive method for measuring sap flow developed by André Granier in the 1980s. The method involves two sensor probes, a reference probe and a heating probe receiving a constant flux of heat (0.2 W) (Granier 1985). Each probe contains a thermocouple and the two thermocouples are wired together in opposition, allowing the system to measure the temperature difference between probes (ΔT : °C). The probes are inserted into the sapwood of the tree with the heating probe positioned above (downstream to) the reference probe (upstream) (Figure 1). The measured temperature difference (ΔT) is influenced by sap flux density (u : $m * s^{-1}$). Increased sap flow during the day decreases ΔT as sap circulating up through the xylem cools the heating probe by convection. At night, when there is little or no sap flow, conduction of heat in the wood around the heating probe is in equilibrium with the energy supplied by the heater, and ΔT reaches a maximum (ΔT_0). Granier developed an empirical relationship between u , ΔT , and ΔT_0 :

$$u = 119 * 10^{-6} K^{1.231} \quad (1)$$

where K is

$$K = (\Delta T_0 - \Delta T) / \Delta T \quad (2)$$

Probes were built using stainless steel medical needles and copper constantan thermocouples, protected within heat-distributing aluminum tubes. The pair of probes were inserted into 3 mm diameter holes drilled 2.5 cm into the tree on its south-facing side at about breast height (1.37 m), with the reference probe 10 cm directly below the heating probe. Defects such as scars and areas below dead limbs were avoided to limit interference in measurements. After installation, the probes were covered with Mylar insulation to minimize thermal gradients from wind and sun flecks. Cables connected to the probes ran from the trees to a data logger (CR1000 and CR800, Campbell Scientific) powered by four 12 V batteries that took measurements every 30 seconds and recorded a mean every 15 minutes. Figure 2 illustrates this setup.

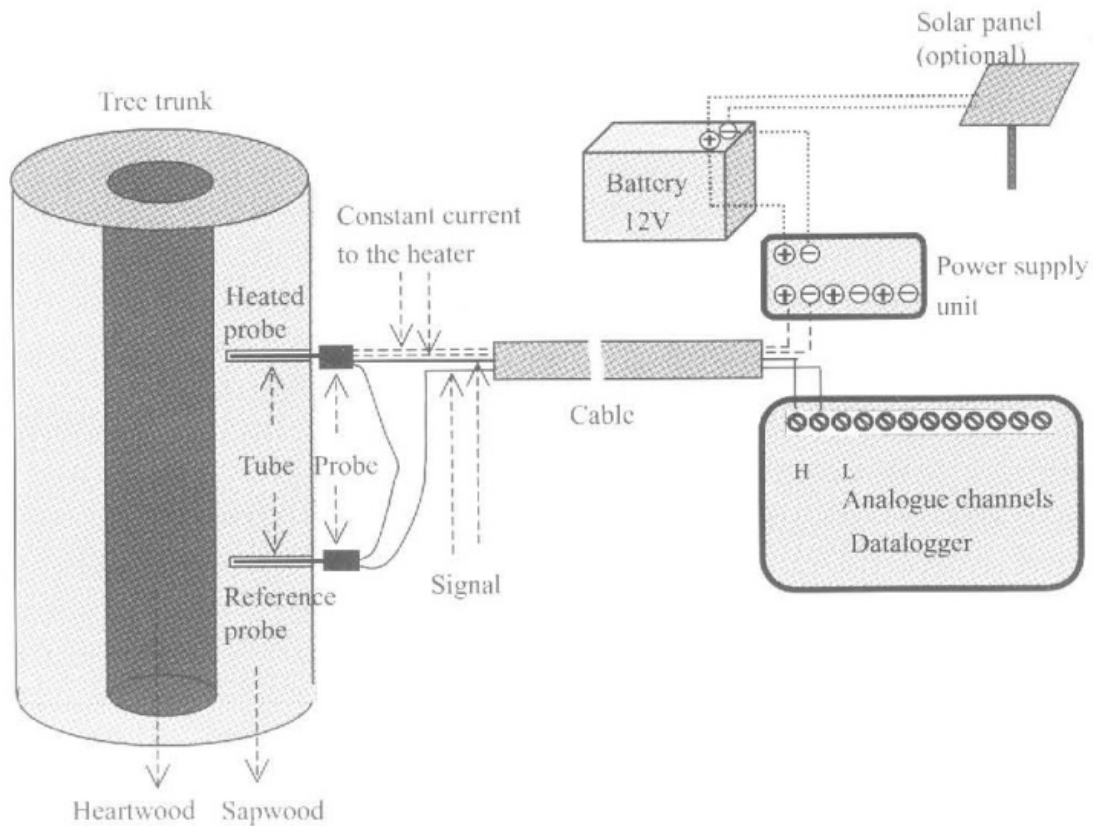


Figure 2. Configuration of the Granier system for sap flow measurement (Lu et al., 2004)

