SUGAR MAPLE LEAF CHARACTERISTICS RESPOND TO DEPTH WITHIN THE CROWN AND TO NITROGEN AND PHOSPHORUS ADDITION

by

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Abstract

A. R. Young. SUGAR MAPLE LEAF CHARACTERISTICS RESPOND TO DEPTH WITHIN THE CROWN AND TO NITROGEN AND PHOSPHORUS ADDITION. 70 pages, 5 tables, 9 figures. 2019. Ecosphere.

Leaf characteristics may differ within tree crowns due to light environment or the availability of nitrogen (N) and phosphorus (P) in the soil, with important considerations for ecosystem budgets. To determine the relationship of leaf characteristics as a function of depth in the crown and increased soil N and P availability, we collected sugar maple leaves and twigs vertically in a full factorial N x P fertilization experiment in three mature forest stands of the multiple element limitation in northern hardwood ecosystems project in central New Hampshire, USA. The addition of N increased the concentrations of many metabolites such as chlorophyll and amino acids, and concentration of toxins such as aluminum (Al) and manganese (Mn). Phosphorus addition dramatically increased foliar P and adjusted the relationship of leaf characteristics with depth in the crown, particularly in leaves low in the crown. Leaf characteristics showed strong relationships with both depth in the crown, and in response to nutrient availability. We did not detect a difference in twig mass or twig growth as a function of depth in the crown, or with N or P addition. Studies that ignore the vertical gradient miss the opportunity to understand the plasticity with which trees can make crown-level adjustments.

Keywords: tree crown, nutrient limitation, leaf metabolism, leaf chemistry

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Chapter 1: Introduction

Background

Leaves receiving full light are often collected to allow for comparisons both within and across species. However, this focus on sun leaves has inhibited our understanding of leaves that do not receive full sun even though the majority of the leaves on a tree are shaded. Plants have strategies to allocate resources to obtain limiting resources such as light, water, and nutrient availability. However, in the temperate forest ecosystem in the northern USA, trees receive adequate levels of precipitation, and the soils are thought to have relatively high P availability relative to N due to the relatively young soil (Walker and Syers 1976).

Decreasing light intensity with depth in tree crowns has strong implications for the photosynthetic capacity of leaves (Vile et al. 2005, Coble and Cavaleri 2017). The gradient of light intensity within tree crowns contributes to contrasting leaf characteristics between the top and bottom of tree crowns. For example, leaves at the top of crowns are small and thick but become larger and thinner with increasing depth in the crown. Other abiotic factors also contribute to the vertical distribution of leaf characteristics including temperature, vapor pressure deficit (Jarvis and Mcnaughton 1986), gravitational constraints (Field 1983, Ellsworth and Reich 1993), and nutrient availability (Grime 1977).

Differences in leaf characteristics from the top to the bottom of tree crowns are examples of phenotypic plasticity that help sun and shade leaves maintain high performance (Poorter et al. 2011, Liu et al. 2016). Specific leaf area (SLA) is calculated by dividing leaf surface area by the dry mass of that leaf (Vile et al. 2005). Changes in SLA are driven by light environment which is highly correlated with photosynthetic capacity and leaf nitrogen (N) globally (Reich et al. 1997, Sack et al. 2006, Liu et al. 2016). The transition in SLA from upper to lower tree crowns enables

efficient light capture throughout the vertical gradient and is a central component of the leaf economic spectrum along with photosynthetic assimilation, leaf N, leaf phosphorus (P), and leaf lifespan (Wright et al. 2004).

The vertical distribution of leaf characteristics

Examining the vertical distribution of leaf characteristics in the crowns of tall trees is challenging due to their size and the difficulty of access, but the vertical gradient of leaf characteristics affords an opportunity to study important biotic and abiotic factors (Nadkarni et al. 2011, Kane et al. 2015, Ishii and Cavaleri 2017). Two primary abiotic factors that drive differences in SLA within tree crowns are light intensity and gravitational hydraulic constraints. Leaves in high light environments such as the top of the crown often have thicker palisade layers and the highest chlorophyll per unit area in a tree crown. Leaves at the bottom of the crown are thinner and weigh less than leaves at the top of the crown, resulting in the specific leaf area increasing with depth in the crown (Coble et al. 2014, Coble and Cavaleri 2017). At the same time, leaves at the top of the crown have higher gravitational hydraulic constraints and have greater cell density and decreased intercellular air space which all contribute to lower SLA (Coble and Cavaleri 2017). Drivers of SLA in tree crowns change seasonally: early stages of leaf development are driven by osmotic potential (Coble et al. 2016). In the mid to late stages of leaf development, light environment is the primary driver of SLA with thick, high SLA leaves at the top of the crown (Evans 1989, Ellsworth and Reich 1993). Leaf temperature and vapor pressure deficit impact the microclimate that leaves experience throughout the vertical gradient, creating opportunities to effectively allocate and acquire resources (Jones and Thomas 2007).

Along with light-mediated changes in leaf physical characteristics within tree crowns, soil nutrient-mediated adjustments in leaf characteristics such as leaf element and leaf metabolite

concentrations may also be related to depth in the crown. Efforts to distinguish which characteristics change with depth in the crown and which are related to nutrient availability would improve ecosystem budgets and sampling methods while providing empirical values for the ranges of leaf characteristics from the top to the bottom of tree crowns (Field 1983, Ishii and Cavaleri 2017).

Carbon and Nitrogen metabolism in tree crowns

Carbon (C) and N metabolism are highly interconnected in plants (Dickson 1989, Nunes-Nesi et al. 2010, Peltoniemi et al. 2012). Foliar N can be used to produce proteins that are essential for C capture including Ribulose-1,5-bisphosphate carboxylase (rubisco) as well as photosynthetic pigments and other essential metabolites (Dickson 1989, Wright et al. 2004). Of the total N in a leaf, up to 60% of it can be stored in protein, with rubisco accounting for 30% of the total foliar N (Perchlik and Tegeder 2018). Within-crown N partitioning is strongly related to light environment and can be quantified by measuring the concentrations of N per unit area or per unit mass. The relationship of N per unit area and N per unit mass with depth in the crown may differ because of the relationship of SLA with depth in the crown (Evans 1989). Foliar N concentrations are highly correlated with chlorophyll concentrations because chlorophyll contains N, and because light availability decreases with depth in the crown.

Polyamines are low molecular-weight aliphatic amines that are involved in a wide range of biological functions such as DNA transcription, response to environmental stress, and regulation of growth (Wuddineh et al. 2018). Three major polyamines found in plant tissues are putrescine (Put), spermine (Spm), and spermidine (Spd), whose concentrations change rapidly in response to external and internal stimuli (Minocha et al. 2000, Singh et al. 2018). Elevated amino acid and polyamine concentrations could be a compensatory mechanism to detoxify leaves from

excessive ammonia concentrations (Ohlson et al. 1995, Huhn and Schulz 1996, Minocha et al. 2015).

Amino acids are both storage compounds and intermediates in metabolic pathways (Singh et al. 2018, Minocha et al 2019). These include glutamate (Glu), alanine (Ala), and arginine (Arg). The initial N assimilation product is Glu, which can be used to store N and donate N to the biosynthesis of many other N-containing compounds such as other amino acids and polyamines. Asparagine (Asp) is also involved in ammonia assimilation and is a N-donor in aminotransferase reactions (Buchanan et al. 2015). High concentrations of Arg may indicate mineral nutrient imbalance and excess N (Minocha et al. 1997). γ-aminobutyric acid (GABA) is a non-proteinogenic amino acid that accumulates rapidly in response to biotic and abiotic stress and promotes the aluminum-activated malate transporter to increase anion transport (Ramesh et al. 2015). In response to many different stimuli, plants adjust biochemical pathways resulting in rapid cycling of amino acids and polyamines. Elevated concentrations of amino acids in leaves are associated with a response to environmental stress (Minocha et al. 2015).

Foliar chemistry and the vertical gradient

The concentrations of elements in leaf tissue influence leaf metabolism and may provide insight into nutrient limitation and nutrient excess. Nutrient availability likely plays a role in optimal resource allocation in northern hardwood trees, especially sugar maple. Sugar maples in good health are thought to have foliar N concentrations between 16 and 23 mg g^{-1} (Kolb and McCormick 1993). Sugar maple photosynthetic rates have been positively correlated with foliar N concentrations (Reich et al. 1991), but, on soils with low Ca availability in Pennsylvania, photosynthetic rates were not strongly correlated with mass-based foliar N concentrations

(St.Clair et al. 2008). Sugar maple seedlings grown in Connecticut had faster growth in soils with high nitrification rates, but only when growing in low light levels (Finzi and Canham 2000).

