# SOIL NUTRIENTS AFFECT SWEETNESS OF SUGAR MAPLE SAP

by

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A thesis submitted in partial fulfillment
of the requirements for the
Masters of Science Degree
State University of New York
College of Environmental Science and Forestry
Syracuse, New York
April 2014

Approved: Department of Forest and Natural Resources Management

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| Ruth D. Yanai, Major Professor | Timothy Toland, Chair<br>Examining Committee |
| David Newman, Department Chair | S. Scott Shannon, Dean<br>The Graduate School |

Acknowledgements

This study would not have been possible without the assistance of my advisor Ruth Yanai who diligently worked with me and provided the opportunity to explore my own interest. My graduate committee members Chris Nowak, Colin Beier and Mike Farrell were always willing to provide assistance. Acknowledgement of Eric Randall for his advice on sap sampling is necessary along with Tim Wilmot, Tim Perkins, Steve Childs, Paul Schaberg, Heidi Asbjornsen, Adan Hernádez, and Sandy Wilmot for their many contributions. Mike Wild willing helped during the last sap sampling period which allowed for a shorter day in the field. Gas exchange measurements could not have been taken without Katherine Sinacore operating the portable photosynthesis system. Chris Costello was always willing to assist me with the snowmobile for sap sampling and ensure I came out of the woods safely. Melany Fisk was gracious in providing the soils data. Members of my lab, Craig See, Franklin Diggs, Yang Yang and Yi Dong were always willing to provide feedback on results. Foliage and tree growth sampling was completed by members of the MELNHE summer field crew including Eric MacPherson who assisted with the canopy assessment. Working with John View, Kevin Reynolds and members of the Mighty Oaks cross-country and track team at ESF kept me sane by providing the opportunity to get away from my research and run.

Funding for this project was provided by a grant from the Northeastern States Research Cooperative, a division of the USDA Forest Service. I could not have completed the project without this funding to cover my many trips to NH. The MELNHE project is funded through the National Science Foundation and the Long Term Ecological Research Network Office.

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Abstract

A. D. Wild. Soil Nutrients Affect Sweetness of Sugar Maple Sap, 49 Pages, 3 tables, 4 figures, 2014.

Understanding how soil and foliar nutrients play a role in sap sweetness of sugar maples is economically important for producing maple syrup. Sugar concentration affects the amount of sap required to produce a gallon of syrup. Sugar maples were sampled for sap sweetness in five stands in the White Mountains of New Hampshire and correlated to foliar and soil nutrients. Treatment plots were fertilized with N, P, N and P, and Ca to test whether a nutrient addition increases sap sweetness. Higher sugar concentration in the sap was correlated with soil nitrogen mineralization. Foliar P had a negative correlation with sap sweetness while trees with higher foliar N:P had sweeter sap. Addition of 30 kg N ha/yr increased sap sweetness two years after initial treatment. By selecting sites with higher soil nitrogen or fertilizing with N, maple producers may be able to collect sweeter maple sap.

Keywords: sugar maple, sap sweetness, sugar concentration, nitrogen, calcium, soil, foliage, tree health

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# Chapter *1*

## I. INTRODUCTION AND INTELLECTUAL MERIT

Sugar maple (*Acer saccharum* Marsh.) trees are valued for their high quality timber, shade, vibrant fall color, and maple sugar throughout the northeastern United States and southeastern Canada. Sap is extracted from sugar maples in the late winter or early spring for producing maple syrup or maple sugar products. Maple syrup or sugar products produced in the United States are valued at over US$81 million a year (Farrell and Cabot 2012) and well over CAN$300 million in Canada (Statistics Canada 2013). Despite the extensive market, factors affecting maple sap sweetness are not fully understood.

Maple sap consists primarily of water with an average of 2.5% sugar (Gabriel and Seegrist 1977). Sap sweetness varies substantially between trees and can reach 10% (Gregory and Hawley 1983). Sucrose is the primary sugar of maple sap with other sugars rarely exceeding 0.05% of the sap (Gregory and Hawley1983). Syrup is produced by boiling sap down to approximately 66% sugar. The amount of sap required to produce a gallon of syrup is dependent upon sap sweetness and can be calculated by dividing the percent sugar concentration into 86 (Taylor 1956, Jones 1967, Perkins and van den Berg 2009). Sweeter sap reduces the amount of energy and labor required to collect and boil the sap into syrup. Trees with sweeter sap also tend to yield a higher volume of sap which increases syrup production (Marvin et al. 1967).

# II. SAP FLOW PROCESS

Sap exudation mechanisms in maples are complex and different from most plants (Tyree 1983). The mechanism for maple sap flow is dependent upon stem pressure, instead of root pressure as in other species (Gregory 1982, Tyree 1983). Sap flow mechanism during the leafless period are different than sap flow during the leaf on period when transpiration within the leaves pulls water up through the stem. Sap flow requires subfreezing temperature at night followed by thawing temperature during the day (Marvin 1958, Marvin et al. 1971). Twig temperature has been found to correlate better with sap flow than root or stem temperature (Marvin 1958). Pressure within stems is lower than atmospheric pressure during freezing temperatures and greater during warmer temperatures (Gregory 1982, Tyree 1983). Greater pressure within stems during sap flow allows sap to exude out of tap holes while atmospheric pressure is lower (Gregory 1982). Rapid flow of sap has been found to occur only 5-60 seconds after the onset of an exotherm, a release of heat from water freezing (Tyree 1983). An osmotically active substance, such as sucrose, is required for maple sap flow and glucose or fructose alone will not work (Marvin 1958).

The way stem pressure pulls sap up into branches through temperature fluctuations is intriguing. Sapwood cells of maples contain $CO_2$ gas bubbles in greater quantity which is different from other species of hardwoods (Gregory 1982, Tyree 1983). Higher density of gas bubbles are present during the dormant season than during the growing season (Sperry et al. 1988). As the temperature starts to cool in a branch, tension of gas particles occurs within cells and $CO_2$ decreases in pressure (Gregory 1982, Tyree 1983) allowing sap to flow into the cell. Water particles crystallize and freeze to the outer wall of the cell and in the process release heat out of the branch and into the atmosphere which further reduces pressure within the stem (Gregory 1982).

Sap continues to be pulled into the cell from warmer inner cells and eventually the roots through water tension and low stem pressure compressing gas particles until the temperature within the cells and the atmosphere equalize (Gregory 1982). Sap flow could also be a result of water moving toward growing ice lenses as studied in soil (Tyree 1983). As temperatures thaw, ice melts and $CO_2$ bubbles increase in pressure and push sap back down the stem (Gregory 1982, Tyree 1983). It is not known where the $CO_2$ gas bubbles come from and why they are abundant in maples (Gregory 1982). More sap is allowed to flow into the branches if temperature slowly drop below freezing than if the temperature fell fast (Tyree 1983). The conversion of starch to sucrose may be a metabolic process that produces $CO_2$ and trees with sweeter sap could create higher pressure and more sap flow from higher metabolism (Gregory 1982). It is also thought that higher amounts of available soil water increases sap flow (Gregory 1982).

# III. CARBOHYDRATES

Carbohydrates are essential for plant development (Magel et al. 2000). Carbohydrates produced within plants are a source of energy used for growth, development, defense, flowering, cold tolerance and survival. Plants store carbohydrates to form new leaves following dormancy and after defoliation. Carbohydrates are molecules of different forms of carbon, hydrogen and oxygen used for structure and energy in plants (Rost et al. 2006).

## *A.Carbohydrate Production*

Carbohydrates are formed by photosynthetic processes in the leaves of plants (Kozlowski 1992, Magel et al. 2000). In addition to leaves, other plant tissues, such as cotyledons, buds, twigs,

stems, flowers and fruits often contain chlorophyll and therefore photosynthesize and produce sugars (Kozlowski 1992). Photosynthesis produces simple sugars such as glucose or fructose through the formation of carbon, hydrogen and oxygen bonding to form rings (Rost et al. 2006). Through a dehydration reaction, two or more simple sugars can bond to form an oligosaccaride such as sucrose (Rost et al. 2006). Sucrose is an abundant carbohydrate in plants often accounting for up to 95% of transported carbohydrates within a plant (Kozlowski 1992). In addition to producing sucrose, long chains of simple sugars can combine to form polysaccharides such as starch (Rost et al. 2006). Starch is the common polysaccharide in plants and accumulates as a reserve carbohydrate when there is a surplus of sugars (Kozlowski 1992).

### *B. Carbohydrate Storage*

Storage of carbohydrates is important for long term survival of plants (Kozlowski 1992, Regier et al. 2010). When in excess, plants accumulate carbohydrates for future mobilization during shortages such as defoliation by insects or herbivory by larger organisms like deer (Kozlowski 1992). Stored carbohydrates also provide energy for root and shoot growth, flowering, and leaf development in the spring. Reserved carbohydrates are also used for flower production of sugar maples who flower before leaf development (Kozlowski 1992). Non-structural carbohydrate reserves make up more than 90% of available carbon in plants (Regier et al. 2010). Sugars produced through photosynthesis enter the phloem for transportation to storage sites (Gifford and Evens 1981). Sugars are typically only in the phloem of plants, however, sugars enter into the xylem during the dormant season of sugar maples (Gifford and Evans 1981; Kozlowski 1992).

### 1.Root Storage

Carbohydrates are often stored in the roots of plants. Root storage is highest during dormancy when plants transport carbohydrates to the roots for overwintering (Regier et al. 2010). In addition to providing energy for growth and development, carbohydrates stored in the roots allow many plants to re-sprout from the base of the tree after damage to above ground tissue (Regier et al. 2010). In addition to roots, carbohydrates are also stored in branches, stems, seeds, fruit and sap (Kozlowski 1992, Wong et al. 2003).

### 2. Ray Cell Storage

Sugar maples store carbohydrates in ray cells of sap wood as starch (Marvin et al. 1971, Gregory 1983, Kozlowski 1992, Liu et al. 1997). In early winter, starch is converted into sugars and released into xylem vessels at ray and axial parenchyma contact cells (Gregory and Hawley 1983, Kozlowski, 1992, Liu et al. 1997, Wong et al. 2003). The rate starch converts to sugars is dependent on temperature and cellular respiratory activity (Kozlowski 1992). The sugars that are tapped for maple syrup are extracted from the xylem.