Statistical Analysis

The ΔT measurement was converted to sap flow (J_s , $g \times m^2 \times s^{-1}$) with BaseLiner software using the relationship $J_s = 119 * ((B/X) - 1)^{1.23}$, where B is a baseline value, which was set at conditions of no-flow for each day, and X is the data point value (Oren and Parashkevov 2012). The baseline was set by visually inspecting the fluctuations in ΔT each day for each individual tree. A baseline was chosen at the flattest, highest point of temperature difference, usually between 1:30- 3:00 AM to be the zeroing out point for that day. If the plot for a tree did not show characteristic diurnal fluctuations on fair weather days, this indicated a problem with the sap flow probes, and the data was not included in statistical analysis. This occurred with three of the original eighteen trees—one sugar maple in the control plot, one sugar maple in the wollastonite plot, and one yellow birch in the wollastonite plot. Data from rainy days that interfered with characteristic diurnal fluctuations were also not used. For statistical analysis, data within a timeframe from 10 am - 8 pm was chosen for analysis to avoid very low or no flow conditions. For each individual tree, the data for each 15-minute increment (within the 10 am - 8 pm timeframe) were averaged over 10 days of fair weather data.

A two-way analysis of covariance (ANCOVA) was conducted using Statistical Analysis System (SAS) software to determine a statistically significant difference in sap flow between treatment (wollastonite and control) and species (sugar maple and yellow birch), controlling for time of day (10 am - 8 pm) as the covariate. The model also contained a treatment by species interaction term. The significance level was set at $p < 0.05$.

Results

Results of the two-way ANCOVA indicate that, after controlling for time, there was a main effect of wollastonite treatment (shortened to "Ca" in tables and figures) on sap flow [F(1, 610)=124.04, $p < .0001$] (Table 2). Trees in the wollastonite addition plot had significantly increased sap flow compared to trees in the control plot (adjusted means 13.64 vs. 9.43). Figure 3 plots mean sap flow (J_s , $g \times m^2 \times s^{-1}$) from 10 am - 8 pm over 10 days for all trees in the wollastonite plot compared to all trees in the control plot.

Table 2.

ANCOVA Summary for Sap Flow by Treatment, Species, and Time

Source	SS	df	MS	F	p value
Treatment	2681.92	1	2681.92	124.04	< .0001*
Species	1529.51	1	1529.51	70.74	< .0001*
Time	798.96	1	798.96	36.95	< .0001*
Treatment x Species	87.46	1	87.46	4.04	0.0447*
Error	13189.25	610	21.62		

Time was used as a covariate. * $p < 0.05$. Analysis of covariance = ANCOVA

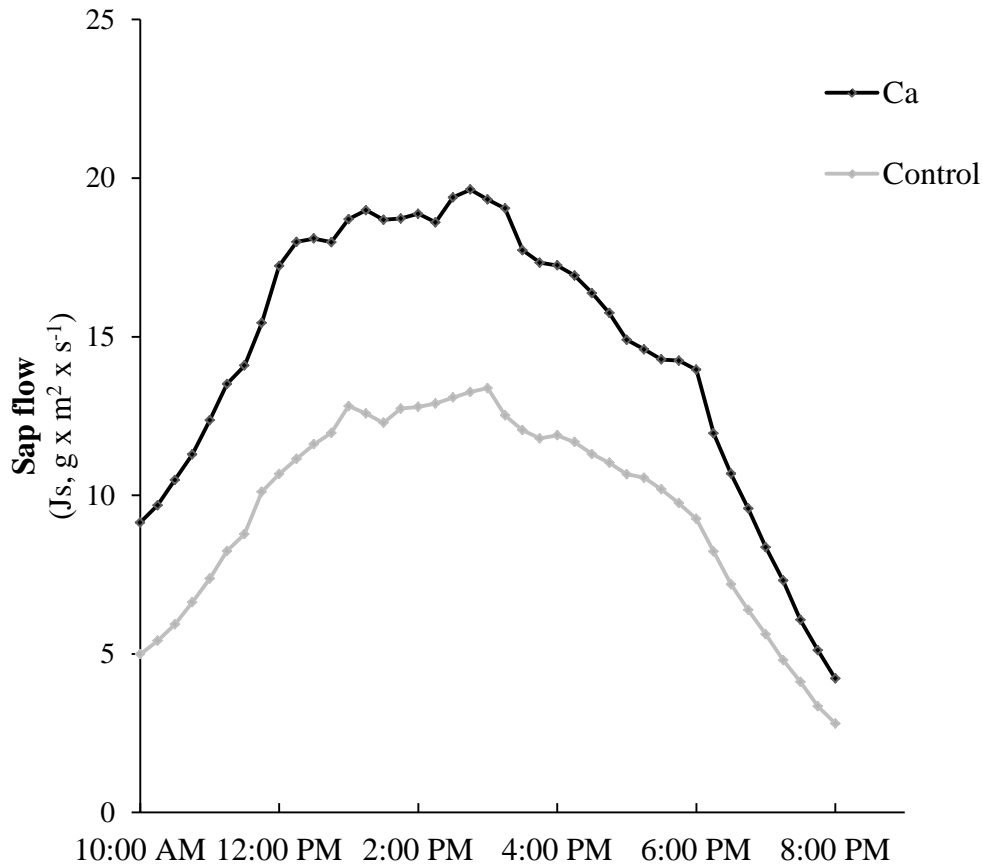


Figure 3. Mean sap flow over 10 days for all trees in the wollastonite plot and all trees in the control plot. After adjusting for time, wollastonite-treated trees had significantly higher mean sap flow than control trees.