Sugar maples do well in soils with high calcium (Ca), and foliar concentrations of Ca are correlated with sugar maple health (Wargo et al. 2002, Gradowski and Thomas 2006, Juice et al. 2006). Foliar Ca concentrations above 5.5 mg g^{-1} and magnesium (Mg) above 0.7 mg g^{-1} are indicators of good health in sugar maple trees (Hallett et al. 2006, Long et al. 2009). Another important foliar element is P, which is involved in energy storage and transfer via ATP, DNA synthesis, and cellular signaling (Murrell et al. 1999, Ellsworth et al. 2015). While low concentrations of foliar N can limit photosynthetic capacity in chloroplasts of plant cells, low P can limit photosynthetic capacity by decreasing the rate at which ADP is converted to ATP (Bauer et al. 2004, Gradowski and Thomas 2006, Ellsworth et al. 2015).

Sugar maples in northern hardwood ecosystems are particularly sensitive to high levels of acidic deposition and soil acidity, which lead to imbalances in soil chemistry, foliar metabolism, reduced growth, and crown dieback (Wargo et al. 2002, St.Clair et al. 2008, Long et al. 2009, Pitel and Yanai 2014, Momen et al. 2015). Soil acidity increases the solubility of elements that are toxic to plants such as aluminum (Al) and manganese (Mn) resulting in impaired root growth (Catovsky et al. 2002) and elevated foliar concentrations of Al and Mn (St.Clair et al. 2008). Foliar Mn concentrations above 1.9 mg g^{-1} and low Ca:Al ratios are associated with sugar maple stress (Cronan and Grigal 1995, Schaberg et al. 2005, Hallett et al. 2006, Long et al. 2009).

Photosynthetic pigments and leaf protein

Photosynthetic pigments require investment in N and P and play important roles in leaf carbon assimilation. Elevated chlorophyll concentrations are advantageous because they decrease the likelihood of photo-inhibition when leaves are inundated with photons and must dissipate

energy by increasing light reflectance or by dissipating heat through the xanthophyll cycle (Leilani et al. 2001). A negative impact of photo-inhibition is a buildup of free radicals and oxidative stress compounds that can impair photosynthetic reactions, particularly at high temperatures at the top of the crown or when the concentrations of foliar nutrients are out of balance due to oxidative stress (Foyer et al. 1994).

The ratio of chlorophyll a to chlorophyll b is an indicator of N partitioning because only chlorophyll a can initiate photosynthetic reactions, whereas chlorophyll b assists photon transport to chlorophyll a but does not increase photosynthetic capacity. When N availability is low, the ratio of chlorophyll a:b is high because plants preferentially synthesize chlorophyll a. However, chlorophyll a:b ratios can also be high in leaves at the top of the tree that receive high irradiance because higher concentrations of chlorophyll a can absorb more light (Hikosaka and Terashima 1995, Kitajima and Hogan 2003). Additionally, if N availability is adequate, chlorophyll a:b ratios may be lower at lower canopy positions because higher chlorophyll b concentrations allow lower canopy leaves to improve light capture (Hidaka and Kitayama 2009). Even though N addition is often thought to lead to increased primary production in temperate forests (Vadeboncoeur 2010), recent evidence from the White Mountain National Forest found that primary production was greater with the addition of P (Goswami et al. 2018).

Foliar N:P ratios and nutrient limitation

Nutrient availability depends on geologic history, parent material, and climate. Younger soils are thought to have high P availability but low N availability, whereas older soils have low P availability and high N availability because the P adsorbed, immobilized, and ultimately lost from the ecosystem (Walker and Syers 1976). Weathering rates impact the availability of P on a global scale with the tropics having less available P and more P limitation (McGroddy et al.

2004, Reich and Oleksyn 2004). However, fertilization studies generally find a stronger growth response to the combined addition of N and P than to either added alone (Elser et al. 2007, Vadeboncoeur 2010, Harpole et al. 2011, Zhang et al. 2018b).

The ratio of leaf N to P reflects soil nutrient availability and can indicate nutrient status for both individual organisms and entire ecosystems (Güsewell 2004). This ratio may also be sensitive to depth within tree crowns, and varies across tree species (Lovett et al. 2004).The ratio of foliar N:P that can be suggestive of nutrient limitation is likely different across ecosystems. For forests, N:P ratios above 20 could be interpreted as P limitation whereas ratios below 10 could indicate N limitation (Güsewell 2004). Elevated N:P ratios indicative of P limitation have been observed in N-addition studies in Ontario (Gradowski and Thomas 2006), California (Menge and Field 2007), and New Hampshire (Gonzales and Yanai 2019). The history of N deposition in the northeast United States (Galloway 2004) may lead to decreased P availability relative to N. This could induce P limitation in northern hardwood forests (Hallett et al. 2006, Elser et al. 2007, Harpole et al. 2011, Goswami et al. 2018).

Leaf-level and crown-level plasticity

If foliar N were optimally distributed, the concentration of N per unit area would be proportional to the light received by a leaf, and leaf N would strongly decline with crown depth (Reich et al. 1991, Peltoniemi et al. 2012). However, field observations have not found foliar N to be proportional to light availability; foliar N does not differ as strongly from the top to the bottom of the crown as light does (Osada et al. 2014). The concentration of foliar N in ecosystems has received attention for its ability to integrate many ecosystem processes. Other nutrients may also be important for allowing trees to adjust leaf characteristics, and these

adjustments may be influenced strongly by depth in the crown as it relates to light availability and shading.

We focused on physical, chemical, and metabolic characteristics of sugar maple leaves at different depths in the crown because most of the leaves on a tree are at least partially shaded but, most studies examining nutrient limitation only examine well-lit leaves. By exploring resource allocation within tree crowns, we will have the opportunity to ask if we are missing treatment responses by only examining leaves from the top of the crown. We repeatedly collected branches from the top to the bottom of tree crowns to increase our understanding of resource allocation to leaf characteristics throughout the crown.

Chapter 2: Sugar maple leaf characteristics respond to depth within the crown and to nitrogen and phosphorus addition

Abstract

The distribution of leaf characteristics within tree crowns may depend on both light environment and the availability of nitrogen and phosphorus in the soil. To explore resource allocation to leaf characteristics throughout tree crowns we collected leaves along a vertical gradient (every 2 m) within mature sugar maple crowns in a full factorial N X P fertilization experiment in three forest stands in central New Hampshire, USA. Plots in each stand were fertilized with 30 kg/ha N as NH_4NO_3, or 10 kg/ha P as NaH_2PO_4, or both at the same rates for seven years prior to sampling. Leaves decreased in mass and increased in area with depth in the crown. Concentrations of chlorophyll increased with depth in the crown, but trees that received N addition had higher chlorophyll concentrations throughout the crown. Trees that received N also had significantly higher concentrations of the amino acids alanine, GABA, isoleucine, glutamate, and valine, but N addition did not change relationships of leaf characteristics with depth in the crown. Trees that received N also had higher concentrations of toxic elements Al and Mn. Trees that received P had higher P concentrations, and P addition altered the relationship of some leaf characteristics with depth in the crown that was most pronounced in the leaves at the bottom of the crown. Understanding the patterns of leaf characteristics at varying depth in tree crowns is useful for modeling crown-level acclimation to increased N and P availability and may improve field sampling designs. Studies that ignore the vertical gradient miss the opportunity to understand the plasticity of traits within tree crowns.

Keywords: vertical gradient, nutrient limitation, leaf chemistry, leaf metabolism

Introduction

Leaves in tree crowns experience heterogenous light environments (Ellsworth and Reich 1993), and the capacity to adjust physical, chemical, and metabolic leaf characteristics may be influenced by soil nitrogen (N) or phosphorus (P) availability (Evans and Poorter 2001, Niinemets et al. 2014). Leaves at the top of crowns shade the leaves lower in crowns reducing light availability for photosynthesis (Hirose and Bazzaz 1998, Le Roux et al. 2001). In response to strong changes in light availability, temperature, and humidity, leaves at the bottom of the crown have larger area but are thinner than leaves at the top of the crown (Reich et al. 1997, Coble and Cavaleri 2017). Many canopy studies have shown that the ratio of leaf area to leaf mass, or specific leaf area (SLA), is a key integrating trait as it represents the biomass cost per unit of light interception (Poorter et al. 2009). Leaves at the top of tree crowns have the highest photosynthetic capacity, denser vascular tissue, and thicker palisade layer than leaves at the bottom of the crown (Hollinger 1989, Ellsworth and Reich 1993, Niinemets and Tenhunen 1997). Leaves in sugar maple crowns display strong gradients of SLA that allow trees to reduce construction cost of leaves that are low in the crown and receive indirect light (Coble et al. 2014, 2016, Coble and Cavaleri 2015, 2017). Plasticity in SLA is a strategy common to shade-tolerant trees but there are likely other leaf characteristics that are plastic in response to light environment and soil nutrient availability (Liu et al. 2016).