The quantity of ray cells are important for maple sap sweetness. Trees with more ray cells and ray cells of larger size are able to store more sugar and have sweeter sap (Morsellie et al. 1978). A tree with sweeter sap does not necessarily produce more sugar throughout the year but is able to store more sugar (Morsellie et al. 1978). Faster growing trees is important for sweet trees as it creates larger and higher quantities of ray cells for storing sugar concentration which increases sap sweetness (Gregory 1977, Morselli et al. 1978). Ray cell abundance can be increased by increasing growth rates of sugar maple through management (Gregory 1982). In addition to higher growth rates, more ray cell area could be a genetic trait as well (Morselli et al 1978).

### *C. Carbohydrate Allocation*

Plants allocate carbohydrates in different ways to ensure long term success of the species. Plants will allocate carbohydrates produced by photosynthesis either to immediate structural components or storage reserves (Kobe 1997). Structurally allocated carbohydrates are used for immediate growth or respiration (Kobe 1997) such as cell development and growth, producing new leaves, buds, fruit, and shoot and root elongation (Kozlowski 1992). Structural allocated carbohydrates are a continuous cycle as growth allows more photosynthesis production and therefore manufacturing of more carbohydrates (Kobe 1997).

There are tradeoffs involved in plant allocation of carbohydrates. When starches and sugars are allocated to structure they are able to produce more areas for photosynthetic production at the cost of losing carbohydrates through respiration (Kobe 1997). Allocation of carbohydrates can vary across seasons.

### *D. Variability in Carbohydrate Storage*

Carbohydrates vary across seasons as photosynthesis and plant development change (Wong et al. 2003). When coming out of dormancy, carbohydrates in branches and stems of sugar maples are reduced and partitioned out to the spring flush of growth and development. Carbohydrate levels remain low during the early part of summer as growth and leaves continue to develop (Wong et al. 2003). Developing leaves use sucrose they produce to finish development along with temporarily storing sucrose for short term purposes (Gifford and Evans 1981). Not till later in the summer and early fall are excess carbohydrates available for accumulation. Carbohydrates of sugar maples reach peak levels in the late fall as leaves are senescing (Wong et al. 2003). Carbohydrate

amounts remain constant throughout dormancy until the following spring when they are used for new tissue development.

Carbohydrates levels are higher during late fall except for a short period right before dormancy. During this time carbohydrates are slightly depleted for the onset of cold tolerance (Wong et al. 2003). Some tree species accumulate sugars in stems during the autumn to protect against freezing during cold periods (Kozlowski 1992, Regier et al. 2010). Sugars accumulate in vacuoles decrease formation of intercellular ice and prevent plants from freezing (Kozlowski 1992).

### *E. Source or Sink*

Stored carbohydrates are considered sources as they provide carbohydrates when photosynthesis is not occurring or photosynthesis is not able to supply adequate amounts of carbohydrates. Sinks are areas within plants that require carbohydrates for processes and activities (Kozlowski 1992). Sinks are often used for cell development, storage and respiration. Carbohydrates used in sinks can come from other stored carbohydrates or directly from photosynthetic production. Reversible sinks are collected carbohydrates in stems or branches that can be remobilized as a carbohydrate source when needed by the plant (Kozlowski 1992). Carbohydrates stored within roots or sap wood of trees can act as a reversible sink to supply sugars for bud break.

### *F. Sink Strength*

Sink strength is the ability of a plant to acquire carbohydrates through competition partitioning. Allocation of carbohydrates to a specific plant organ sink is determined by the potential sink strength of the tissue or organ (Kozlowski 1992, Magel et al. 2000). Sink strength is

best determined by calculating the sum of net carbon gain minus carbon loss to respiration (Kozlowski 1992). Growth rate of a tree can determine the sink strength of plant tissue (Kozlowski 1992). Demands of carbohydrate sinks have the ability to regulate photosynthetic supply (Gifford and Evans 1981). Greater sink strengths from plant organs, such as fruit, can reduce leaf growth (Gifford and Evans 1981). However, the demand and characteristic of each sink is dependent on the type of carbohydrate sink such as sucrose, glucose or starch (Gifford and Evans 1981).

### *G. Soil Impact on Carbohydrate Storage*

Soil nutrients may contribute to carbohydrate transport within plants. Studies have found that increases in available phosphate limits carbon transport in roots (Magel et al. 2000). Plants with ample supply of nitrogen allocate carbohydrates to producing enzymes and growth (Magel et al. 2000). As a result, less carbohydrates were allocated to storage and roots did not store as much carbohydrates thus having a negative effect on mycorrhiza (Magel et al. 2000).

## IV. SAP SWEETNESS VARIABILITY

Variability of sap sweetness of maple sap from sampling dates and from tree to tree is commonly known among maple producers and documented by researchers (Wiley 1885, Morse and Wood 1895, Taylor 1956, Larochelle et al. 1997). However, ranking of sap sweetness among trees does not usually vary between sampling dates and seasons (Taylor 1956). Understanding what controls sap sweetness is important for increasing sugar production and reducing energy cost.

### *A. Genetic Influence*

Variability of sap sweetness of trees in a stand with similar site factors presents the idea that sap sweetness is controlled by genetics. Links have been made with ray cell area and sap sweetness as a reason for increases in sap sweetness (Gregory 1977, Morselli et al. 1978). Higher ray cell area is a result of faster growing trees (Gregory 1977) but is also an inherited trait (Morselli et al. 1978, Gregory 1982). Producing a genetically sweeter tree has been considered for many years. Wiley's report on the sugar industry of the United States in 1885 said, "There is every reason to believe that a race of maples yielding a large percentage of sugar could be developed as easily as a race of cows yielding large quantities of butter. Among the maples there may yet be a race of Jerseys" (Wiley 1885, p. 209). Attempts to understand genetic influence have been made through clonally reproducing sugar maples although it has not been as easy as Wiley thought. Published studies of clone reproduction has been limited to bud or scion grafting onto a seed grown rootstock. Sap sweetness among ramets of grafted trees selected for sap sweetness still have high variability (Santamour and Cunningham 1964, Demeritt 1985). Measurements of sap sugar concentration above and below the graft union had a difference in sap sweetness on the same tree (Demeritt 1985). These results showed that rootstock influences sap sweetness collected in the stem and that grafting is not the best way to propagate sweet trees (Santamour and Cunningham 1964, Demeritt 1985).

Seed selection from sweet trees is another possibility for producing trees with sweeter sap. However, maternal seed selection has not proven to be effective as there remains a large sap sweetness variability among propagules (Gabriel 1972, Kriebel 1989 & 1990). Biparental seed selection through crosses in a seed orchard of trees grafted for superior sweetness has proven to

produce offspring of high sap sweetness (Kriebel 1990). Therefore, genetics substantially influences sap sweetness although an efficient way to propagate genetically sweeter trees has not been developed. Rooted cuttings would be the most effective way to mass produce genetically sweeter trees and ensure heritability of traits but an effective way to root sugar maple cuttings is still being developed (Santamour and Cinningham 1964). A study on variability of sap sweetness of sugar maples reproduced through rooted cuttings has not been published.

### *B. Environmental Influence*

Site factors such as soils and exposure to weather patterns have been considered as possible factors affecting sap sweetness. Light is important for sap sweetness as photosynthesis in leaves produce sugars. Sugar maples that are healthier have higher net photosynthesis than unhealthy trees (Liu et al. 1997). Open grown trees with larger canopies typically have sweeter sap than forest-grown trees (Morrow 1955). Larger canopies have sweeter sap as there is more leaf area for photosynthesis and light is allowed to reach lower parts of the tree (Morrow 1955). Diameter of the canopy is more important than height of the canopy in forest-grown trees as light is limited to the top of the canopy (Morrow 1955). It is commonly advised to thin a sugar bush to allow superior sweet trees to increase canopy size and sugar production (Kriebel 1990, Wilmot and Perkins 2004). It would be expected that trees with higher photosynthesis would have sweeter sap but this has not been tested.

Choosing sites with good soil quality has been advised as a management strategy but little has been studied on the influence of soils and nutrients on sap sweetness. Even in 1885 it was advised to choose sites "high in loam" and sites with slate or limestone parent material were

preferable to granite parent material (Wiley 1885). Morrow (1955) recommended choosing sites with "good soils" but did not define good soils and admitted that the influence of soils was unknown. Higher foliar N and Ca has been found to correlate with higher photosynthesis (Ellsworth & Liu 1994). If N and Ca increase photosynthesis it seems logical that higher amounts of N and Ca could increase sugar production through increasing photosynthetic rates. The ability of Ca to increase photosynthesis is not surprising as Ca is used for plant cell integrity and cell wall growth (Jones and Lunt 1967, Ellsworth & Liu 1994, White and Broadley 2003). On the contrary foliar P has been found to be higher in declining sugar maples which may have a negative effect on photosynthesis (Liu et al 1997).

Faster growing trees often have sweeter sap (Taylor 1956, Gregory 1977) and tree growth depends on adequate nutrients (Horsley et al 2002, Juice et al 2006). Temperature has also been found to influence sap sweetness within a season (Marvin 1958, Marvin et al. 1971) which could be affected by slope exposure to sun and wind. Temperature accounts for variability among sampling dates, however, it does not necessarily account for inter tree variability and is not controllable without high cost.

# V. SUGAR MAPLE HEALTH AND DECLINE

Sugar maple decline has been observed the past 50 years across the northeastern and midwestern United States (Horsley et al. 2002, Bailey et al. 2004). Sugar maple decline could be a result of abiotic factors such as late spring frost, freeze and thaw cycles, or nutrient deficiency (Horsley et al. 2002, Bailey et al 2002). Decline of sugar maples could also be a result of biotic factors such as invasive insects, fungus or diseases (Horsley et al. 2002, Bailey et al 2004).

Atmospheric deposition is often considered as the reason for sugar maple decline but decline is most likely a result of multiple factors and can vary by site (Horsley et al. 2002).