Species was also a main effect [F (1, 610)=70.74, p<.0001] after controlling for time, with sugar maple (*Acer saccharum*, ACSA) having greater mean sap flow than yellow birch (*Betula alleghaniensis*, BEAL) (adjusted means 13.13 vs. 9.94) (Table 2). Figure 4 plots mean sap flow (J_s , $g \times m^2 \times s^{-1}$) from 10 am - 8 pm over 10 days for all sugar maple (ACSA) trees compared to all yellow birch (BEAL).

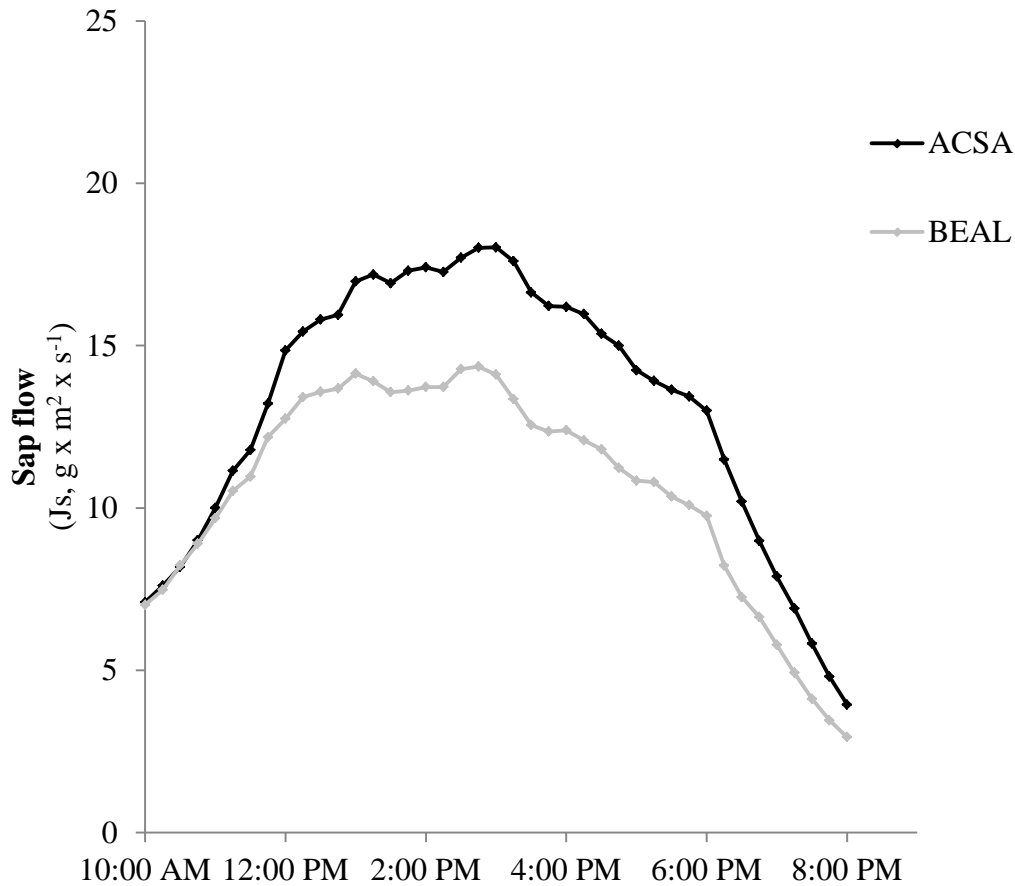


Figure 4. Mean sap flow over 10 days of data for all sugar maple trees (ACSA) and all yellow birch trees (BEAL). After adjusting for time, sugar maple had significantly higher mean sap flow than yellow birch.

Additionally, a significant treatment by species interaction effect was found [F(1, 610)=4.04, p=0.0447] (Table 2). These results suggest that sugar maple and yellow birch responded differently to the wollastonite addition. Sugar maple showed a more substantial increase in sap flow following a wollastonite addition than yellow birch. Table 3 lists adjusted means (controlled for time) for wollastonite sugar maple, control sugar maple, wollastonite yellow birch, and control yellow birch, and Figure 4 plots them.

Table 3

Adjusted Means for Sap Flow by Treatment and Species

Treatment	Species	Sap Flow LSMeans
Control	ACSA	10.64
Ca	ACSA	15.61
Control	BEAL	8.22
Ca	BEAL	11.67

ANCOVA adjusted means, controlling for time.