Studies that examine within-crown variation in leaf characteristics are important because they provide information on phenotypic plasticity and can improve our understanding of tree functional traits, which has broad implications for carbon sequestration and tree physiology (Baldocchi and Harley 1995, Bonan et al. 2012). A majority of tree foliage research has focused on obtaining and comparing sun leaves, even though most leaves are at least partially shaded

(Keenan et al. 2016). Capturing the relationship between a leaf characteristic and depth in tree crowns is important for accurately scaling estimates of whole canopy photosynthesis; ignoring the seasonal and vertical gradient of N per unit area sugar maple crowns led to a ~60% under-estimate of whole crown photosynthesis across a growing season (Coble et al. 2016). Studying resource allocation to leaf characteristics within crowns is difficult because strategies of acclimating photosynthetic capacity to light environment differ across plant functional types (Niinemets et al. 2014). Across species, leaf characteristics that are related to light capture show stronger relationships per unit area basis than per unit mass basis (Reich 2014, Díaz et al. 2016). However, within a species, leaf characteristics per unit mass are useful because they reflect the biomass cost of investment (Niinemets et al. 2014, Keenan et al. 2016). Additionally, woody species with low rates of leaf turnover use structural adjustments to acclimate to light availability, whereas plant species with high leaf turnover like herbs acclimate to light environment by translocating nutrients within a growing season (Niinemets et al. 2014). Since deciduous trees flush leaves at the same time, adjustments in physical leaf characteristics within the crown are likely related to acclimation to light availability.

Soil nutrient availability may also impact the vertical distribution of leaf characteristics in tree crowns. Changes in soil nutrient availability may cause shifts in resource allocation, particularly if a previously limiting nutrient becomes more available resulting in physical, chemical, or metabolic leaf characteristics to adjust in release from a nutrient limitation (Elser et al. 2007, Harpole et al. 2011). In addition to soil nutrients affecting resource allocation, differences in light availability impacts N partitioning between primary and secondary photosynthetic pigments (Hikosaka and Terashima 1995). The amount of N invested in photosynthetic pigments is driven both by light and soil N in a way that makes it important to

collect leaves at different heights (Hollinger 1996, Kitajima and Hogan 2003). A meta-analysis of tree responses to N addition suggests that leaf characteristics including leaf area index, foliar N content, and net photosynthetic rates increase with N addition, whereas SLA, stomatal conductance, and transpiration rates did not meaningfully differ with N addition (Zhang et al. 2018a).

Leaf tissue chemistry has been used to infer plant health, with numerous studies on acid rain focused on Ca depletion and growth reductions (St.Clair et al. 2008). The concentrations of foliar Ca and Mg are important for photosynthesis, while Al and Mn are toxic to leaves at high concentrations. Foliar B is a micro-nutrient that improves the structural integrity of the cell wall, and evidence of B deficiency has been documented in sugar maple (Bal et al. 2015). The concentrations of nutrients and toxins influence leaf performance which can be further explored by examining the concentrations of foliar metabolites.

Leaf metabolism likely differs with depth in the crown and with soil nutrient availability, with some leaf metabolites more sensitive to soil nutrient availability than light environment. Polyamines are aliphatic amines that mediate cell C and N metabolism and initiate cellular response to abiotic stress (Minocha et al. 1997). Their concentrations in plant tissues can change within seconds to minutes in response to abiotic and biotic stimulus and thereby promote plant defense mechanisms. Elevated concentrations of leaf metabolites can also be indicative of chronic abiotic stress (Minocha et al. 2014, Singh et al. 2018). Three common polyamines in plants are putrescine (Put), spermidine (Spd) and spermine (Spm). The concentrations of polyamines are essential for photosynthesis, and fluctuate rapidly to maintain homeostatic conditions in the symplasm (Wuddineh et al. 2018). Elevated concentrations of Put can indicate foliar nutrient imbalance and Put concentrations can increase in orders of magnitude in response

to abiotic stress (Minocha et al. 2014). Previous studies suggest that Spm and Put show similar responses to environmental stress such as inadequate soil calcium (Ca), excess soil aluminum (Al), and chronic N accumulation (Minocha et al. 1997, 2000, 2010, 2015, Wargo et al. 2002). Under conditions of stress, concentrations of these three polyamines increase and confer greater stress tolerance (e.g. through lowering NH_3 toxicity and scavenging free radicals). Additionally, elevated concentrations of amino acids, particularly the amino acids glutamate (Glu), alanine (Ala), and arginine (Arg), may indicate elevated N metabolism because they store N. In contrast, the accumulation of the branched-chain amino acid valine (Val) may indicate increased cellular respiration (Kochevenko et al. 2012). The non-proteinogenic amino acid γ-aminobutyric acid (GABA) is higher in leaves that experience foliar element imbalance and abiotic stress and activates inter-membrane transporters that shift solute concentrations (Bouche and Fromm 2004, Kochevenko et al. 2012, Ramesh et al. 2015). Finally, GABA is a signaling molecule and a transcription factor (Bown and Shelp 2016).

Forests on geologically young soils are thought to be N-limited, but decades of elevated N deposition (Galloway et al. 2003) may have alleviated N limitation in northern hardwood forests. Recent studies in the Multiple Element Limitation in Northern Hardwood Ecosystems project have reported foliar N:P ratios of unfertilized trees that are suggestive of P limitation, and increased aboveground biomass production with the addition of P (Goswami et al. 2018). Additionally, trees that did not receive N or P addition had greater resorption of P than N, and high N:P ratios (from 20 to 31) in green leaves of trees that received N (Gonzalez and Yanai 2019). However, it remains to be determined if nutrient addition alters resource allocation patterns as a function of depth in the crown, or if some leaf characteristics are more strongly driven by nutrient availability.

The goal of this research was to describe the relationships of physical, metabolic, and chemical leaf characteristics as a function of depth within tree crowns and in response to N and P addition. We measured leaf characteristics of mature sugar maples in the Bartlett Experimental Forest in the White Mountains of central New Hampshire. We predicted that leaf characteristics would differ substantially in their relationships with depth in tree crowns: leaf characteristics could increase, decrease, or stay consistent as a function of depth in the crown. We predicted that the concentrations chlorophyll would show strong relationships with depth in tree crowns; that particular N-rich compounds such as chlorophyll, amino acids, and polyamines would be higher with N addition; and that the addition of P would increase concentrations of foliar P.

Methods

Field site and sample collection

Trees in this study were located in three mature forest stands in the Bartlett Experimental Forest, NH and are part of the Multiple Element Limitation in Northern Hardwood Ecosystems project (MELNHE). These stands regenerated following harvest ~ 1890 and are dominated by American beech (*Fagus grandifolia Ehrh*), sugar maple (*Acer saccharum Marsh.*), and yellow birch (*Betula alleghensiensis* Michx.) (Goswami et al. 2018). Soils in these sites are well drained Spodosols formed in granitic glacial drift (Vadeboncoeur et al. 2012, 2014). The regional climate is humid continental with an average annual precipitation of 127 cm and average monthly temperatures range from 14 C to 27 C (Adams et al. 2003). Since 2011, annual additions of N (30 kg/ha NH_4NO_3), P (10 kg/ha NaH_2PO_4), and a combined treatment of N + P at the same rates are applied to experimental treatment plots, in addition to a control that does not receive N or P treatments (Fisk et al. 2014). Treatment plots have a 30 m by 30 m measurement area with a 10 m buffer.

Branches from canopy-dominant trees were collected using a pole pruner and minimally invasive rope access techniques on July 31st and August 1st 2017 (Jepson 2000, Anderson et al. 2015). Leaves free of herbivory and physical damage were selected for study by cutting branches every two meters from the top to the bottom of tree crowns. Once on the ground, ~200 mg of leaf discs were collected in pre-weighed 2 ml microfuge tubes with 1 ml of 5% perchloric acid (PCA) for amino acids and polyamine analyses and rest of the leaf discs placed in a separate microfuge tubes for chlorophyll and soluble protein analyses. All samples were stored on ice until frozen at -20˚C (Minocha et al. 2000).

Sample processing

Physical leaf characteristics

Ten leaves from each branch were pressed for two days then imaged with an OLYMPUS TG4 camera and ImageJ software was used to measure leaf surface area. After imaging, these same 10 leaves were oven dried at 60˚ C. Leaf surface area and leaf dry mass are reported as the average of 10 leaves.

Total leaf element concentrations

Additional leaves from each branch were oven dried at 60˚ C and ground using a Wiley mill through a 40-mesh screen. Leaf N concentrations were quantified using a CN analyzer (FlashEA 1112 analyzer, Thermo Scientific). Apple leaves (1515, 1545, and 1575) were run as a tissue standard. Leaf element concentrations for Ca, Mg, Mn, P, Al, Mn, and B were quantified by microwave digestion using ~0.25 g of oven dried leaf tissue in 10 ml of 15.8 N nitric acid, followed by inductively coupled plasma optical emission spectroscopy (ICP-OES) (MARS6 Microwave digestion system CEM). One duplicate sample, one blank, and two replicates of a standard (NIST 1515) were processed with each group of 25-36 samples. During ICP-OES a

calibration blank and in-house quality control were run after every 5 samples. We re-calibrated the machine if drift exceeded 5% of the in-house quality control. Tissue standard and recovery values were within 10% of the certified values for N (average recovery within 6%), 3% for Ca (average recovery within 2%), 16% for Mg (average recovery within 10%), and 11% for P (average recovery 7%) (Appendix 1).