### *A. Soil Acidification & Cations*

Soil acidification from atmospheric deposition is a result of inputs of sulfur dioxides, and nitrogen oxides from gaseous emissions (Driscoll et al 2003). These inputs displace base cations (Ca, Mg, K) which causes them to leach from the soil (Driscoll et al. 2003). Sites on granite parent material often do not have enough Ca to buffer a Ca depletion. Sugar maples depend upon Ca and Mg (Horsley et al 2002) and sugar maple health can be predicted by soil nutrients (Bailey et al. 2004). Sugar maples growing on soil with lower Ca and Mg are often not as healthy as those on soils with higher cations (Horsley et al. 2000, Bailey et al. 2004, Schaberg et al. 2006). Reproduction of sugar maples is lower or nonexistent on sites with low Ca (Sullivan et al. 2013). As a result, atmospheric deposition depleting cations essential to sugar maple health is typically implicated as the primary reason for sugar maple decline (Horsley et al. 2002).

### *B. Al & Mn Toxicity*

Sugar maple decline could also be a result of Al or Mn toxicity. Acid deposition causes mobilization of Al and Mn which allows them to enter into plants as part of the soil solution (Driscoll et al. 2003, Kogelmann and Sharpe 2006, Schaberg et al 2006). Manganese and Al have a negative effect on sugar maple health (Kogelmann and Sharpe 2006, Schaberg et al 2006, Long et al. 2009). Foliar Mn decreases photosynthesis in sugar maples as well (Kogelmann and Sharpe 2006).

### *C. Ca Additions*

Calcium additions have been found to increase the health of sugar maples. Additions of lime to forest on the Allegheny Plateau of Pennsylvania increased the health and growth of sugar maples and reduced the negative effect of Al and Mn (Long et al. 1997, Horsley et al. 2002). Growth and fitness of sugar maple trees and seedlings increased in a $CaSiO_2$ watershed addition in the White Mountains of New Hampshire (Juice et al 2006). These Ca additions provided further confirmation that sugar maples respond to increases of Ca. Atmospheric deposition may deplete base cations in the soil which could stress sugar maples allowing them to be more susceptible to insect or disease and increase decline and mortality.

### *D. N & P Influence*

Nitrogen is typically the most limiting nutrient in northern temperate forest (Aber et al., 1998). Compared to other dominant species in the northeastern United States, sugar maples do not have unusually high foliar N concentration but they do have high N mineralization in the soils surrounding them (Lovett & Mitchell 2004). Sites with higher N or additions of N increased growth of sugar maples (Liu et al. 1997, Thomas et al. 2010). Sugar maple leaves with higher foliar N directly correlate with higher photosynthesis (Ellsworth & Liu 1994) as chlorophyll contains high amounts of N. In contrast, addition of ammonium nitrate increased crown dieback and decline of sugar maples while reducing foliar and soil Ca and Mg (Moore and Houle 2013). Phosphorus was limiting to sugar maples on soils sufficient in N and Ca in Ontario (Casson et al. 2012, Gradowski & Thomas 2006). Adding P increased growth of sugar maples in Ontario (Gradowski & Thomas 2008). However, higher foliar P was found on sites with declining sugar maples in northwestern Vermont (Liu et al. 1997).

# VI. NUTRIENT ADDITION STUDIES TO INCREASE SAP SWEETNESS

Increasing sap sweetness through fertilizing has been attempted a few times with mixed results (Yawney & Walters 1973). One of the first documented fertilizer trials for increasing sap sweetness found that adding wood-ash and plaster increased sugar production on soils in Vermont (Wiley 1885). Fertilizer trials for sap sweetness usually add a combination of nutrients which limit the ability to identify effects of specific nutrients. LaValley (1969) fertilized with two applications of 20-10-10 (percent N,P,K) and three applications of 16-16-16 fertilizer for a total of 1000 lb of fertilizer to an acre on soils with sufficient N in the Adirondack Mountains of New York. Sap sweetness increased 11% two years post treatment and maintained this sweetness for at least five years post treatment. The sweeter sap increased production of syrup by 23% for every tap. Kriebel (1961) increased sap sweetness two years after fertilizing two stands with 600 lb per acre of ammonium nitrate in Ohio. Addition of NPK and NP increased sap volume yield but NPK was the only treatment able to increase sap sweetness and fertilizing with NK or NCa did not increase sap sweetness in Ontario (Leech & Kim 1990). Only plants deficient in nutrients may increase sap sweetness after a nutrient addition. A fertilization trial of KCaMg was the best for increasing the sap sweetness in northern Vermont (Wilmot and Perkins 2004) which could have been a result of decreased cations to acid deposition.

Fertilizing a sugar bush does not always increase sap sweetness. Sap sweetness decreased in trees severely damaged by an ice storm in Ontario and the addition of lime, PK, or lime and PK did not increase sap sweetness (Noland et al. 2006). Wilmot et al. (1995) fertilized stands in Vermont with a mix of CaKMg, lime plus CaKMg, and a commercial Maple Gro fertilizer of NPK

but did not see a change in sugar concentration. Fertilizer trials for sap sweetness can also decrease sap sugar concentration. Addition of P decreased sugar yield while N and K applied separately had no affect on sap sweetness in central New York (Watterston et al. 1963). Addition of NPK was able to increase sugar yield the second year following treatment but did not make up for the large decrease in sap sweetness the first season (Watterston et al. 1963). Addition of N, NK, NMg, or CaMgK in healthy stands of sugar maples in Ontario decreased total sugar production (sap volume x sugar concentration) the first year after fertilizing and slightly increased the second year, but sap sweetness did not change (Bary & Roy 1998). When the same nutrients were added to declining maple stands, sap sweetness was increased (Bary & Robichaud 1994).The potential to increase sap sweetness through fertilizing is still possible although the proper nutrient to add may depend on the stand.

# Chapter 2

## I. INTRODUCTION

Production of maple syrup is a long-standing tradition of economic important in the northeastern United States and southeastern Canada. Maple sugar, a non-timber forest product, is produced by boiling sap collected from sugar maple trees (*Acer saccharum* Marsh.). Sap collected from maple trees is primarily water with sugar concentration of 1-5% (Gregory and Hawley 1983, Larochelle et al 1997). The amount of syrup produced from maple sap is determined by the sugar concentration of the sap. Gallons of sap required to produce a gallon of syrup can be calculated by dividing 86 by the percent sugar of the sap (Taylor 1956, Jones 1967). Increasing sap sweetness directly affects the amount of time and energy required to boil the sap into syrup (LaValley 1969).

Sugar bush management is suggested as the best way to increase sap sweetness (Morrow 1955, Wilmot & Perkins 2004). Thinning of a sugar bush is the most common management recommendation (Kriebel 1961, Kriebel 1990, Laing & Howard 1990, Wilmont & Perkins 2004). In addition to thinning, selecting sites with favorable soil qualities for a sugar bus is recommended but the impact of soil quality on sap sweetness is limited (Morrow 1955). Further understanding of how soil nutrients affect sap sweetness will inform sugar bush managers of ideal soil qualities to look for when selecting a sugar bush (Morrow 1955) or whether nutrients need to be added.

Photosynthesis produces sugars within maples (Noland et al. 2006). Sugars produced by photosynthesis are stored within ray cells and roots as starch (Marvin et al. 1971, Gregory 1983, Liu et al. 1997). During late fall and early winter, starches are converted into sucrose and flow through the xylem where sap is collected for syrup production in the spring (Marvin et al. 1971,

Gregory 1983, Liu et al. 1997, Wong et al. 2003). Healthier trees with larger canopies and greater growth rates have higher sugar concentration (Morrow 1955, Taylor 1956, Blum 1973, Laing & Howard 1990). Faster growing trees is especially important as it creates larger and higher quantities of ray cells for storing sugar and increasing sap sweetness (Gregory 1977, Morselli et al. 1978).

Sugar maple decline has been observed the past 50 years in forest of the northeastern United States (Horsley et al. 2002, Bailey et al. 2004). Sugar maple health can be predicted by available soil nutrients and nutrient deficiency can be implicated as a reason for sugar maple decline (Bailey et al. 2004). Nutrient deficiency in sugar maple health is a result of base cation displacement and leaching from acid deposition (Horsley et al. 2002, Driscoll et al. 2003). Sites with lower available soil calcium (Ca) and magnesium (Mg) support fewer healthy sugar maples (Horsley et al. 2000, Bailey et al. 2004, Schaberg et al. 2006, Sullivan et al. 2013). Adding Ca to declining stands has increased the health, growth and fitness of trees and seedlings (Long et al. 1997, Horsley et al. 2002, Juice et al 2006). Addition of Ca also mediates the toxicity effects aluminum (Al) and manganese (Mn) have on sugar maples (Schaberg et al 2006, Long et al. 2009). It has not yet been shown that adding Ca without K or Mg could increases sap sweetness.

Soil nitrogen (N) mineralization is higher around sugar maples than other hardwood species (Lovett & Mitchell 2004). Sites with higher N exhibit higher growth rates of sugar maples (Thomas et al. 2010) and healthier canopies (Liu et al. 1997). Higher photosynthetic potential of sugar maples correlate with higher foliar N (Ellsworth & Liu 1994) suggesting that adding N could increase sap sweetness. On the other hand, excessive soil N can cause canopy dieback and reduced foliar Ca (Aber et al. 1998, Moore & Houle 2013). In the past century, N deposition has increased N in terrestrial ecosystems in the northeastern United States (Aber et al. 1998, Driscoll et al. 2003,

Thomas et al. 2010). Foilar P was the most limiting nutrient by DRIS analysis on acidic sites in Ontario with sufficient N (Casson et al. 2012) and soil P explained 74% of variation in sugar maple growth (Gradowski & Thomas 2006). However, compared to healthy trees, sites with declining sugar maples in northwestern Vermont have higher foliar P (Liu et al 1997).

Because of its economic importance, there have been several sugar bush fertilization trials but it has not yet been shown what specific nutrients affect the sugar concentration of maple sap as fertilization trials to increasing sap sweetness have produced mixed results (Yawney & Walters 1973). A majority of fertilizer addition studies have added a combination of nutrients which limits our ability to identify effects of specific nutrients. On soils sufficient in N, complete fertilizer additions of NPK increased sap sweetness in the Adirondacks (LaValley 1969) and NPK increased sugar yield on co-dominant trees in southern Ontario (Leech & Kim 1990). Addition of NPK decreased and increased sap sweetness in different years in central New York (Watterston et al. 1963) or had no affect on sap sweetness in northern Vermont (Wilmot et al. 1995). Calcium, potassium (K) and Mg increased sap sweetness in northern Vermont (Wilmot and Perkins 2004) but had no affect with healthy sugar maples in northwestern New Brunswick (Bary & Roy 1998). Combinations of N with K, P, Ca, or Mg did not increase sap sweetness in Ontario or New Brunswick (Leech & Kim 1990, Bary & Roy 1998, Noland et al. 2006). Adding N by itself increased sap sweetness in Ohio (Kriebel 1961) but had no affect on sugar concentration of sap in central New York and healthy trees in New Brunswick (Watterston et al. 1963, Bary & Roy 1998). Adding P by itself decreased sap sweetness of trees in central New York (Watterston et al. 1963). These studies show mixed results and do not provide a conclusive answer for what nutrients control sap sweetness.