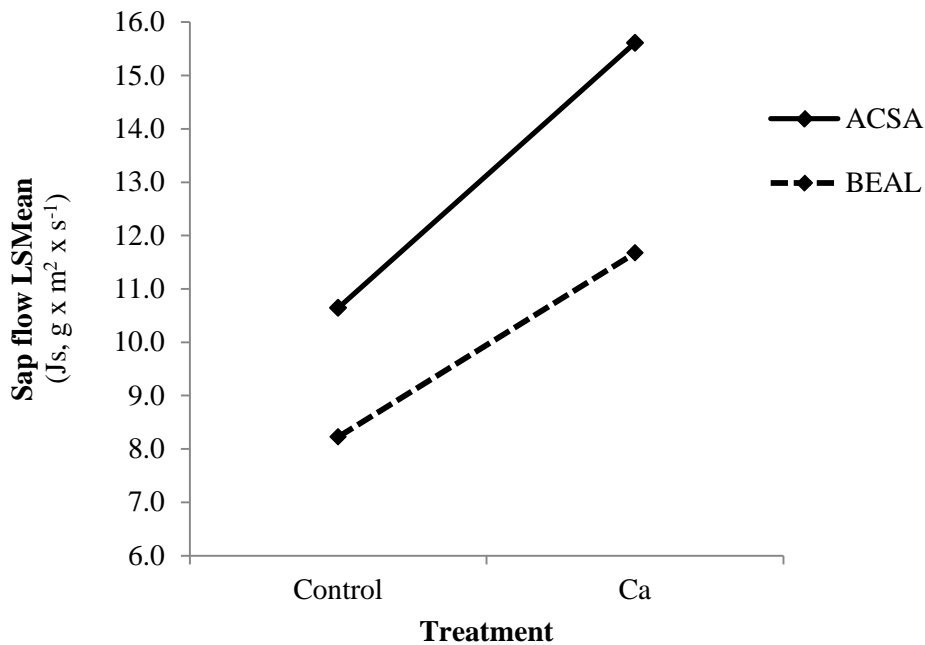


Figure 4. Adjusted sap flow means show a significant interaction effect between treatment and species.

Discussion

The ANCOVA results support the hypothesis that a wollastonite addition increases sap flow. Sap flow increased significantly in response to wollastonite ($p < 0.001$) (Table 2 and 3), which can be visually observed in Figure 3. This is an important finding, as it confirms the transpiration response at HBEF Watershed 1 that was once attributed to measurement error.

Green et al. (2013) stated that the W1 response was likely part of a "whole-forest fertilization of physiological activity" leading to rapid calcium uptake and increased primary production, which would be expected if the watershed forest was calcium-limited. The evidence supporting this conclusion included increased canopy reflectance, leaf area index (LAI), aboveground biomass, foliar calcium accumulation, soil water calcium, rapid streamwater Ca:Si ratio change, and enhanced ET. Researchers suggested that increased transpiration could occur from an increased number of stomata from new foliage, evidenced by increased LAI, crown density, foliar biomass, and seedling regeneration (Green et al., 2013).

At a mechanistic level, added calcium could be inducing primary production and thus transpiration and sap flow in a number of ways due to its varied role in plant function (McLaughlin and Wimmer, 1999). Calcium is necessary for the activation of enzymes involved in biosynthesis of membranes and cell walls and also stabilizes these structures (Brummel and MacLachlan, 1989). A study by Lautner et al. (2007) found that *Populus tremula* × *Populus tremuloides* clones supplied with a nutrient solutions of varying calcium concentration resulted in significant reduction in xylem fiber length correlated with reduction in calcium content. Xylem fibers are one of three general cell types found in the xylem of vascular plants; they have thick secondary cell walls to provide structural support (Ragni et al., 2011). Lignin is an integral component of secondary cell walls, and calcium plays a role in lignin synthesis (Eklund and Eliasson, 1990). Additional available soil calcium in the wollastonite addition plot could be contributing to more stable xylem tissues, allowing trees to transport water upwards at a faster rate.

Calcium also functions in fine root production and mycorrhizal colonization. Naples and Fisk (2010) showed that fine root length was significantly increased in soil cores taken from a

calcium addition plot compared to control cores in HBEF. Juice et al. (2006) found that the Watershed 1 wollastonite addition had significantly increased mycorrhizal colonization of sugar maple germinants compared to reference plots when it was measured in both 2003 and 2004. These results suggest that calcium could also be stimulating sap flow by enhancing the ability of tree roots to uptake water. However, mycorrhizal colonization could be a consequence rather than a cause of generally increased forest growth following the wollastonite addition (Juice et al., 2006). Pinpointing specific mechanisms by which wollastonite mediates transpiration will require considerable further study.

Additional considerations are also the possible effects of pH, Si, or a combined effect of Si and Ca on sap flow. In Watershed 1 both the streamwater and soil water Ca:Si ratios and mass balances suggested that Ca from wollastonite was strongly retained relative to Si, but an effect cannot be ruled out with certainty (Cho et al., 2010).