Quantification of polyamines and amino acids

Previously frozen leaf samples (~200 mg) were repeatedly frozen and thawed (3X) and centrifuged at 13,000 g for 10 minutes. The supernatants were used to determine the dilute-acid extractable concentrations of polyamines (Put, Spd, and Spm) and amino acids (Ala, GABA, Glu, Ile, Val). A dansyl group was added to the amino acids to improve separation (Minocha and Long 2004) with modifications for polyamines described here. Samples were incubated at 60°C for 30 min, cooled for 3 min and then microfuged at 14,000 x *g* for 30 sec. Dansylation was terminated by the addition of 45 µl of glacial acetic acid. Sample tubes were kept open for 3 min under a flow hood to allow CO_2 bubbles to escape. Acetone used to dissolve dansyl chloride was evaporated using a SpeedVac Evaporator (Savant, Farmingdale, NY, USA) for 5 min. Finally, filtered HPLC grade methanol was added to bring the volume to 2 ml, and polyamines were separated by injecting 20 µL of standards and samples into a linear gradient flow from 40% acetonitrile to 100% acetonitrile at a flow rate of 2.5 mL min^{-1} (in 10 mM heptane sulfonic acid). Data were processed using Perkin Elmer TotalChrom software (version 6.2.1).

Chlorophyll, carotenoids, and soluble protein

To determine the concentrations of chlorophyll a, chlorophyll b, and carotenoid pigments, 5-10 mg of leaf tissue were thawed and frozen three times. Samples were then incubated in the dark for 16 hours then centrifuged. Absorption at 664 and 649 nm was converted to concentrations of chlorophyll a, b, and carotenoids (Lichtenthaler and Buschmann 2005,

Minocha et al. 2009). Dilute-acid soluble protein was prepared by adding 50 mg of thawed leaf tissue to 500 µl of extraction buffer (Jones 1989) and quantified using the absorbance at 595 nm (Bradford 1976).

Data analysis

The experimental units were the twelve trees, with one tree per treatment plot and four treatment plots within three stands (Table 1). Each stand was considered a block. The number of samples within each tree varied from 3 to 7 depending on crown depth. The total number of samples was 60.

A total of 21 leaf characteristics were examined in this study. Response variables related to physical leaf characteristics were leaf area, leaf mass, and specific leaf area. Cellular metabolites included concentrations of total photosynthetic pigment (chlorophyll A +B), carotenoids, and concentrations of polyamines Put, Spd, and Spm and amino acids Ala, GABA, Glu, and Val. Response variables related to leaf chemistry were concentrations of leaf Ca, Mg, N, P, Al, and Mn.

Patterns in leaf characteristics were examined by fitting a linear model of each leaf characteristic as a function of depth in the crown for each of the 12 trees. We scaled depth in the crown from 0 to 1, with 0 being the top of the crown and 1 being the bottom of the crown. To test if a leaf characteristic changed significantly as a function of depth in the crown we used a t-test comparing the 12 values of slope to zero. To test if nutrient addition influenced the relationship of leaf characteristics with depth in the crown, a N by P factorial ANOVA blocked by stand was used to compare the slopes and intercepts of the linear models, as well as the average value of the samples from each tree. The residuals of the N X P ANOVA passed a Shapiro-Wilks test for normality with all $p > 0.05$ (Appendix 2).

R version 3.5.1 (R Core Team 2018) and ggplot2 (Wickham 2016) were used for this analysis.

Results

Leaf characteristics relation to depth in the crown

Many leaf characteristics differed significantly from the top to the bottom of sugar maple crowns. Twelve out of 21 leaf characteristics had significant relationships with depth in the crown, with 10 of the 12 increasing as function of depth in the crown (Table 2). Leaf area and SLA significantly increased with depth in the crown (Figure 1). This change in SLA with depth in the crown was driven by strong increases in leaf area ($p < 0.01$), and small decreases in leaf mass ($p = 0.09$; Figure 1).

Our analysis of leaf characteristics demonstrates that some leaf characteristics vary substantially with depth in the crown. For example, the photosynthetic pigments chlorophyll a, chlorophyll b, and carotenoids had positive slopes with crown depth indicating that they were lower at the top of the crown than the bottom. In contrast, concentrations of leaf metabolites did not show clear consistent relationships with depth in the crown (Figure 2).

Physical leaf characteristic response to N and P addition

The relationship of leaf mass and area with depth in the crown was different for trees that received P compared to trees that did not receive P. Leaf mass decreased with canopy depth more steeply in trees that received P (main effect of P on the slope: $p = 0.05$, Figure 1). Leaf area had a less steep increase from the top to the bottom of the crown in trees that received P ($p = 0.09$) (Figure 1). With N addition, trees had larger leaves throughout the canopy ($p < 0.01$). Although experimental additions of P altered the relationship of both leaf area and leaf mass as a function of depth in the crown, addition of N increased leaf size at all depths in the canopy.

Foliar chemistry response to depth in the crown and N and P availability

The concentrations of foliar elements increased with depth in the crown (Figure 2), significantly in the case of N ($p < 0.01$), P ($p = 0.04$), Mn ($p = 0.04$), Mg ($p < 0.01$), and B ($p = 0.01$) (Table 2, Figure 2). The concentrations of Al, Fe, and the ratio of N:P did not have a detectable relationship with depth in the crown (Table 2).

The concentration of foliar elements also reflected nutrient availability, with N increased the concentrations of N ($p = 0.01$), Al ($p = 0.05$), and Mn ($p = 0.01$) at the top of tree crowns (Figure 5, 6). The addition of P increased the concentrations of P at the top of the tree ($p = 0.03$), which contributed to a steeper increase of P as a function of depth in the crown ($p = 0.03$). The addition of P also affected leaf Al ($p = 0.05$) and leaf B ($p = 0.01$) by having more steep increases as a function of depth in the crown (Figure 6). The addition of P also dramatically lowered the N:P ratio to ~10 in trees that received P alone (Fig. 5).

Leaf metabolite response to depth in the crown and N and P availability

As expected, concentrations of photosynthetic pigments and amino acids increased with N addition. Total chlorophyll ($p = 0.05$) and carotenoid ($p = 0.02$) concentrations increased as a function of depth in the crown per unit mass, and trees that received N had higher chlorophyll and carotenoids at any height in the crown ($p < 0.01$, Table 2) (Figure 3). The chlorophyll a:b ratio was not strongly influenced by N or P addition ($p \geq 0.22$), suggesting that trees adjust the concentration of photosynthetic pigments in concert, rather than selectively increasing a particular pigment as a function of depth in the crown.

Amino acid concentrations were either higher throughout the crown or were higher at the top of the crown with N addition. Trees that received N had higher concentrations of Ala ($p <$

0.04), GABA, ($p = 0.01$), and Ile ($p = 0.03$) throughout the crown. Glutamine ($p = 0.02$) and Val ($p = 0.04$) increased with N addition, but only at the top of the crown (Figure 4).

In contrast to the metabolites that responded to N addition, concentrations of the polyamine Spd were higher throughout tree crowns that received P ($p = 0.02$) but concentrations of Put and Spm were not detectably influenced by the addition of N or P. Only Put concentrations decreased significantly with depth in the crown ($p = 0.01$, table 2).

Discussion

Within-crown plasticity in leaf characteristics

In sugar maple crowns the increase in leaf area from the top to the bottom was greater than the decrease in mass, resulting in increasing SLA with depth in the crown. For this reason we chose to examine resource allocation per unit mass but we recognize that area-based traits may be appropriate when examining light-mediated plasticity and its effect on photosynthesis (Niinemets et al. 2014, Keenan et al. 2016). The increase in SLA and chlorophyll as a function of depth in the crown is primarily due to increasing leaf thickness and vascular tissue associated with well-lit leaves at the top of the crown (Figure 2). The relationship of SLA and depth in the crown was not strongly impacted by N or P addition, emphasizing the commonality and importance of reducing construction costs for leaves lower in the crown. We predominately found differences in chemical and metabolic characteristics with N and P addition rather than adjustments in physical leaf characteristics.

Models commonly assume an exponential distribution of foliar nitrogen down the crown (Peltoniemi et al. 2012, Campany et al 2016), because light availability declines exponentially

through the crown (Ellsworth and Reich 1993). However, we found that foliar nitrogen concentrations had linear relationships with depth in the crown. Other studies have also documented linear relationships of foliar nitrogen with depth in the crown, attributing this to multiple contributing factors such as mesophyll conductance leading to co-optimum distribution of foliar N (Coble and Cavaleri 2017, Johnson et al. 2010).