The objective of this study was to determine which nutrients affect the sugar concentration of maple sap. This study tests the effect of N, P, N and P, and Ca on sap sweetness through separate fertilizer applications. Site characteristics of soil, sap and foliar nutrient concentrations, growth rates, canopy health, and photosynthesis were measured and correlated to sap sweetness to determine whether nutrient concentrations or site factors affect sap sweetness. I predicted that soil and foliar Ca and N would be limiting nutrients for sap sweetness and that Ca would increase sap sweetness.

## II. METHODS

### *A. Site Description*

Forest stands for this study are located in the White Mountains of New Hampshire, United States. Three sites were located in the Bartlett Experimental Forest and two sites were located at Jeffers Brook on the western edge of the White Mountain National Forest (Table 1). Soils at both sites are Spodosols developed in glacial till. The bedrock at Bartlett is granitic while the bedrock at Jeffers Brook is metamorphic. Temperature in the White Mountains can reach over 30°C in the summer and winter temperature can reach -35°C with an average annual temperature of 5.5°C. Average annual precipitation in the Bartlett Experimental Forest is 127 cm with snow often accumulating 150-180 cm.

Stands varied in age, dating 28-130 years since harvest (Table 1). Average basal area for mature stands was 33.9 ($m^2$/ha) and the average basal area for the two mid-aged stands was 31.0 ($m^2$/ha). Overstory vegetation at Bartlett is primarily sugar maple, yellow birch (*Betula alleghaniensis* Britton), and American beech (*Fagus grandifolia* Ehrh.) with occasional white ash (*Fraxinus americana* L.) and red maple (*Acer rubrum* L.). Vegetation of the older stand at Jeffers

Brook is primarily sugar maple and yellow birch while vegetation of the younger stand at Jeffers Brook was a mix of sugar maple, yellow birch, and pin cherry (*Prunus pennsylvanica* L.f.) None of the stands had ever been thinned or tapped for maple sap.

The study was part of the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) study. Stands contained treatment plots for addition of Ca, N, P, N and P, and a control. Calcium was applied once in the fall of 2011 as $CaSiO_3$ at the rate of 1150 kg Ca ha and N and P were applied twice a year in 2011, 2012 and 2013. Nitrogen was applied as $NH_4NO_3$ at the rate of 30 kg N ha/yr, and P was applied as $NaH_2PO_4$ at the rate of 10 kg P ha/yr.

Treatment plots were 50x50 m and trees were selected for sampling from the inner 30x30 m of the plot leaving a 10 m buffer with the exception of the younger stand at Jeffers Brook where the plots were 30x30 m with a 5 m buffer. Both stands at Jeffers Brook and Bartlett C8-mautre had all five treatments while stand C9 mature at Bartlett did not have a Ca plot. Stand C6 mid at Bartlett had all treatments plots but only the Ca plot with control trees chosen around the outside of the plot, at least 15 m from the plot edge, were sampled as there was not enough sugar maples in the other treatment plots. All sugar maples with a diameter at breast height (1.37 m) of 10 cm or greater were selected (10-18 trees per plot) with the exception of the older stand in Jeffers Brook where 25 trees were chosen from the inventory list using a random number generator because there was high abundance of sugar maples.

### *B. Maple Sap Sampling*

Sap flow during the tapping season requires freezing nights followed by thawing daytime temperatures (Edson 1910, Tyree 1983). Each site was sampled three times except for stand C8 mature at Bartlett, which was sampled four times. All site at Bartlett were sampled on the same day

and sites at Jeffers Brook were sampled either the following day or the day before Bartlett was sampled. The two back-to-back sampling days were considered one sampling period. Sampling periods were February 24 (Bartlett C8 mature only), March 10th and 11th, March 24th and 25th, and March 30th and 31st. This time period covered the majority of the sap flow season during 2013. Each sample from a tree was taken at least one week apart from another and there were days in between when the temperature remained below freezing and sap did not run. The order that sites and plots were sampled was switched at every sampling date to distribute what time of the day sap was sampled.

Sap was sampled from each tree using a mini-tapping procedure (Gabriel 1982). A 2-mm diameter hole was drilled approximately 1 cm into the south side of the tree approximately 1.3 m above the ground. A 16-gauge syringe needle was then inserted into the tree as a sap spile. When sampling, a plastic 50 ml test tube (with a hole drilled into the side of the test tube) was hung on the end of the syringe needle to collect sap. If rain or snow was falling, the top of the test tube was covered to prevent dilution. After the test tubes had been hanging on the tree for at least 15 minutes and at least 1.5 ml of sap was collected in the test tube, sap was measured with a MISCO PA201 digital temperature-compensating refractometer (MISCO, Cleveland OH) providing the sugar concentration of the sap (Gregory 1983) on a Brix scale. The refractometer was calibrated with de-ionized water prior to sampling and after sampling every ten trees. The prism of the refractometer was cleaned after every sample with isopropyl alcohol and dried with a tissue wipe.

A composite sap sample from each plot was collected by gathering approximately 2 ml of sap from each tree and freezing immediately upon returning from the field. Sap samples were later filtered through #1 Whatman filter paper to remove debris and two drops of nitric acid ($HNO_3$) were added for stabilization. The sap samples were then analyzed by inductively coupled plasma-

optical emission spectrometry (ICP-OES) using a PerkinElmer Optima 3300 DV (PerkinElmer inc., Waltham MA) for the concentration of P, Ca, Mg, K, and Mn.

### *C. Soil Nutrients*

Soil nutrients were measured in late June 2009 as reported by Fisk et al. (2014). The Oi (litter layer) was removed before approximately 30 soil cores that were 2 cm diameter and 10 cm deep were sampled in each plot. Samples were taken from four plots at each site in late June 2009. Soil nutrients were not available for the plot sampled at Bartlett C6 mid. Each core was separated into Oe, Oa, and mineral soil. Mineral soil included the E horizon if present. Soil cores, separated by horizon, were composited from each plot.

Nitrogen mineralization rate was determined by 21 day laboratory incubations (Fisk et al., 2014). Soil P was analyzed by extracting with pH 8.5 bicarbonate (DeForest and Scott 2010). Exchangeable soil Ca was determined by extracting with $NH_4Cl$ (Naples & Fisk, 2010). Extractable soil Ca and P concentrations and N mineralization rates were averaged across horizons for a nutrient characteristic of each plot.

### *D. Foliage Collection and Gas Exchange Measurements*

Foliar nutrients and gas exchange were measured for the two trees with the highest average sugar concentration and the two trees with the lowest average sugar concentration in each plot. Trees were chosen randomly if there were more than two trees with the same sap sweetness. At least 15 sun-exposed leaves from the upper canopy were collected from each tree using a shotgun. Only leaves free from shot holes, disease and insect herbivory were used. Foliage was sampled between 10 am and 2 pm on sunny days to ensure the leaves were dry and photosynthesizing. The

removed branches were immediately placed in water to sustain transpiration for gas exchange measurements.

A single healthy leaf from each tree was sampled for potential photosynthesis and conductivity while it was still attached to the branch within a few minutes of being shot down (Schaberg et al. 1997). Gas exchange measurements were taken using an LI 6400 (LI-COR Inc., Lincoln, NE) by shining an LED light in a sealed chamber of known $CO_2$ and $H_2O$ (Schaberg et al.1997). Ten measurements were taken for each leaf and averaged for potential photosynthesis and conductivity.

### *E. Foliage Nutrients*

After gas exchange measurements, leaves were dried at 65$^{o}$C for 24 hours and ground using a Wiley Mill 40-mesh screen (Thomas Scientific, Swedesboro, NJ). Foliar P, Ca, Mg, K and Mn concentrations were determined by ashing ~0.25 g of sample in a muffle furnace at 470 °C overnight, digesting in 6N $HNO_3$ on a hot plate for 20 minutes, and filtering the digested solution through Whatman #42 filter paper and rinsing with de-ionized water (Wilde 1979). The digested solution was analyzed by ICP-OES for P, Ca, Mg, K, and Mn concentrations. Nitrogen was analyzed by combusting ~2.5 mg of sample with a C and N elemental analyzer (Flash EA 1112 series, CE Elantech Inc., Lakewood, NJ).

### *F. Tree Health and Growth*

Canopy health of all sampled trees was assessed using the procedure developed by the North American Maple Project (Miller et al. 1991). Two individuals were trained and certified through the Vermont Department of Forest, Parks and Recreation to perform the assessment by visualizing a tree from two different angles. The assessment rated the tree on characteristics of

overall tree vigor, percent dieback, canopy transparency, live crown ratio, and the overall density of the canopy. In addition to assessing canopy health, growth was determined by measuring DBH of each tree in 2008, 2011 and 2013. The growth rate for each tree was fit by regression to the three observations of diameter for growth rate by year.

### *G. Data Analyses*

In total, 314 trees were tapped with 298 providing sap for sampling. Sugar concentration samples from each date were averaged from individual trees for a single sap sweetness characteristic for each tree. Foliar nutrient concentrations, photosynthesis, and stomata conductivity of individual trees were correlated with the average sugar concentration from the same tree for a total of 84 experimental units. A sample for foliar N leaked during analysis and was not usable. Individual tree growth measurements, stem diameter, and canopy assessment was also correlated with individual sap sweetness from all 298 trees. Previous diameter measurements were not available for all trees therefore growth measurements could only be calculated for 256 trees.