Species significantly affected sap flow as well. Sugar maple had higher mean sap flow than yellow birch, averaged across treatment ($p < 0.001$) (Table 2 and 3, and Figure 4). This is not surprising, as tree species vary widely in phenology and physiological characteristics. More interesting, however, is that the response to wollastonite was greater in sugar maple than in yellow birch trees. According to Green et al. (2013), weathering of wollastonite increased available calcium most in the organic soil horizon of W1 (approximately double the pretreatment concentrations). Sugar maple roots are most abundant in this horizon and can grow quickly in response to changes in nutrient resources (Naples and Fisk, 2010). If wollastonite is influencing increased transpiration via fine root growth, this might explain a stronger response in sugar maple compared to yellow birch. Sugar maple also has relatively high calcium requirements

(Hallett et al., 2006). Sugar maple may have been more limited than yellow birch in depleted calcium conditions, leading to a stronger response to wollastonite.

Another aspect to consider is the transpiration response timeline. In W1, evapotranspiration increased for three years following the addition in October 1999, then stopped increasing two years and seven months later in May 2002 and began to decrease compared to a reference watershed. It would thus be of interest to continue monitoring sap flow in Jeffers Brook Forest in the future to determine whether trends in transpiration are following a similar timeline. Compared to Hubbard Brook Experimental Forest, Jeffers Brook has a greater natural abundance of soil calcium (Bae, unpublished manuscript). It might then be hypothesized that a transpiration response to wollastonite would occur more slowly in JB compared to HBEF if the forest were less calcium-limited. Indeed, in this study sap flow measurements were taken about two years and nine months after the initial wollastonite addition in October 2011, which is two months beyond the comparative point in time when transpiration stopped increasing in HBEF W1. Green et al. (2013) suggested that the transience of the transpiration response in W1 could be attributed to drastically improved water use efficiency due to enhanced stomatal function. Ca also plays a role in control of leaf gas exchange through its regulation of stomatal guard cell turgor relations and stomatal opening and closing (McAinsh et al., 1996). Continued study of sap flow and other measures in Jeffers Brook to investigate this hypothesis is recommended.

Conclusion

Overall, this study suggests soil calcium supply can substantially influence forest water cycles. A wollastonite addition in Jeffers Brook Forest significantly increased sap flow, and sugar maple trees showed a stronger response than yellow birch. The results verify a transpiration response observed following a whole-watershed wollastonite addition in Hubbard Brook Experimental Forest. Forests provide a great range of ecosystem services that impact human well-being, from raw materials to water purification, and forest health is highly dependent on the flow and balance of nutrients driven by water movement. The outcome of this study provides knowledge of forest water use, which may have important implications for forest management amidst continued acid deposition, deforestation, and other threats to calcium reserves.

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References

- Bae, K. et al. (2014). Soil nitrogen availability affects soil respiration and belowground carbon allocation in northern hardwood forests of New Hampshire. Unpublished manuscript.
- Battles, J.J., Fahey, T.J., Driscoll, C.T., Blum, J.D., and Johnson, C.E. (2013). Restoring soil calcium reverses forest decline. *Env. Sci and Tech. Letters*, 1, 15–19.
- Brummel D., and MacLachlan, J. (1989). Calcium antagonist interferes with auxin-regulated xyloglucan glycosyltransferase levels in pea membranes. *Biochemistry and Biophysics*, 1014, 298–304.
- Cho, Y., Driscoll, C.T., Johnson, C.E., Blum, J.D., and Fahey, T.J. (2012). Watershed-level responses to calcium silicate treatment in a northern hardwood forest. *Ecosystems*, 15, 416–434.
- Cogbill, C.V., and Likens, G.E. (1974). Acid precipitation in the northeastern United States. *Water Resource Research*, 10(6), 1133–1137.
- Currie, H.A., and Perry, C.C. (2007). Silica in plants: Biological, biochemical, and chemical studies. *Annals of Botany*, 100(7), 1383–1389.
- Driscoll, C.T. (2000). Summary of the wollastonite (CaSiO₃) addition to Watershed 1 at the Hubbard Brook Experimental Forest. *Hubbard Brook Ecosystem Study*. http://www.hubbardbrook.org/research/longterm/calcium/w1_overview/w1rshxx.htm .
- Driscoll, C.T., et al. (2001). Acidic deposition in the northeastern United States: Sources and inputs, ecosystem effects, and management strategies. *BioScience*, 51(3), 180–198.
- Driscoll, C.T., et al. (1989). Changes in the chemistry of surface waters: 25-year results at the Hubbard Brook Experimental Forest, NH. *Environ Sci Technol*, 23, 137–143.
- Eklund L., and Eliasson, L. (1990). Effects of calcium ion concentration on cell wall synthesis. *Journal of Experimental Botany*, 41, 863–867.
- Federer C., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, R.S., Smith, C.T. (1989). Long-term depletion of calcium and other nutrients in eastern U.S. forests. *Environmental Management*, 13, 593–601.
- Granier, A. (1985). Une nouvelle methode pour la mesure du flux deseve brute dans le tronc des arbres. *Annales Sciences Forestieres*, 42, 193–200.
- Granier, A. (1987). Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiology*, 3, 309–320.