Sugar maple leaves respond to N addition

In general, leaf response to N addition did not impact the relationship of a leaf characteristic as a function of depth in the crown. Concentrations of chlorophyll, amino acids, and polyamines were higher in trees that received N compared to those that did not receive N. Some N-rich compounds were different at the top of the crown (e.g. Glu, Ile, Leu) and other metabolites were higher on average (e.g. GABA, Ala) in tree crowns that received N, but there was no detectable difference in the relationship with depth in the crown with N addition. This change in amino acid concentrations may indicate precursors to altered patterns of growth (Goswami et al. 2018). The allocation of N to chlorophyll can be directly related to light availability; but some metabolites related to defense or N storage may be more related to soil nutrient availability than light availability (Minocha et al. 1997, Ramesh et al. 2015).

Trees that received N also had higher concentrations of toxic elements Al and Mn, presumably due to increased solubility of these cations in soil. The increases we observed in the defense compound GABA may be a response to these toxins (Johnson et al. 2010, Minocha et al. 2015). Producing defense compounds may incur a cost to growth (Galloway et al. 2004, Perchlik and Tegeder 2018). These results suggest that increased soil N may not have been beneficial for sugar maples, because the addition of N increased toxins and defense metabolites throughout the crown. Given the response of foliage to N addition, it is clear that sugar maple trees are

responding to the addition of N, however, it remains to be seen how the consequences of these foliar adjustments will manifest given the legacy of N deposition in the northeast USA.

Sugar maple leaves respond to P addition

Adjustments in resource allocation as a function of depth in the crown were observed in trees that received P. Trees that received P had higher foliar P concentrations throughout the crown, and this effect of increased P concentrations was strongest at the bottom of the crown resulting in a significantly greater slope of leaf P with depth in the crown. Similar trends were observed with B, where trees that received P had significantly more B, particularly in the leaves at the bottom of the crown (Brown and Shelp 1997).

Even though chlorophyll concentrations are important for photosynthetic activity, our study suggests that the increased growth with P addition observed in these stands (Goswami et al. 2018) was not accomplished by changing the concentration of photosynthetic pigments. Increased P availability caused sugar maples in this study to adjust resource allocation to leaves low in the crown, but it is not clear if this response is related to tree growth. Additionally, this effect of P addition would be unlikely to be observed with traditional foliage sampling of well-lit leaves at the top of the crown. We do not completely understand the physiological consequences of the vertical distribution of P.

Leaf collection strategies

By prioritizing sampling of sun-lit leaves, we have neglected most of the vegetation on the earth. The emphasis on sun-lit leaves is reasonable because the leaves at the top of the crown have the highest photosynthetic activity, and these leaf characteristics can be directly interpretable for remotely sensed observations. Although leaf characteristics that are involved in light capture like concentrations of chlorophyll, Ca, and Mg are likely sensitive to light

environment, leaf characteristics related to soil nutrient availability like the N:P ratio and abiotic stress metabolites may be relatively insensitive to crown position. Collecting leaves from multiple branches in a tree is time-intensive and comes at the cost of reduced spatial extent and statistical replication. Previous research in the MELNHE study has only examined one branch from each tree for a given growing season, which has obscured the effect of P addition on leaves low in the crown. Research regarding resource allocation to physical, chemical, and metabolic leaf characteristics throughout the crown of these trees increases understanding of resource partitioning within tree crowns.

Conclusion

Historically, researchers have used sun-exposed leaves of forest trees as a standard for reliable comparisons across species, space, and time. However, these studies miss the opportunity to study the prevailing light conditions in the crown leading to bias and neglect of shade leaf characteristics (Le Roux et al. 2001, Keenan et al. 2016). Trees that received additional N had higher concentrations of chlorophyll, Al and Mn, and abiotic defense compounds. Trees that received P increased foliar P, and had different relationships with depth in the crown that were particularly pronounced in leaves low in the canopy. Accounting for the physical heterogeneity within tree crowns is important for accurate estimates of whole-canopy photosynthesis and for revealing differences in phenotypic plasticity related to changes in soil N and P availability (Raulier et al. 1999, Dai et al. 2004, Jones and Thomas 2007).

Chapter 3: Twig growth in sugar maple crowns

Introduction

Tree growth is commonly measured as diameter increment, but trees also grow by increasing twig length. Twig growth is important for positioning leaves to areas of higher light availability. Twig growth is also important because longer twig segments can have multiple flushes of leaves within a growing season and wider inter-node spacing can reduce self-shading (Kozlowski and Pallardy 1997). Growth of twigs occurs as elongation (primary growth) and as increases in diameter (secondary growth) and mass.

Nutrient limitation may be an important constraint to ecosystem productivity in temperate forests. Tree growth on relatively young soils is thought to be N-limited (Elser et al. 2007), but anthropogenic atmospheric deposition has shifted patterns in ecosystem stoichiometry, which likely influences patterns of resource allocation in trees (Goswami et al. 2018, Gonzales and Yanai 2019). Base cation losses associated with acid rain reduced sugar maple growth (Kolb and McCormick 1993, Momen et al. 2015), regeneration (Cleavitt et al. 2014), and fine twig dieback (St. Clair et al 2008). Increased fine twig die-back was an indicator of sugar maple decline (Kolb and McCormick 1993, Hallett et al. 2006).

To explore an alternative method to detect N or P limitation of aboveground production in sugar maple, we measured annual twig growth at multiple depths within crowns. Previous measurements of aboveground productivity in the northern hardwood system that I studied suggested that the addition of P allowed trees to grow more, but this was detected at the ecosystem level (Goswami et al. 2018). By collecting many measurements of twigs from sugar maple we hope to detect changes in growth related to increased N and P availability.

Understanding how trees allocate resources is important because stoichiometric disturbance from forest management activities, anthropogenic emissions, and climate change will likely alter the resource economics of trees and result in different limitations to forest growth. Although twigs can provide more information about growth over time than a single bole diameter measurement, there is also high variability in twig growth that is likely driven by heterogenous light environments within tree crowns.

Here we examine whether variation in twig growth and twig biomass investment can be explained by depth in the crown, age of the twig, identity of each tree, or experimental additions of N and P in a factorial combination. We expected that twig mass would be significantly explained by twig age because secondary growth increases the mass of previous twig segments, whereas twig length would be better explained by depth in the crown due to the differences in light availability.

Methods

Twig collection & measurement

Branches from canopy-dominant sugar maples were collected on July 31st and August 1st 2017. Branches were collected every two meters from the top of the crown to the lowest available branches. Three twig sections were selected from each branch and pooled to provide an average twig length and mass per year for each sampling height. Branches varied widely in the number of years represented, with the oldest twig segment dating back to 1991 and, at the other extreme, one tree only had twigs going back to 2012.

Annual twig growth can be measured using the distance between the bud scars on a twig, which mark the beginning and end of the growing season. Bud scars differ from leaf scars in that

they are cylindrical and have abrupt start and end points. The distance between bud scars was measured with digital calipers to the nearest 0.01 mm. Each annual twig increment was then cut, oven dried at 60˚ C, and weighed to the nearest 0.0001 gram.

We excluded all years before N and P addition in 2011 because there were large differences in the amount of data available pre- and post-treatment. The data available prior to fertilizer addition in 2011 were severely unbalanced, with 2 out of 12 trees accounting for 82% of the total number of twig segments (Table 3).

Statistical analysis

We used a linear mixed effect model with tree as a random effect to determine if twig growth could be significantly explained by forest stand, year, N*P nutrient additions, or depth in the crown. Depth in the crown was scaled from 0 to 1 with 0 as the top of the crown. The number of samples in a tree varied from 3 to 7 depending on the crown depth of each tree (Table 1). The total number of sampling locations was 60, and there was a total of 2,429 observations of twig segments from 2011 to 2017. Response variables included twig length and twig mass. The residuals of the linear mixed effect models were non-normally distributed as assessed by visual observation, having large tails even after log-transforming the response variables (Appendix 9). The p-values for the log transformed Shapiro-Wilks tests were all < 0.01, indicating non-normal distributions.

We relied on a model selection approach to compare 14 candidate models with every combination of stand, year, N+P addition, and depth in the crown to identify explanatory variables for twig growth in sugar maple crowns. Model selection was performed using AICc with the best model having the lowest AIC score and the highest weight. We also calculated the

minimal detectable difference in twig length and mass required to produce a significant difference related to treatment (Zarr 1984).

R version 3.5.1 (R Core Team 2018) and the packages lme4 (Bates et al. 2015), MuMIn (Barton 2018) and ggplot2 (Wickham 2016) were used for this analysis.

Results

Twig growth was highly variable within and among tree crowns, such that twig length and mass did was not detectably different with N or P addition (Figures 8 and 9). The average annual twig length for the 12 trees was 22.8 mm, and average twig mass was 0.09 g. Trees varied in the number of twig segments for each twig age, with up to 20% of the total number of twig segments coming from one of the twelve trees, and as few as 3% of the total number of twig segments coming from the tree with the fewest years of twig growth (Table 3).