Soil nutrient characteristics were available for individual plots therefore the average sap sweetness of each tree in a plot were averaged for the average sap sweetness of each plot. Average sap sweetness of each plot was correlated with soil Ca, P, and N mineralization from 16 plots. The 16 plots represented four of the stands with four replicate plots at each stand. Sap nutrient concentrations of each plot were averaged across the season and correlated with average sap sweetness of all of the 21 plots sampled from five stands. An alpha level of 0.10 ($\alpha \leq 0.10$) and an r-value of 0.25 or greater ($r \geq 0.25$) was considered as criteria for testing significance for the correlations.

Test for a nutrient treatment effect on sap sweetness was performed by comparing plot means of sap sweetness through randomized incomplete block design analysis of variance. To adjust for nonhomogeneous differences between plots, pretreatment soil N mineralization was used as a concomitant in an analysis of covariance. Criteria for including a pretreatment covariate required variables to be correlated with sap sugar concentration with at least an r-value of 0.30 ($r \geq 0.30$) and an alpha value of 0.10 or less ($\alpha \leq 0.10$) (Cochran 1957). Pretreatment soil N mineralization was the only value that fit this criteria. A Dunnett's test was used to test for significance between treatments. Calcium addition plots and Bartlett C6-mid was not used for analysis of covariance as pretreatment soil N mineralization was not available. Therefore, analysis of covariance was performed as a randomized complete block design on four treatments in four stands. For both analysis of variance and analysis of covariance stand was considered as a blocking variable. An alpha level of 0.10 ($\alpha$=0.10) was used as the critical value for testing significance of treatment response. Analysis of variance and analysis of covariance were tested using a Proc GLM in SAS version 8 (SAS Institute Inc., Cary, NC). Correlations were also tested using SAS. Figures were produced using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA) and Minitab 16 (Minitab Inc., State College, PA).

## III. RESULTS

### *A. Soil and Foliar Nutrients*

Correlation results of sap sweetness are presented in table 2. Trees in sites with higher soil N mineralization had higher sugar concentration in the sap ($p= 0.007$, $r = 0.65$ Figure 1). Although it was expected that higher soil Ca would correlate with higher sugar concentration this did not

hold true (p=0.83, r = -0.06, Figure 1). Soil P did not have a significant relationship with sugar concentration either (p=0.42, r = -0.22, Figure 1).

Although there was a strong relationship with soil N, foliar N was not related to sap sweetness (p=0.14, r = 0.16). Sap sweetness was lower when foliar P was higher (p=0.02, r=-0.25, Figure 1). As with soil Ca there was no correlation between sap sweetness and foliar Ca (p=0.79, r=-0.03, Figure 1). Trees with higher foliar N and lower foliar P had sweeter sap as shown by the ratio of foliar N:P (p<0.001, r=0.38, Figure 2).

Comparison of foliar nutrients to published nutrient concentrations for healthy sugar maples (Kolb and McCormick 1993, Vizcayno-Soto and Côté 2004) showed that 76% of the trees had sufficient foliar Ca concentration while 24% were deficient in Ca (Figure 1). 35% of the trees were deficient in N while all of the trees had sufficient P (Figure 1). The trees were the most deficient in Mg as 61% of the trees were below the Mg threshold although Mg did not correlate with sap sweetness. Only 8 % of the trees were deficient in K. A minimum Mn deficiency threshold does not apply to Mn as too much can become toxic to sugar maples. All of the trees were below the Mn toxicity threshold.

### *B. Fertilizer Treatment Effect*

Treatment had no affect on sap sweetness when all treatments plots were analyzed and pretreatment soil nutrients were not considered (p=0.21, Figure3, Table 3). A balanced design of only N, P, N and P, and control plots also had no affect on sap sweetness (p=0.23, Table 3). Addition of N increased sap sweetness when pretreatment soil N mineralization was used as a concomitant to adjust for differences between plots in an analysis of covariance (p=0.09, Table 3). Sap sweetness in N-addition plots was 0.25% higher than control plots (Table 3). Phosphorus or

the addition of N and P together did not increase sap sweetness (Table 3). Similarly, Ca addition plots did not have higher sap sugar concentration (Table 3).

### *C. Growth & Canopy Health Assessment*

Trees with greater diameter growth rates or greater basal area growth had no correlation with sap sweetness (Table 2). There was also no correlation with sap sugar concentration and stem diameter ($p=0.997$, Table 2). Measures of canopy health did not predict sap sweetness (Table 2).

### *D. Gas Exchange*

Trees with higher potential photosynthesis in the leaves was not considered to have higher sugar concentration in the sap ($p=0.04$, $r=0.23$, Table 2). Conductivity of the leaves did not correlate with sugar concentration ($p=0.57$, $r=-0.06$, Table 2). Foliar N correlated positively with higher photosynthetic potential ($p<0.001$, $r=0.40$, Figure 5) and conductivity ($p=0.02$, $r=0.26$).

### *E. Sap Nutrients*

Sap nutrients varied widely from plot to plot and across the season with each plot varying considerably. Average sap nutrient concentrations did not correlate with average sap sugar concentration (Table 2). Nutrient concentrations of sap does not predict sap sweetness and is not consistent at different sampling dates (data not shown).

## IV. DISCUSSION

### *A. Soil and Foliar Nutrient Effect on Sap Sweetness*

Results of the study point toward N as the limiting nutrient for sap sweetness. Soil N mineralization correlated with higher sap sweetness and N addition increased sap sweetness (Figure 1, Table 2). In addition, trees with higher foliar N:P had sweeter sap. The importance of N

to sweeter sap can be understood through the effect N has on photosynthesis. Although the correlation of sap sweetness and potential photosynthesis was not considered to be statistically significant the trend does suggest that higher photosynthesis increases sap sweetness. Higher foliar N increased the rate of photosynthesis (Figure 4) as would be expected (Ellsworth and Liu 1994, Liu et al. 1997) which presumably allowed for higher production of sugars.

A decrease in sap sweetness when foliar P is higher was surprising (Figure 1 and 2). Understanding of why higher P was correlated with lower sap sweetness is unknown although the negative effect of P could be a result of lower N when P is higher. Foilar P was the most limiting nutrient by DRIS analysis on acidic sites in Ontario with sufficient N (Casson et al. 2012) and soil P explained 74% of variation in sugar maple growth (Gradowski & Thomas 2006). However, compared to healthy trees, foliar P was higher on declining sugar maples (Liu et al. 1997) and addition of P to sugar maple stands decreased the sugar concentration of maple sap (Watterston et al. 1963). Trees with higher foliar N:P having sweeter sap also suggests that P could have a negative effect on sap sweetness.

It was surprising that soil or foliar Ca did not correlate with sweeter sap (Figures 1). Higher Ca abundance is known to increase the health of sugar maples (Horsley et al 2002, Schaberg et al 2006, Sullivan 2013) and healthier trees have higher sap sugar concentration (Noland et al. 2006). The most likely reason for Ca not increasing sap sweetness is because only 24% of the trees were in the deficiency range for foliar Ca concentrations (Figure 1). It could be that the trees in this study are the most deficient in N and have enough Ca. Comparison of foliar N concentrations with deficiency thresholds revealed that 35% of the trees were in the deficiency range for N (Figure 1). Measurements of canopy health confirms that a majority of the trees were healthy.

### *B. Fertilizer Treatment Effect*

Nitrogen was the only nutrient that significantly increased sap sweetness when plots were neutralized with pretreatment soil N mineralization (Table 3). Consistent with soil N mineralization correlating with higher sap sweetness, addition of N to the soil allowed trees to increase in sap sweetness. The N addition most likely contributed to increasing photosynthesis (Figure 4), which allowed for an increase in sugar production. Increasing sap sweetness following N fertilization was also reported by Kriebel (1961) but in contrast, N fertilization trials have not increased sap sweetness (Watterston et al. 1963, Bary & Roy 1998). On average, addition of N increased sap sugar concentration 0.25% over control trees and eliminated 4 gallons of sap required in the production of 1 gallon of syrup. The application rate of N was rather small and future sampling is needed to determine whether adding more N will further increase sap sweetness or whether the current application rate will continue to increase sap sweetness.

Phosphorus could have a negative effect on N as the addition of N and P was unable to increase sap sweetness. In a fertilization trial P was reported to decrease sap sweetness (Watterston et al. 1963). It could also be that the nutrients are co-limited as found in a separate study in our stands (Fisk et al. 2014).

Calcium was applied at a much higher application rate than N or P therefore it is surprising that there was not a treatment response in the foliage. Foliage of sugar maples have taken up silicate in the $CaSiO_3$ addition (data not shown). Calcium could be allocated to shoot growth instead of increasing sap sweetness. More time is needed to determine whether a Ca treatment response to increase sap sweetness will develop.

Most sap sweetness fertilizer addition trials only monitor for two years after treatment (Kriebel 1961,Watterston et al. 1963, Bary & Roy 1998) with a maximum of five years (LaValley 1969). A decrease in sap sweetness has been noticed the first year after fertilizer addition (Watterston et al. 1963, Bary & Roy 1998). A decrease in sap sweetness could be a result of allocating additional nutrients to shoot and root growth. An increase in roots and shoots would then allow for higher sugar production and storage in years following. Prolonged sampling is needed to further understand the affect fertilization has on sap sweetness.

### *C. Sap Nutrients*

It is known that sap nutrient concentrations vary within trees (McCormick 1997, Perkins & van den Berg 2009) and can vary between sampling dates (McCormick 1997, Leaf and Watterson 1964). The variation between sampling dates in the tapping season is most likely a phenological response of warming weather and timing of bud break (McCormick 1997). Nitrogen concentrations in sap can increase throughout the tapping season and could be the reason why sap has a buddy flavor later in the season (Holgate 1950, Leaf and Watterson 1964). Calcium and Mg have been found to increase throughout the tapping season and then quickly decrease at the end while P and K increase through the first half of the season, decrease the second half, and then quickly increase right before bud break (Leaf and Watterson 1964). Due to the substantial variation I noticed from compositing sap in a plot, it best to measure sap nutrient concentrations of individual trees.

### *D. Tree Health and Growth*

None of the measurements of canopy health correlated with sap sweetness. This was surprising as previous studies have shown that healthier canopies typically have sweeter sap

(Taylor 1956, Blum 1973). Wilmot and Brett (1995) were also surprised not to find a correlation between canopy dieback and sugar yield. However, there was little variation in tree health across our stands.