- Green, M.B., et al. (2013). Decreased water flowing from a forest amended with calcium silicate. *Proceedings of the National Academy of Sciences*, 110(15), 5999–6003.
- Hallett R.A., Bailey, S.W., Horsley, S.B., Long, R.P. (2006). Influence of nutrition and stress on sugar maple at a regional scale. *Can J For Res*, 36(9), 2235–2246.
- Hepler, P.K., Wayne, R.O. (1985). Calcium and plant development. *Annual Review of Plant Physiology*, 36, 397–439.
- Horna, V., et al. (2011). Environment and tree size controlling stem sap flux in a perhumid tropical forest of Central Sulawesi, Indonesia. *Annals of Forest Science*, 68(5), 1027–1038.
- Hubbard Brook Ecosystem Study. Site Description. Hubbard Brook Ecosystem Study. <http://www.hubbardbrook.org/overview/sitedescription.shtml>.
- Joly, J. and Dixon, H.H. (1894). On the ascent of sap. *Annals of Botany*, 8, 468–470.
- Juice, S.M., et al. (2006). Response of sugar maple to calcium addition to northern hardwood forest. *Ecology*, 87(5), 1267–1280.
- Kirkby, E.A., Pilbeam, D.J. (1984). Calcium as a plant nutrient. *Plant, Cell and Environment*, 7, 397–405.
- Lautner, S., et al. (2007). Calcium nutrition has a significant influence on wood formation in poplar. *New Phytologist* 173(4), 743–752.
- Likens, G.E., et al. (1998). The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry*, 41(2), 89–173.
- Lu, P., Urban, L., and Zhao, P. (2004). Granier's thermal dissipation probe (TDP) method for measuring sap flow in trees: Theory and practice. *Acta Botanica Sinica*, 46(6), 631–646.
- Marion, G.M., Van Cleve, K., Dyrness, C.T., Black, C.H. (1993). The soil chemical environment along a forest primary successional sequence on the Tanana River floodplain, interior Alaska. *Canadian Journal of Forest Research*, 23, 914–922.
- Martin, C.W., Hornbeck, J.W., Likens, G.E., and Buso, D.C. (2000). Impacts of intensive harvesting on hydrology and nutrient dynamics of northern hardwood forests. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, 19–29.
- McAinsh M.R., Clayton, H., Mansfield, T.A., and Hetherington, A.M. (1996). Changes in stomatal behavior and guard cell cytosolic free calcium in response to oxidative stress. *Plant Physiology*, 111, 1031–1042.
- McLaughlin, S.B., and Wimmer, R. (1999). Calcium physiology and terrestrial ecosystem processes. *New Phytologist*, 142, 373–417.

- Meinzer, F., et al. (2004). Dynamics of transpiration, sap flow and use of stored water in tropical forest canopy trees. *Tree Physiology*, 24, 901–909.
- Miller, R. W., and Donahue, R. L. (1990). *Soils: an introduction to soils and plant growth* (6th ed.). Englewood Cliffs, NJ: Prentice Hall.
- Miransari, M. (2013). Soil microbes and the availability of soil nutrients. *Acta Physiol Plant*, 35, 3075–3084.
- Multiple Element Limitation in Northern Hardwood Ecosystems. Overview. MELNHE.
<http://www.esf.edu/melnhe>.
- Naples, B.K., and Fisk, M.C. (2010). Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry*, 97(2), 109–121.
- Nezat, C.A., Blum, J.D. and Driscoll, C.T. (2010). Patterns of Ca/Sr and ⁸⁷Sr/⁸⁶Sr variation before and after a whole watershed CaSiO₃ addition at the Hubbard Brook Experimental Forest, USA. *Geochim Cosmochim Acta*, 74, 3129–3142.
- Oren, Parashkevov, and Duke University. (2012). BaseLiner (Version 2.4.2).
<http://ch2oecology.env.duke.edu/orenlab/software.html>
- Oyonarte, C., et al. (1994). Factors affecting soil organic matter turnover in a Mediterranean ecosystems from Sierra de Gador (Spain): An analytical approach. *Communications in Soil Science and Analysis*, 25, 1929–1945.
- Peters, S.C., Blum, J.D., Driscoll, C.T., and Likens, G.E. (2004). Dissolution of wollastonite during the experimental manipulation of Hubbard Brook Watershed 1. *Biogeochemistry*, 67(3), 309–329.
- Ragni, L., et al. (2011). Mobile gibberellin directly stimulates *Arabidopsis* hypocotyl xylem expansion. *The Plant Cell*, 23(4), 1322–1336.
- Roberts, D.M., Harmon, A.C. (1992). Calcium-modulated proteins: Targets of intracellular calcium signals in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 375–414.
- Schwarz, P.A., Fahey, T.J., and McCulloch, C.E. (2003). Factors controlling spatial variation of tree species abundance in a forested landscape. *Ecology*, 84(7), 1862–1878.
- Smith, M., and Martin, M.E. (2001). A plot-based method for rapid estimation of forest canopy chemistry. *Canadian Journal of Forest Research*, 31, 549–555.
- Taiz, L., and Zeiger, E. (2010). *Plant physiology*. (5th ed.). Sunderland, MA: Sinauer Associates.