The best performing models for twig length and twig mass had twig age with no additional terms (Table 4). The weights for each of these candidate models was also the highest, indicating that additional terms in the model were not justified. Annual twig length increment increased with twig age ($p = 0.03$). Twig mass was lowest in the youngest twigs segments ($p = <0.01$), because older twig increments accrue secondary growth and increase in diameter (Table 5).

The minimum detectable difference necessary to observe a significant treatment response was a 13 mm or 58% difference in twig length, or a 0.06 g or 66% difference in twig mass. It would require much higher replication to detect ecologically relevant adjustment in twig length and mass in response to treatment.

Discussion

Our measurements of resource allocation to twig growth and light exploration offer a novel view of within-individual resource optimization. While many characteristics of leaves showed consistent responses to depth in the crown, twig growth was best explained by twig age. Variation related to increased N and P availability was not sufficient to be detected given our sampling intensity.

Chapter 4: Conclusion

Adjustments in leaf characteristics within tree crowns to both light environment and soil nutrient availability are critical for trees, with changes in leaf characteristics more apparent than changes in twig growth. This study had the advantage of exploring both within-crown variation and response to increased soil nutrient availability. We saw that leaf characteristics related to N availability responded to the soil nutrient treatments, whereas characteristics related to light availability had strong relationships with depth in the crown. While many leaf characteristics changed with N addition, trees that received P had different patterns of resource allocation with depth in the crown and dramatically lower N:P ratio, possibly indicating an alleviation of P limitation (Goswami et al. 2018, Gonzales and Yanai 2019).

Adjustments in resource allocation with depth in the crown are useful for accounting for leaf characteristics across a gradient of light availability. Repeated sampling from an individual provides information on phenotypic plasticity.

Studies that focus only on well-lit leaves neglect most of the leaves in a crown, which may create a bias when measuring leaf characteristics that have strong relationships with depth in the crown.

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Tables

Table 1. Characteristics of the three forest stands and 12 trees used in this study. These mature stands in the Bartlett Experimental Forest, NH are part of an ongoing nutrient limitation research project on Multiple Element Limitation in Northern Hardwood Ecosystems. Tree diameter, height, and crown depth varied among the 12 trees.

Stand	Year clearcut	Elevation (m ASL)	Aspect	Slope (%)	DBH (cm)				Height (m)				Crown depth (m)			
					C	N	P	N+P	C	N	P	N+P	C	N	P	N+P
C7	1890	440	ENE	5–10	59.3	42.6	64.5	49.3	24	20	24	20	14	8	8	6
C8	1883	330	NE	5–35	50.7	40.8	49.5	52.9	25	23	24	25	10	6	10	10
C9	1890	440	NE	10–35	55.7	44.0	59.8	53.8	25	24	23	22	10	12	10	12

Table 2. Leaf characteristics respond to depth in tree crowns and experimental additions of N and P. The slopes of leaf characteristics as a function of depth in the crown were examined by fitting a linear model for each of the 12 trees. To compare the slope in common units for each leaf characteristic, we normalized by dividing the slope for each tree by the inter-quartile range of the values for that tree. Relationships of leaf characteristics with depth in the crown were evaluated using a t-test asking if the values differed from 0, and an N by P factorial ANOVA blocked by stand. The intercept and average values for each leaf characteristic also evaluated using an N by P factorial ANOVA blocked by stand. The degrees of freedom for the t-tests are 11. The degrees freedom for the sources of variation in the ANOVA are Stand: 2, N: 1, P: 1, and N*P: 1. Values that are underlined are $p \leq 0.05$.

	Depth in crowns t-test on slope		Slope p-values			Intercept p-values			crown average p-values		
	slope	p-value	N	P	N*P	N	P	N*P	N	P	N*P
Physical characteristics											
Area	1.93	< 0.01	0.83	0.09	0.65	0.39	0.39	0.23	< 0.01 +	0.72	0.14
Mass	-1.16	0.04	0.62	0.05	0.52	0.44	0.54	0.63	0.28	0.39	0.31
SLA	1.93	< 0.01	0.95	0.18	0.85	0.14	0.50	0.22	0.44	0.54	0.63
Photosynthetic pigments											
Carotenoids	1.69	< 0.01	0.11	0.29	0.31	0.02 +	0.34	0.82	0.25	0.87	0.88
Chlorophyll A	2.19	< 0.01	0.70	0.27	0.44	0.04 +	0.30	0.98	0.17	0.51	0.33
Chlorophyll B	2.29	< 0.01	0.92	0.43	0.66	0.07	0.27	1.00	0.57	0.67	0.66
Metabolites											
Alanine	0.39	0.41	0.11	0.41	0.81	< 0.01 +	0.34	0.84	0.04 +	0.88	0.41
GABA	-0.75	0.10	0.38	0.43	0.33	0.07	0.38	0.88	0.01 +	0.71	0.02
Glutamate	1.65	< 0.01	0.13	0.88	0.94	0.02 +	0.44	0.57	0.37	0.33	0.90
Valine	-0.85	0.08	0.10	0.38	0.50	0.04 +	0.29	0.78	0.88	0.77	0.25
Polyamines											
Putrescine	-2.00	< 0.01	0.18	0.65	0.17	0.51	0.79	0.32	0.34	0.53	0.71
Spermidine	-0.72	0.08	0.78	0.39	0.43	0.43	0.30	0.79	0.23	0.02 +	0.41
Spermine	0.46	0.70	0.11	0.59	0.98	0.31	1.00	0.67	0.10	0.09	0.08
Elements											
Aluminum	0.12	0.78	0.30	0.05	0.42	0.05 +	0.50	0.54	0.51	0.81	0.99
Boron	1.51	< 0.01	0.98	0.04	0.44	0.84	0.38	0.49	0.17	0.47	0.38
Manganese	0.77	0.12	0.58	0.25	0.49	0.01 +	0.74	0.29	0.27	0.89	0.33
Nitrogen	1.39	< 0.01	0.92	0.14	0.78	0.01 +	0.96	0.36	0.23	0.46	0.56
N:P ratio	-0.41	0.46	0.54	0.40	0.39	< 0.01 +	< 0.01 -	0.02 -	0.83	0.36	0.20
Phosphorus	1.14	0.07	0.48	0.03	0.31	0.16	0.03 +	0.12	0.50	0.42	0.34

Table 3. The number of twig segments from each year of growth for each tree expressed as a proportion of the total number of twig segments. If each of the trees had equal representation, there would be ~8% for each tree and ~10% for each twig age.

	Tree and Treatment												
Year	Con C8	Con C7	Con C9	N C8	N C9	N C7	NP C7	NP C9	NP C8	P C9	P C8	P C7	Proportion
2007	1	36	8	3	8	7	0	17	9	0	7	17	4%
2008	2	37	9	5	8	8	0	17	11	0	7	19	4%
2009	2	41	9	5	10	8	0	18	11	2	10	21	5%
2010	4	41	11	5	12	9	0	18	13	2	9	21	5%
2011	5	42	16	6	16	10	0	20	14	2	11	21	6%
2012	7	57	20	6	24	16	4	25	21	10	15	35	8%
2013	12	63	30	7	34	21	8	33	31	14	20	39	11%
2014	13	68	37	10	42	22	11	37	35	19	25	40	12%
2015	18	69	46	13	51	24	15	40	39	28	25	43	14%
2016	19	70	49	19	53	28	21	43	48	30	26	43	15%
2017	24	72	53	23	54	31	31	43	51	32	35	46	17%
Proportion	4%	20%	10%	3%	11%	6%	3%	11%	10%	5%	6%	12%	

Table 4. Model selection for twig length and mass was performed for 14 candidate models.

Twig length model selection

Model	Stand	Age	N*P	Depth in the crown	df	AICc	ΔAICc	weight
l2		x			4	535.8	0.0	0.6
l1	x				5	537.5	1.7	0.2
l3			x		6	539.1	3.4	0.1
l4				x	4	540.9	5.1	0.0
l5		x	x		6	541.6	5.8	0.0
l8		x	x		7	543.3	7.5	0.0
l6	x		x		8	544.6	8.8	0.0
l9		x		x	5	545.0	9.2	0.0
l7	x			x	6	546.7	10.9	0.0
l10			x	x	7	548.4	12.7	0.0
l11	x	x	x		9	548.8	13.1	0.0
l12	x	x		x	7	550.8	15.1	0.0
l13		x	x	x	8	552.6	16.8	0.0
l14	x	x	x	x	10	558.2	22.4	0.0

Twig mass model selection

Model	Stand	Age	N*P	Crown depth	df	AICc	ΔAICc	weight
m2		x			4	-1275	0.0	1.0
m5	x	x			6	-1259	16.1	0
m1	x				5	-1259	16.4	0
m8		x	x		7	-1252	22.7	0
m3			x		6	-1252	23.0	0
m11	x	x			9	-1237	38.4	0
m6	x		x		8	-1236	38.8	0
m9		x		x	9	-1235	40.1	0
m4				x	8	-1234	41.3	0
m12	x	x			11	-1219	56.4	0
m7	x			x	10	-1217	57.7	0
m13		x	x	x	12	-1212	63.2	0
m10			x	x	11	-1211	64.5	0
m14	x	x	x	x	14	-1196	79.1	0

Table 5. Linear mixed effect model results for twig length and twig mass.