Live crown ratio was expected to affect sap sweetness as canopy influences sap sweetness (Morrow 1955, Taylor 1956). However, crown diameter, is probably more important than the height of the crown because sun does not reach lower parts of the tree in a closed canopy forest (Morrow 1955).

## V. CONCLUSION

Proper management of a sugar bush through thinning and selection of genetically superior trees may be more beneficial than fertilizing (Kriebel 1990, Wilmot & Perkins 2004, Perkins & van den Berg 2009). Selecting sites for a sugar bush is a possibility for maximizing sap sweetness. By choosing sites with higher N, maple producers can collect sweeter sap which reduces energy needed to boil sap into syrup. Stands that are lower in N may benefit by an addition of N. Fertilizing with 30 kg N ha/yr increased sap sweetness 25%. Prior to fertilizing a sugar bush it is important to assess the nutrients of the stand to determine deficiencies (Wilmot & Perkins 2004). Adding too much N could be detrimental to the health of sugar maples (Moore & Houle 2013). Sites with high P could have a negative effect on sap sweetness and addition of P with N was not able to increase sap sweetness although N alone increased sap sweetness. Adding Ca in our study did not increase sap sweetness, at least within two years of treatment. Further study of individual nutrients and sap sweetness is needed for understanding the affect site has on sugar concentration but results of this study show that N is the most important nutrient for explaining sap sweetness.

# 3. TABLES

Table 1. Site Description of all five stands in the Bartlett Experimental Forest and Jeffers Brook.

| Site | Stand | Treatments | Year of Harvest | Coordinates | Elevation (m) | Slope | Slope Aspect | BA ($m^2$/ha) | BA of Sugar Maple ($m^2$/ha) | DBH of Tapped Trees (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Bartlett | C8 Mature | Control, N, P, NP, Ca | 1883 | 44° 03' N 71° 18' W | 330 | 5-35% | NE | 34.7 | 14.1 | 34.9 |
| | C9 Mature | Control, N, P, NP | 1890 | 44° 03' N 71° 17' W | 440 | 10-45% | NE | 32.7 | 17.1 | 29.3 |
| | C6 Mid | Control, Ca | 1975 | 44° 02' N 71° 16' W | 460 | 13-20% | NNW | 34.6 | 1.2 | 18.3 |
| Jeffers Brook | Mature | Control, N, P, NP, Ca | ~1900 | 44° 03' N 71° 88' W | 730 | 30-40% | WNW | 34.2 | 27.0 | 27.0 |
| | Mid | Control, N, P, NP, Ca | 1985 | 44° 03' N 71° 88' W | 730 | 25-35% | WNW | 27.3 | 3.5 | 13.7 |

Table 2. Correlation of sap sugar concentration with soil, foliar, growth, photosynthesis, growth, and canopy health measurements.

| Variable | Units | df | r-value | p-value |
|---|---|---|---|---|
| Soils | | | | |
| Extractable soil Ca | ug/g | 16 | -0.06 | 0.83 |
| Soil N mineralization | ug/g | 16 | 0.65 | 0.007 |
| Extractable soil P | ug/g | 16 | -0.22 | 0.42 |
| Foliage | | | | |
| Foliar Ca | mg/g | 84 | -0.03 | 0.79 |
| Foliar N | mg/g | 83 | 0.16 | 0.14 |
| Foliar P | mg/g | 84 | -0.25 | 0.02 |
| Foliar N:P | mg/g | 83 | 0.38 | <0.001 |
| Foliar Mg | mg/g | 84 | -0.11 | 0.32 |
| Foliar Mn | mg/g | 84 | -0.17 | 0.12 |
| Foliar K | mg/g | 84 | -0.22 | 0.05 |
| Potential photosynthesis | $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ | 84 | 0.23 | 0.04 |
| Stomata conductivity | $mol\ H_2O\ m^{-2}\ s^{-1}$ | 84 | -0.06 | 0.57 |
| Stem Growth | | | | |
| Stem Diameter | cm | 298 | <0.001 | 0.997 |
| Diameter growth | cm | 256 | 0.12 | 0.06 |
| Relative diameter growth | cm | 256 | 0.08 | 0.19 |
| Basal area growth | $cm^2$ | 256 | 0.08 | 0.2 |
| Sap Nutrients | | | | |
| Sap Ca concentration | mg/L | 21 | 0.28 | 0.22 |
| Sap P concentration | mg/L | 21 | -0.29 | 0.19 |
| Sap Mg concentration | mg/L | 21 | 0.08 | 0.72 |
| Sap Mn concentration | mg/L | 21 | 0.08 | 0.73 |
| Sap K concentration | mg/L | 21 | 0.24 | 0.29 |
| Sap Al concentration | mg/L | 21 | 0.34 | 0.14 |
| Canopy Health | | | | |
| Vigor | rating scale | 298 | 0.03 | 0.61 |
| Crown Dieback | rating scale | 298 | 0.02 | 0.77 |
| Transpiration | rating scale | 298 | -0.05 | 0.41 |
| Live Crown Ratio | rating scale | 298 | -0.02 | 0.70 |
| Crown Density | rating scale | 298 | -0.03 | 0.65 |

Table 3. Analysis of variance sources of variability, p-values, and LS means for the treatment effects on sap sweetness. Model 1 represents a randomized incomplete block design ANOVA with all five treatments, control, N, P, N and P, and Ca. Model 2 is a balanced ANOVA with four treatments of control, N, P, and N and P. Model 3 is a balanced ANCOVA using pre-treatment soil N mineralization as a covariate.

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| Source | df | p-value | df | p-value | df | p-value |
| Stand | 4 | <0.001 | 3 | 0.005 | 3 | 0.12 |
| Soil N mineralization | | | | | 1 | 0.11 |
| Treatment | 4 | 0.21 | 3 | 0.23 | 3 | 0.09 |
| Error | 12 | | 12 | | 8 | |

| LS Means & Error* | Model 1 LS Means | Model 2 LS Means | Model 3 LS Means** |
|---|---|---|---|
| Control | 2.31 (0.066) | 2.24 (0.081) | 2.27 (0.075) b |
| N | 2.56 (0.078) | 2.47 (0.081) | 2.52 (0.077) a |
| P | 2.38 (0.078) | 2.29 (0.081) | 2.26 (0.074) b |
| NP | 2.35 (0.078) | 2.26 (0.081) | 2.21 (0.078) b |
| Ca | 2.34 (0.076) | | |

*Standard error is presented in parentheses
** Values sharing different letters are statistically significant from one another

# 4. Figures

Figure 1. (A) Sap sweetness as a function of exchangeable soil Ca, potential N mineralization, and extractable P. Plots with higher potential N mineralization had trees with sweeter sap. (B) Sap sweetness as a function of foliar Ca, N, P, by tree. The vertical dashed line represents the threshold for nutrient deficiency (Kolb & McCormick 1993).

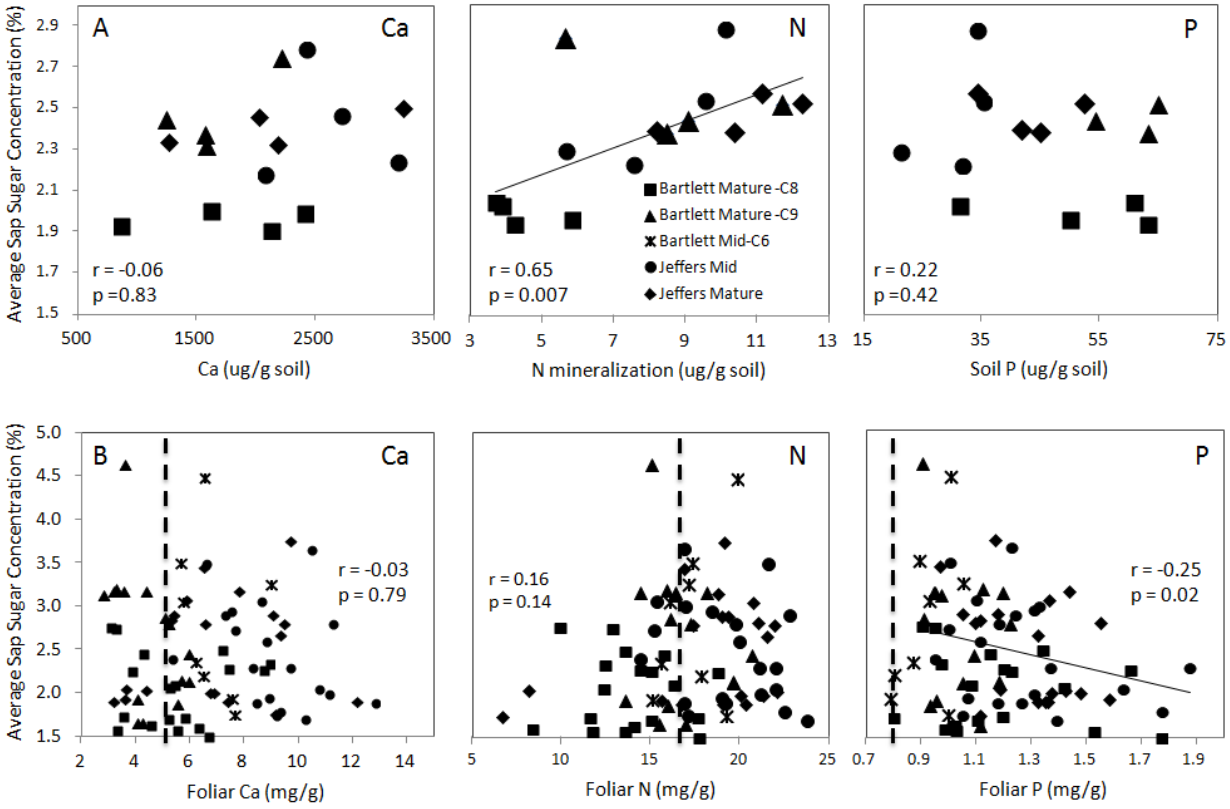


Figure 2. Trees with a higher ratio of N:P had sweeter sap ($p<0.001$).