- Tomlinson, G.H. (2003). Acidic deposition, nutrient leaching, and forest growth. *Biogeochemistry*, 65(1), 51–81.
- Ulrich, B., Matzner, E. (1986). Anthropogenic and natural acidification in terrestrial ecosystems. *Experientia*, 42, 344–350.
- White, P.J., and Broadley, M.R. (2003). Calcium in plants. *Annals of Botany*, 9(4), 487–511.
- Vadeboncoeur, M.A. (2014). All NH sites. MELNHE. http://www.esf.edu/melnhe/restricted/maps_catalog.htm. Unpublished data.

Appendices

Data

Time	Calcium								Control							
	ACSA1	ACSA2	ACSA3	ACSA5	BEAL1	BEAL2	BEAL3	BEAL4	ACSA7	ACSA8	ACSA9	ACSA10	BEAL6	BEAL7	BEAL8	
10:00 AM	12.4278	3.6953	10.7630	8.3752	8.7656	11.2018	8.7575	4.4362	6.3294	4.8693	3.9339	6.3906	8.2003	1.7187	3.4702	
10:15 AM	12.8479	4.1508	11.7320	8.6894	9.1513	12.0658	9.1492	5.0842	7.1958	5.1904	4.3151	6.7094	8.6586	1.9636	3.8587	
10:30 AM	13.3400	5.1923	12.5435	8.9819	9.6672	13.9026	9.7484	5.6686	7.3561	5.7221	5.0140	7.3285	9.3313	2.1281	4.6253	
10:45 AM	14.4702	5.6512	14.0545	9.5577	10.4939	13.9157	10.8930	6.1017	8.0887	6.3619	5.7535	8.1048	10.2407	2.3868	5.4492	
11:00 AM	15.8785	7.1214	15.1581	10.4391	11.3018	14.9827	11.6659	6.7630	9.1312	7.0389	6.4276	8.8288	11.0047	2.8787	6.2700	
11:15 AM	17.5385	8.0387	16.6050	11.5709	11.6427	15.9968	13.1753	7.4263	10.3053	8.0345	7.3131	9.7534	11.6784	3.3516	7.2784	
11:30 AM	18.3092	8.4338	17.6365	12.0437	11.8245	16.6604	13.7086	7.6589	10.6309	8.7254	8.0678	10.4345	11.9830	3.6075	8.0125	
11:45 AM	20.5985	10.2456	19.0728	12.5245	12.2893	17.7655	15.5536	8.1172	12.0704	10.1498	9.3854	11.6614	13.3809	4.3457	9.7599	
12:00 PM	24.4500	11.5756	21.3426	14.1868	13.2557	18.1423	17.6897	8.7314	12.9134	11.2347	10.6274	12.5143	13.8767	4.9098	8.6216	
12:15 PM	24.0698	12.7930	22.5758	15.3807	13.3293	19.5902	18.1909	8.9037	13.4265	11.6955	11.0184	12.5366	14.0057	5.0487	10.3219	
12:30 PM	23.3296	13.9189	23.0515	15.2665	13.1825	20.2129	17.7196	9.0412	14.4169	12.0138	11.7534	12.6953	14.1468	5.1819	11.0357	
12:45 PM	23.2950	14.0640	23.1743	15.1999	12.9726	19.0111	18.1521	9.3772	13.9540	12.3086	12.4221	13.1617	14.4178	5.6632	11.8507	
1:00 PM	24.1032	16.6072	24.2284	15.6793	13.4152	18.1062	18.8149	9.3801	14.7679	12.9835	13.4509	14.0097	15.1113	6.2960	13.0886	
1:15 PM	24.6743	17.3943	25.0867	15.6319	13.5187	17.9987	18.6312	9.2605	14.3032	13.0819	13.5774	13.7850	14.7721	5.8797	12.6330	
1:30 PM	24.4583	15.5777	25.4256	15.9794	13.3692	17.6822	18.3134	9.4313	14.2564	13.0542	13.2942	13.3445	14.5898	5.4939	11.9502	
1:45 PM	24.7197	17.0498	25.1439	15.7791	13.1857	17.0755	18.1139	9.8004	14.9651	13.3817	13.7627	13.6839	15.0715	5.8357	12.4139	
2:00 PM	24.6366	17.6855	25.3272	15.7027	13.4606	17.4238	17.8970	9.7505	14.6351	13.3785	13.8738	14.0563	15.2904	5.7316	12.5386	
2:15 PM	24.2767	16.2098	25.2597	15.5347	13.6471	18.1586	17.1490	9.5906	14.6409	13.8072	14.3693	14.0480	15.3681	5.5385	12.5027	
2:30 PM	25.6326	16.7470	25.0784	16.5426	14.4285	19.0815	18.2477	10.0255	14.7910	13.7754	14.5100	14.5880	15.2423	5.8761	12.7800	
2:45 PM	26.5244	15.7480	26.4507	16.9654	15.8637	18.0450	17.8594	10.0782	15.0766	13.8884	14.5426	14.9063	15.2308	6.3152	12.8268	
3:00 PM	26.1461	16.4084	25.3965	16.9542	15.1877	17.4168	17.7602	9.7963	15.5200	14.3210	14.6792	14.8091	15.1814	6.3130	12.8147	
3:15 PM	25.2159	16.0766	26.8963	16.6833	14.7423	16.7534	16.9293	9.4759	13.9891	13.5949	14.2545	14.0837	14.4747	5.5378	11.6600	
3:30 PM	24.1171	13.4655	25.3873	16.3935	13.1727	15.6146	15.9384	9.2723	13.4097	12.8494	13.7205	13.7830	13.8833	5.7296	10.9750	
3:45 PM	24.2978	13.4374	23.8704	15.7165	12.7892	14.8351	16.3869	9.2920	12.8462	12.4027	13.4741	13.7026	13.6867	5.7712	10.6711	
4:00 PM	23.8345	14.3037	23.2562	15.3671	12.5767	15.1573	16.2264	9.3034	13.2248	12.3353	13.3606	13.8965	13.7596	5.9666	10.6790	