	Source	Sum squares	Numerator DF	Denominator DF	F value	p value
Twig length	Twig age	1.05	1	368.3	5.01	0.03
Twig mass	Twig age	0.09	1	368.04	15.04	< 0.01

Figures

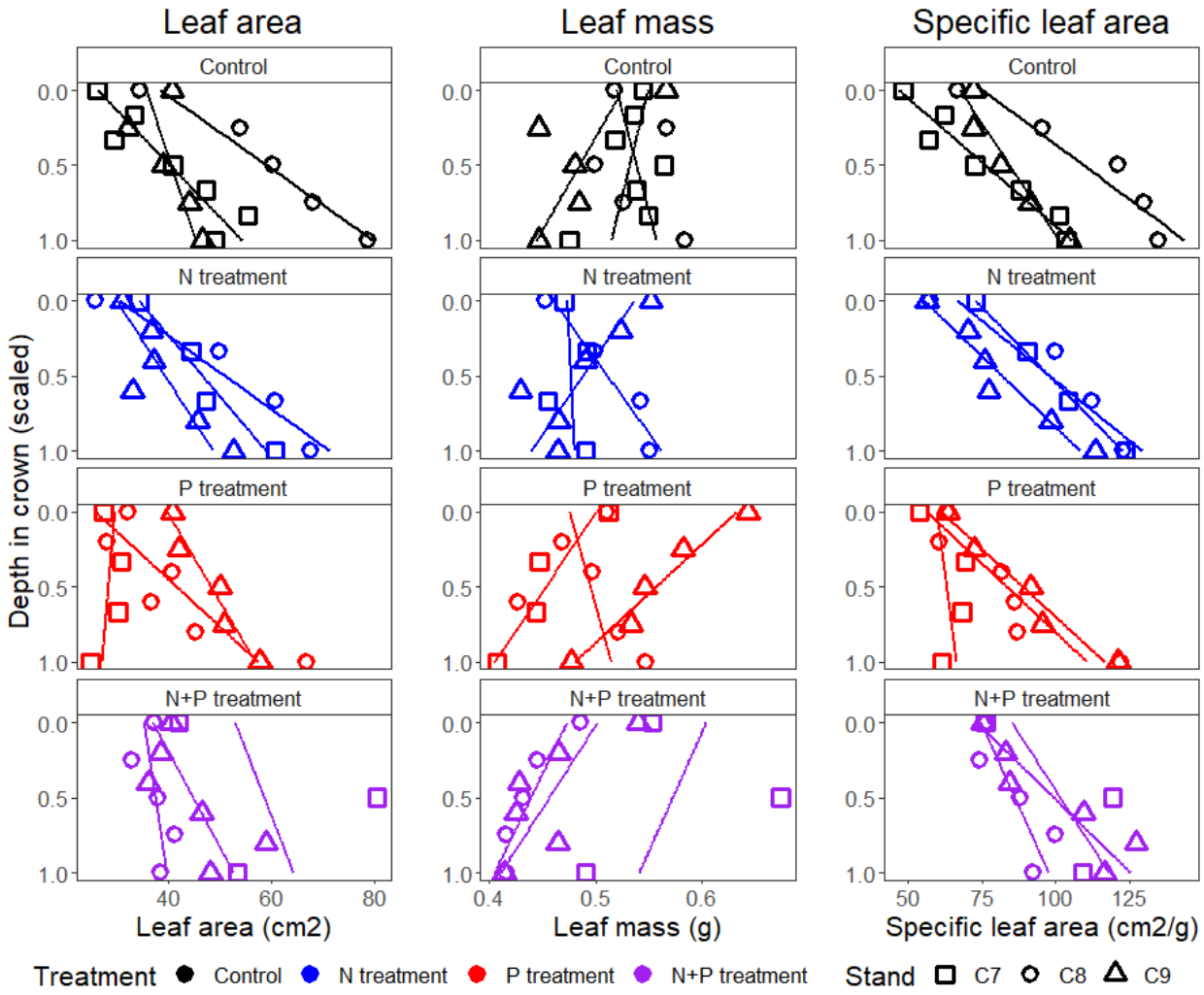


Figure 1. Leaf area, leaf mass, and specific leaf area as a function of depth in the crown for mature sugar maple trees at the Bartlett Experimental Forest, NH. Data points represent leaf measurements from each of the 12 trees (lines). Height in the canopy was scaled from the top of the crown (0) to the bottom (1). Leaf area and mass had different relationships with increasing crown depth with P addition, but these canceled out such that specific leaf area as a function of crown depth did not change with experimental additions of N and P.

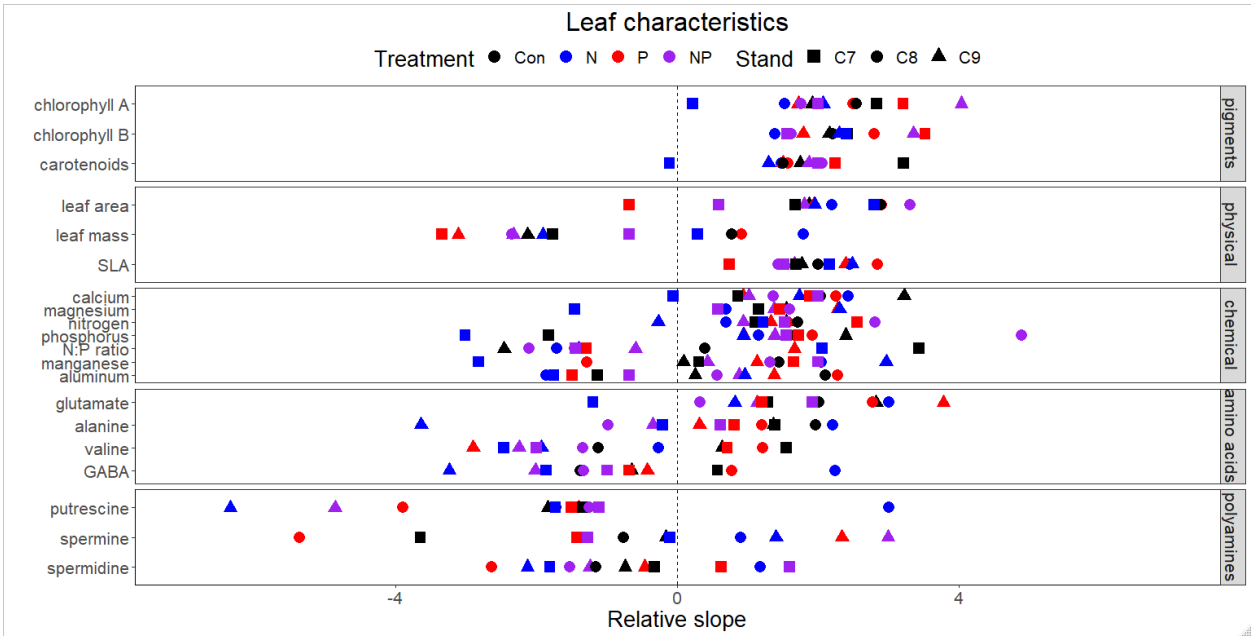


Figure 2. Leaf characteristics had both positive and negative relationships with depth in the crown. Leaf characteristics related to light capture such as the concentration of photosynthetic pigments all increased with depth in the crown. Foliar element concentrations in general showed increases with depth in tree crowns, whereas amino acids and polyamines decreased slightly with depth in the crown.