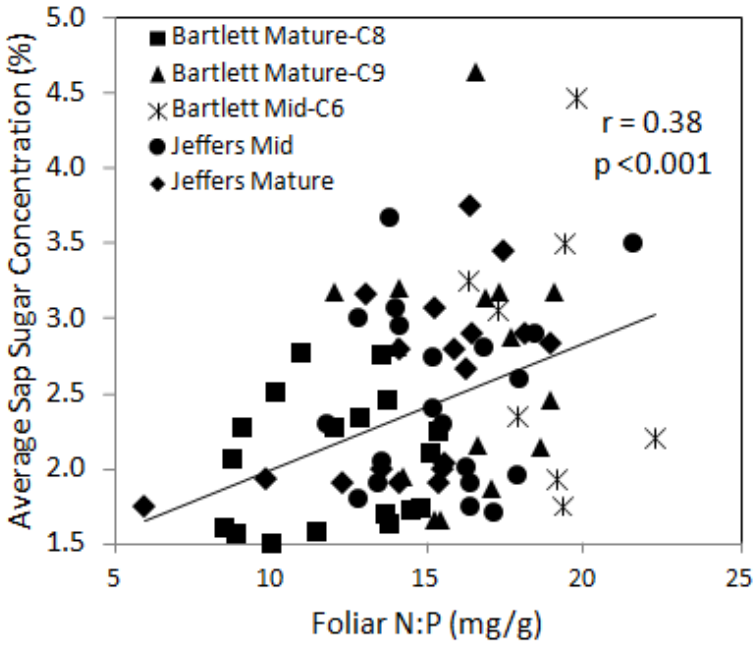


Figure 3. Sap Sweetness two years after addition of N (30 kg/ha/yr), P (10 kg/ha/yr), N (30 kg/ha/yr) and P (10 kg/ha/yr), and Ca (1150 kg/ha). The control is represented by "C."

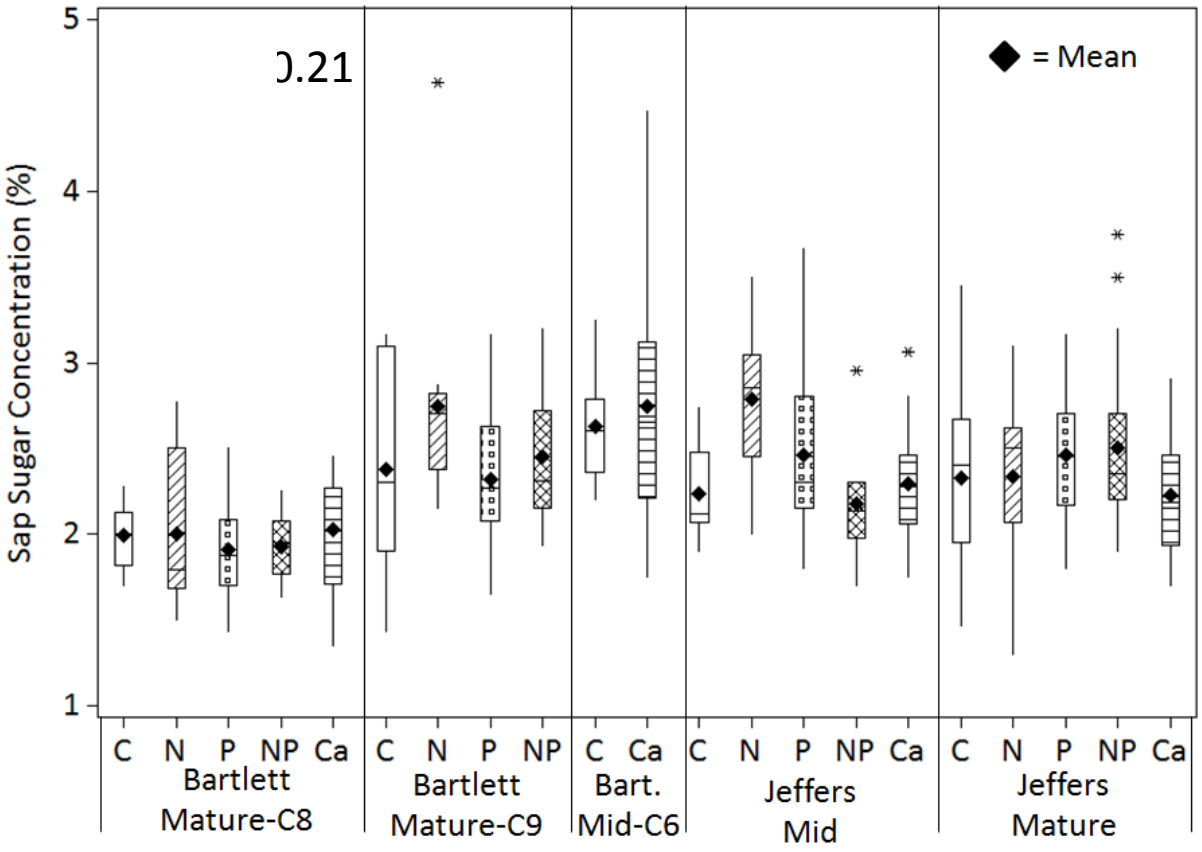
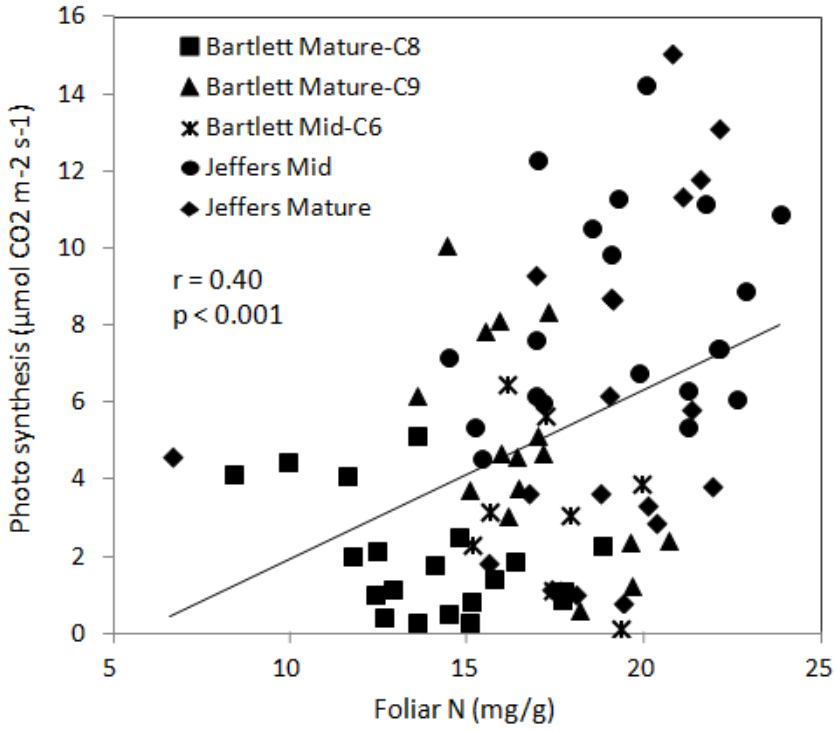


Figure 4. Leaves with higher N concentration rates had higher photosynthesis (p=0.007).



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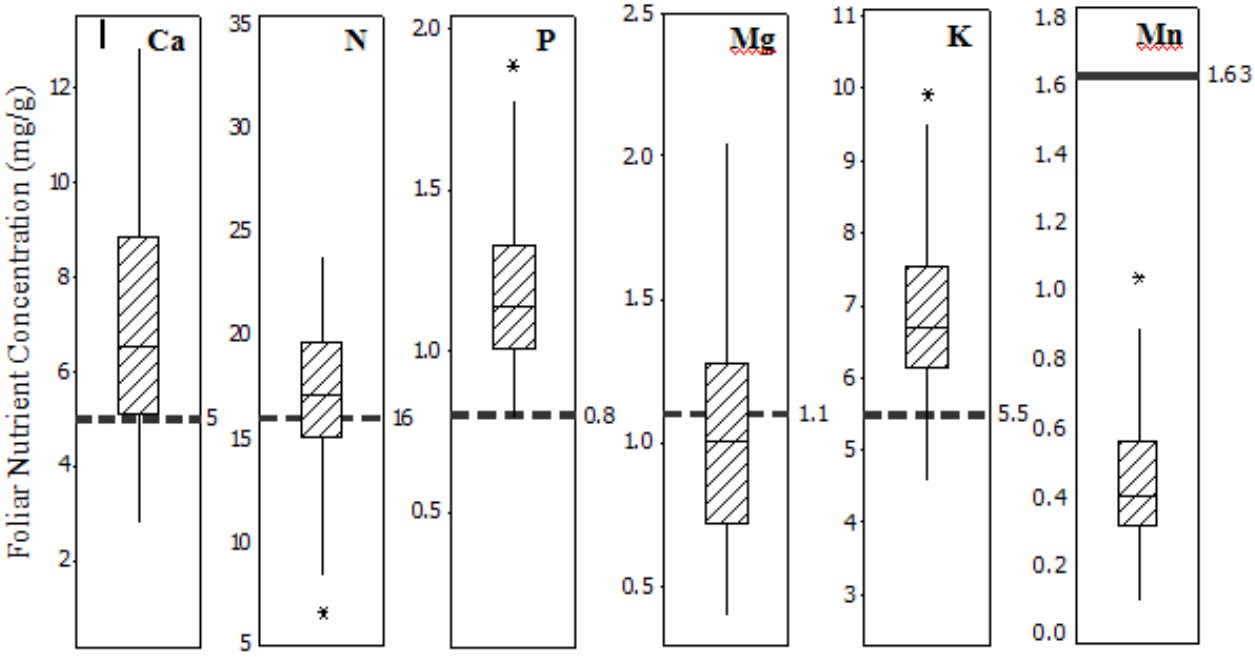
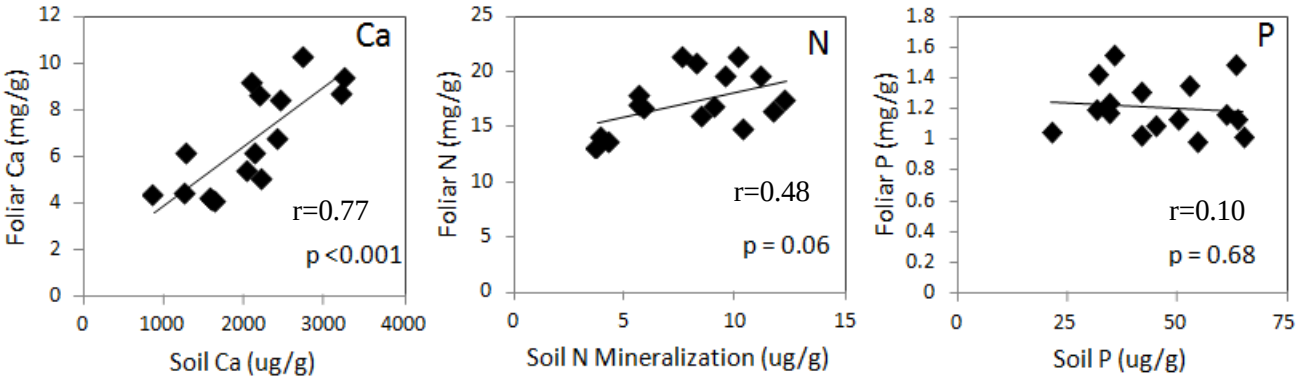
# 6. Appendix