4:15 PM	22.8237	13.3631	24.2625	15.4015	12.3045	14.9707	15.3974	9.0666	13.0406	12.2805	13.1604	13.4409	13.6070	5.7857	10.4461
4:30 PM	22.1951	13.0172	22.2845	14.9713	11.9849	14.9107	15.2698	9.0802	12.9321	11.8229	12.4135	13.2487	13.5786	5.5074	9.6067
4:45 PM	21.0577	12.7957	22.5201	14.3228	11.5106	14.2122	13.7796	8.6979	12.8033	11.5836	11.7928	13.0887	13.5155	5.1467	9.2269
5:00 PM	20.2894	10.9335	21.3654	13.7038	11.1973	13.9718	12.8509	8.3620	12.5302	10.9612	11.2002	12.9457	13.2284	5.2455	8.5527
5:15 PM	19.7037	9.4890	21.2407	13.5321	11.2649	13.9905	12.9788	8.0224	12.5437	10.7849	10.9204	13.0838	13.0828	5.1266	8.3366
5:30 PM	19.9367	8.6544	20.7983	13.3280	10.8932	13.8211	12.5252	7.5544	12.0839	10.6826	10.7782	12.8784	12.5722	4.4938	7.8121
5:45 PM	19.9825	8.4262	20.6231	13.7078	10.9729	13.3813	12.6321	7.6025	11.6566	10.4873	10.1052	12.4875	11.9123	4.1916	7.4310
6:00 PM	19.3641	7.9503	19.9236	14.0685	10.6414	13.1589	12.6167	7.7774	11.3432	10.0504	9.5561	11.7219	11.3291	4.1529	6.6478
6:15 PM	16.4698	6.8554	17.5499	12.6833	9.0697	11.1346	9.9005	6.8154	10.2786	9.0977	8.4936	10.5028	10.3791	3.3505	5.5098
6:30 PM	14.2444	6.0143	16.2579	11.5313	8.0239	9.6424	9.0468	6.5875	8.8170	8.0266	7.1592	9.5357	9.3361	2.7918	4.6926
6:45 PM	12.6566	5.1004	14.7519	9.8383	6.8430	9.3841	8.4691	5.5236	7.8329	6.9553	6.0807	8.6771	8.5619	2.5508	4.0274
7:00 PM	11.1202	4.5744	12.8930	8.5999	5.9666	7.4037	7.9731	5.2236	7.0478	5.9375	5.0391	7.9129	7.6508	2.2366	3.4696
7:15 PM	9.2104	4.8122	11.3135	7.4843	4.9372	6.7471	6.6794	4.3529	6.1636	5.0149	4.1576	7.1309	6.6272	1.7586	2.7763
7:30 PM	7.9547	4.1236	9.2101	6.2107	3.9585	5.2652	5.7443	3.9280	5.3729	4.1192	3.4005	6.1677	5.6638	1.7018	2.3731
7:45 PM	7.0367	3.3666	7.6517	5.0704	3.1524	4.6190	4.8673	3.5714	4.4467	3.3155	2.6305	4.9095	4.8243	1.4672	1.8200
8:00 PM	5.7030	3.7514	5.7773	3.8654	2.3841	3.9401	4.1281	3.0713	3.9004	2.6522	2.0298	3.8567	4.1370	1.5381	1.5220