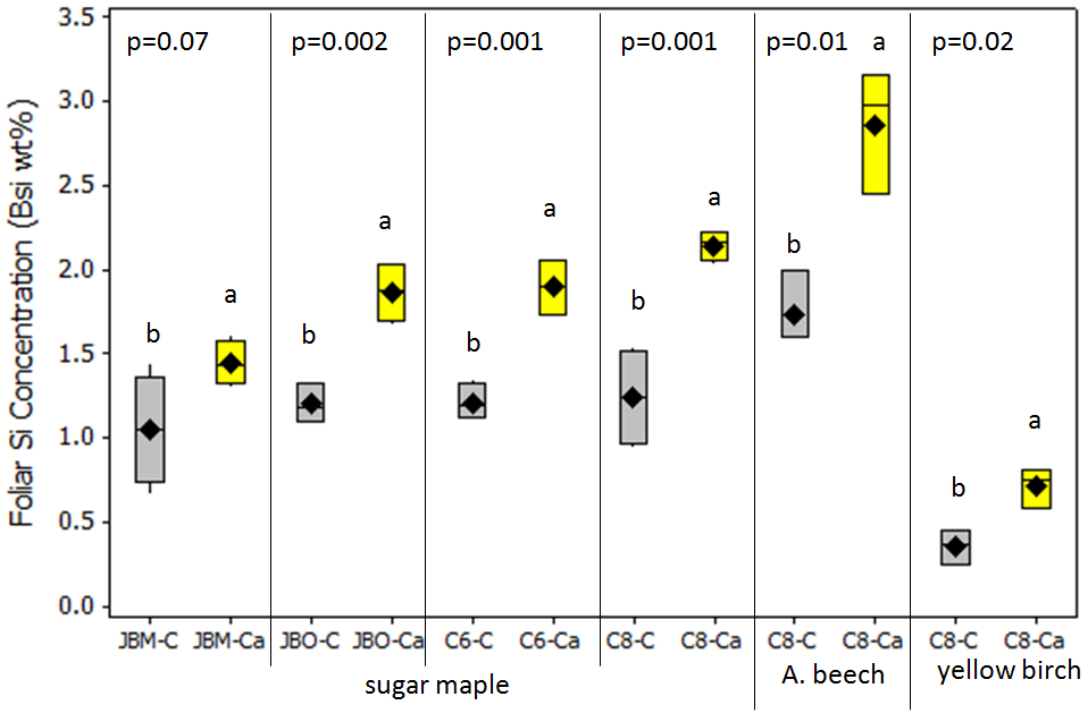



Appendix 1. Foliar nutrient concentrations for Ca, N, P, Mg, K, and Mn (mg/g) representing four sampled trees in each of the sampled treatment plots. The thick dashed line represents the nutrient deficiency threshold for healthy sugar maples. The solid line for Mn represents the threshold at which Mn becomes toxic to sugar maples. None of the trees showed luxury consumption. Threshold levels are from Kolb & McCormick 1993.

Appendix 2. Relationship of soil nutrients to foliar nutrients. Soil Ca and N mineralization is correlated with foliar Ca and N while P is not.




Appendix 3. Foliar uptake of silicate two growing seasons after a $CaSiO_3$ addition. All species are sugar maple with the exception of American beech and yellow birch in C8 control and Ca plots. One-way ANOVA was performed for each stand.



# *7. Curriculum Vitae*


Adam D. Wild

**Address:**
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**Current Position:**
Master's Student
Teaching & Research Assistant
Asst. Cross-country & Track Coach
State University of New York
Environmental Science & Forestry
Major Professor: Ruth Yanai


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**EDUCATION**

State University of New York at Cobleskill, Cobleskill, NY
**Bachelors, Plant Science & Landscape Management,** May 2012
GPA: 3.89, High Honors

State University of New York, Environmental Science and Forestry
**Masters of Science, Forest Ecology & Ecosystem Science**
Thesis: "Soil Nutrients Affect Sap Sweetness of Sugar Maple"
Anticipated, May 2014

**HONORS**

Coached USCAA Men's Cross Country National Champions - 2012 & 2013
SUNY Cobeskill Outstanding Student in the Landscape Major - 2012
SUNY Cobleskill Nick Iorio Men's Scholar Athlete Award - 2012
Who's Who Among Students in American Universities and colleges - 2012
Merrill Family Foundation Scholarship Recipient – Fall 2011
Albert "Nick" Iorio Community Service Scholarship Recipient – Fall 2011
SUNY Cobleskill Cross Country Coaches Award – 2011 & 2012
NEAC Scholar-Athlete Award – 2011 & 2012
NEAC All Conference Cross Country Runner - Fall 2010
PLANET AEF Scholarship Recipient - 2010 & 2011
Deans List – Fall 2008, Spring 2009, Fall 2009, Spring 2010, Fall 2010, Spring 2011, Fall 2011, Spring 2012
SUNY Cobleskill Academic Transfer Scholarship – 2009

**GRANTS**

SUNY 4E Network of Excellence Award "Aphid-like Biosensors for Ecosystem Studies: NANAPHID Proof of Concept" January 2014
Northern States Research Cooperative Graduate Research Grant "Sugar Content of Maple Sap after N, P, or Ca Fertilization" January 2013-May 2014 - P.I.

**RELATED WORK EXPERIENCE**

**Teaching Assistant, SUNY ESF:** January 2013 - Present

- APM 391, Introduction to Probability and Statistics
  - Instruct computer labs in Minitab

- Grade lab assignments and exams
-Instruct lecture when professor was not present
- Office hours for students seeking extra instruction

- FOR 321/521 Forest Ecology and Silviculture
  - Assist with field labs
  - Grade quizzes and projects
  -Instruct lecture when professor was not present
  - Field prep for labs
  - Office hours for students seeking extra instruction
- FOR 207, Introduction to Economics
  - Prepare and Grade exams
  - Office hours for students seeking extra instruction
  - Lead review before exam
  - Instruct lecture when professor was not present

**Research Assistant, SUNY ESF: December 2012-Present**

- Data entry and Analysis
- Sample processing for foliar nutrients
- Writing and figure preparation for publication

**Quantifying Uncertainty in Stream Loads:** September 2012-Present
Funded by LTER: Working lab group to analyze & quantify uncertainty in solute loads of streams

- Regression analysis using SAS software

**Multiple Element Limitation of Northern Hardwood Forest Ecosystems**
**Forest Research Field Crew Leader:** Summer 2012 & 2013

- Hired and supervised individuals for the summer field crew
- Organized and carried out scientific research projects in the White Mountains
- Organized daily task for up to 15 undergraduates, graduate and middle school teachers
- Managed crews to complete research task in the field and lab
- Used various scientific instruments for data collection
- Data entry, organization and presentation
- Collaborated and organized tasks among multiple principle researchers
- Developed a program to incorporate local high school students interested in science to work with the research crew
- Worked with undergraduates to create summer research projects

**Center for Academic Support and Excellence State University of New York at Cobleskill, Student Tutor:** January-May 2012

- Tutored students individually in horticulture related courses
- Held reviews for exams
- Conducted field plant identification review for students

**PRESENTATIONS:**

Hubbard Brook Committee of Scientist Spring Meeting, April 2014
"First Signs of a Foliar Treatment Response in the Multiple Element Limitation in Northern Hardwood Forest Ecosystem Study"
New York Society of American Foresters, January 2014
"Do Nutrients Make Maple Sap Sweeter?"
Rochester Academy of Science Fall Paper Session, November 2013
"Project Sweeter Sap: Do Soil Nutrient Make Maple Sap Sweeter?"
Hubbard Brook Cooperators Meeting, July 2013
"Sweet Times in the MELNHE Plots: Do Soil Nutrients Make Maple Sap Sweeter"
Hubbard Brook Cooperators Meeting, July 2012
"Third Time's the Charm: Remeasuring the Federer Chronosequence"

**POSTERS:**

SUNY ESF Research Spotlight, April 2014
"Is Sap Sweetness of Sugar Maples Genetically Controlled?"
New York Society of American Foresters, January 2014
"Do Nutrients Make Maple Sap Sweeter?"
Syracuse University Life Sciences Research Showcase, March 2013
"Project Sweeter Sap: Increasing the Sugar Concentration of Sugar Maple Sap Through Nutrient Additions"

**CONFERENCES & MEETINGS:**

SUNY Research Foundation 4E Network of Excellence Funded Projects Meeting, May 2014
Hubbard Brook Committee of Scientist Meeting, April 2014
New York Society of American Foresters, January 2014
Hubbard Brook Committee of Scientist Meeting, January 2014
New York State Maple Conference: January 2014
Rochester Academy of Science Fall Paper session: 2013
Syracuse University Life Sciences Research Showcase, 2013
Hubbard Brook Cooperators Meeting: 2012 & 2013
Professional Landcare Network (PLANET) Student Career Days: 2010 & 2011
PLANET Green Industry Conference: Fall 2011

**POTENTIAL PUBLICATIONS**

Soil Nutrient Affect on Sugar Concentration of Maple Sap - First Author
Variability of Maple Sap Sweetness Across 10 Different Genetic Sugar Maples: Selecting Genetically Sweeter Trees For Maple Syrup Production - First Author
Regeneration of Sugar Maples and American Beech Across an 18 Year Chronosequence inventory - First Author
Creating the Best Model for Predicting Stream Solutes - Coauthor