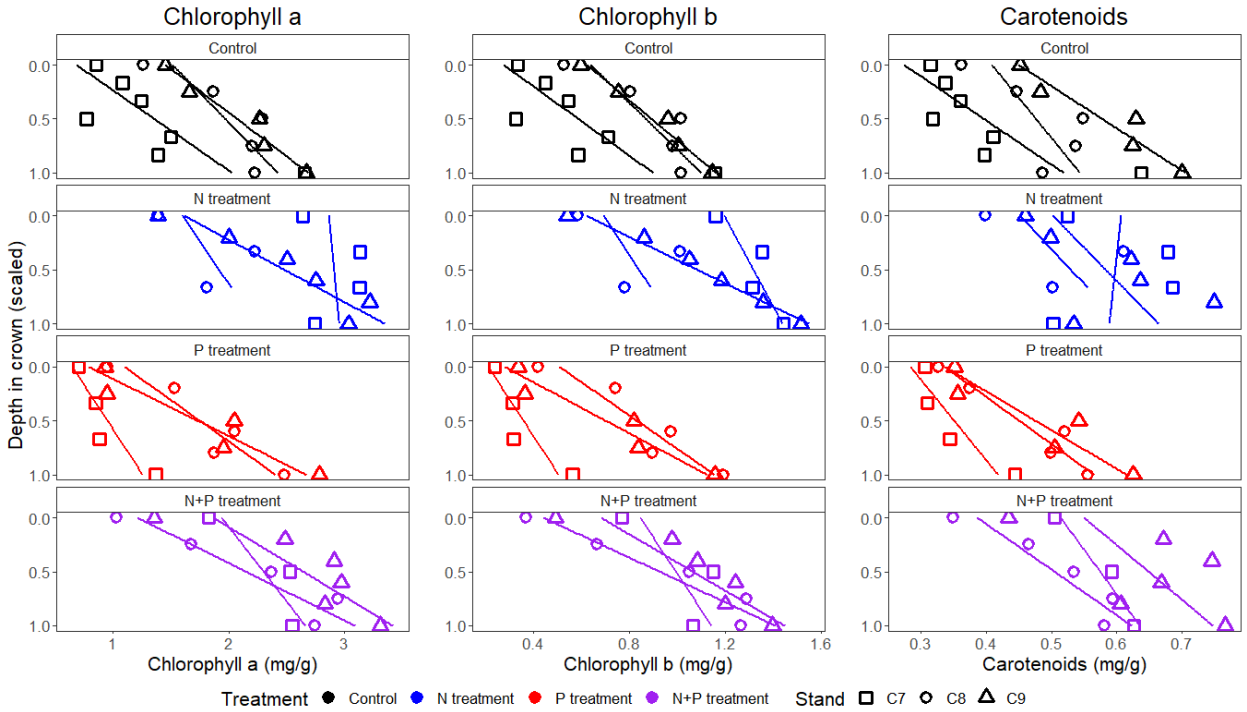


Figure 3. Photosynthetic pigment concentrations on a leaf mass and leaf area basis as a function of depth in the crown for sugar maple trees at the Bartlett Experimental Forest, NH. Trees that received N had higher concentrations of chlorophyll a ($p = 0.07$) and chlorophyll b ($p = 0.05$), and carotenoids ($p = 0.03$) at the top of their crowns. The increase in photosynthetic pigments can be seen throughout the crown and N addition did not strongly change the relationship of photosynthetic pigments and depth in the crown. Data points represent leaf concentrations from each of the 12 trees (lines).

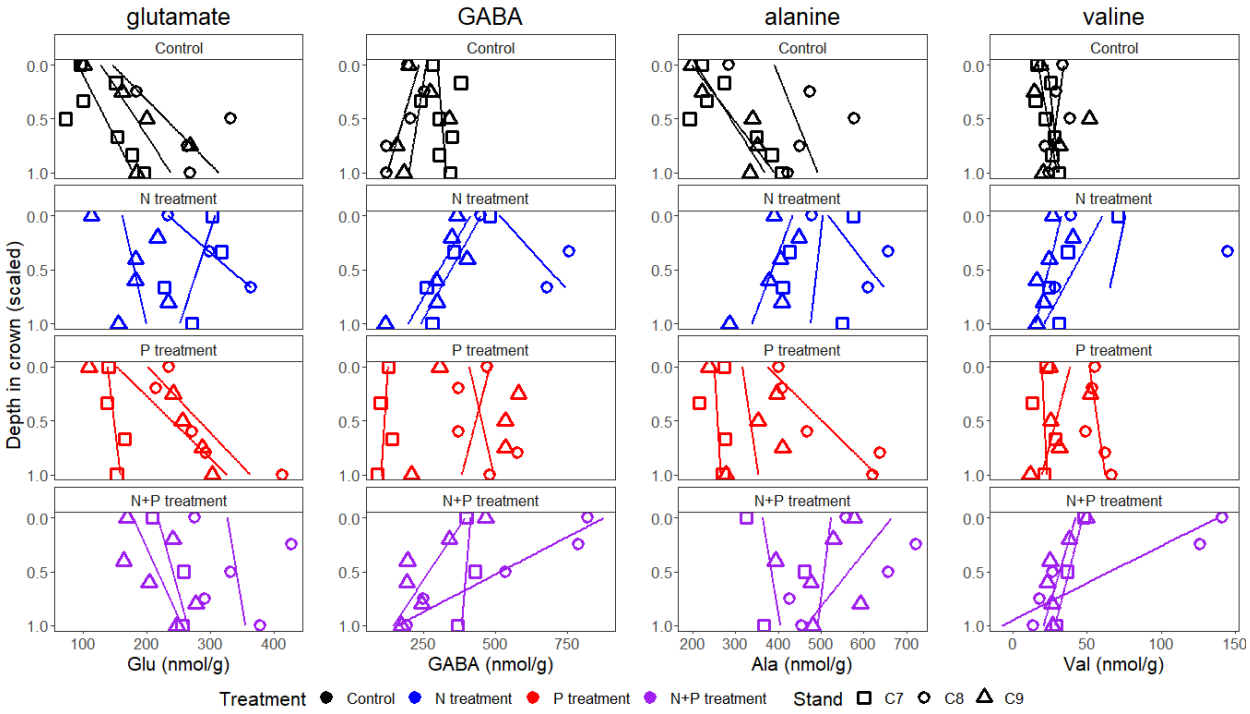


Figure 4. Foliar concentrations of amino acids Glu, Ala, GABA, and Val as a function of depth in the crown for mature sugar maple trees at the Bartlett Experimental Forest, NH. Glutamate was the only amino acid to have a strong relationship with depth in tree crowns ($p \leq 0.01$). The amino acids Glu, GABA, Ala, and Val all increased with N addition ($p < 0.07$).

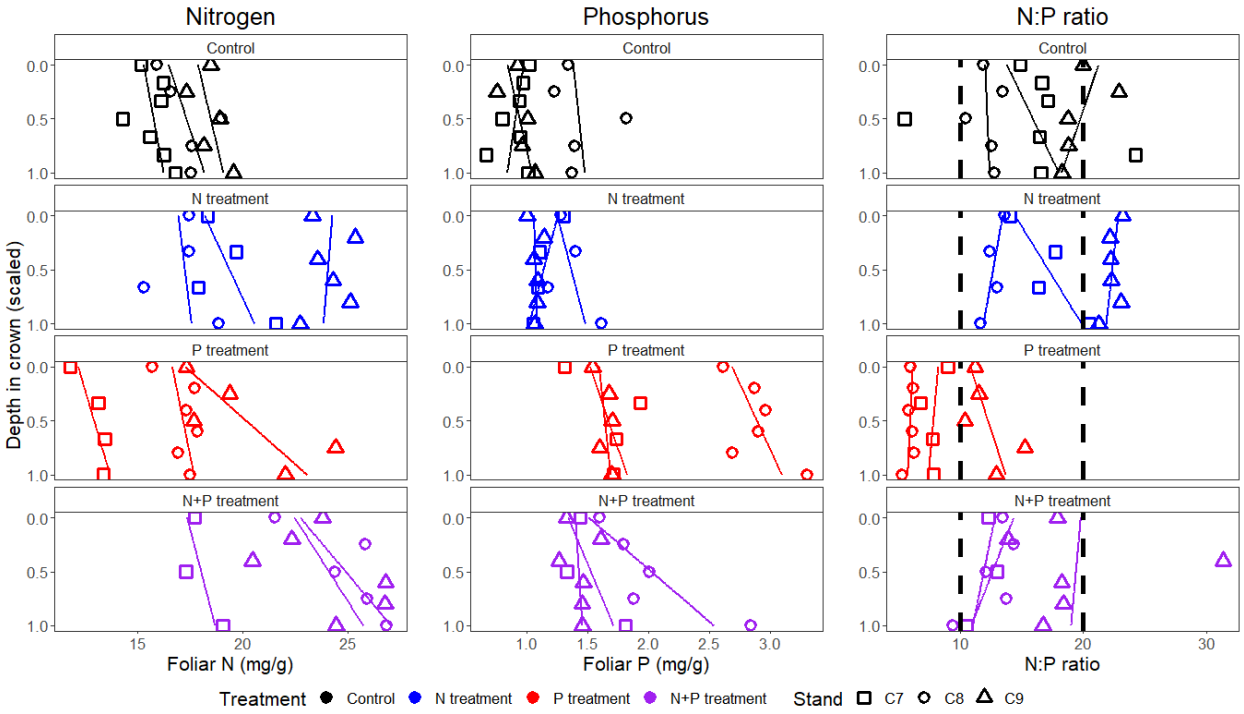


Figure 5. Foliar N and P increased with depth in the crown and also increased in response to N and P addition. Trees that received N had higher foliar N at the top of tree crowns ($p = 0.01$), and trees that received P had higher foliar P at the top of tree crowns ($p = 0.03$). Trees that received P also had a steeper increase in leaf P as a function of depth in the crown which was most pronounced in the leaves at the bottom of the crown ($p = 0.03$). The rate of change of N:P from the top to bottom leaves did not differ strongly with crown depth ($p = 0.46$), but the addition of P alone strongly decreased the N:P ratio in leaves at the top of the crown ($p = 0.02$).

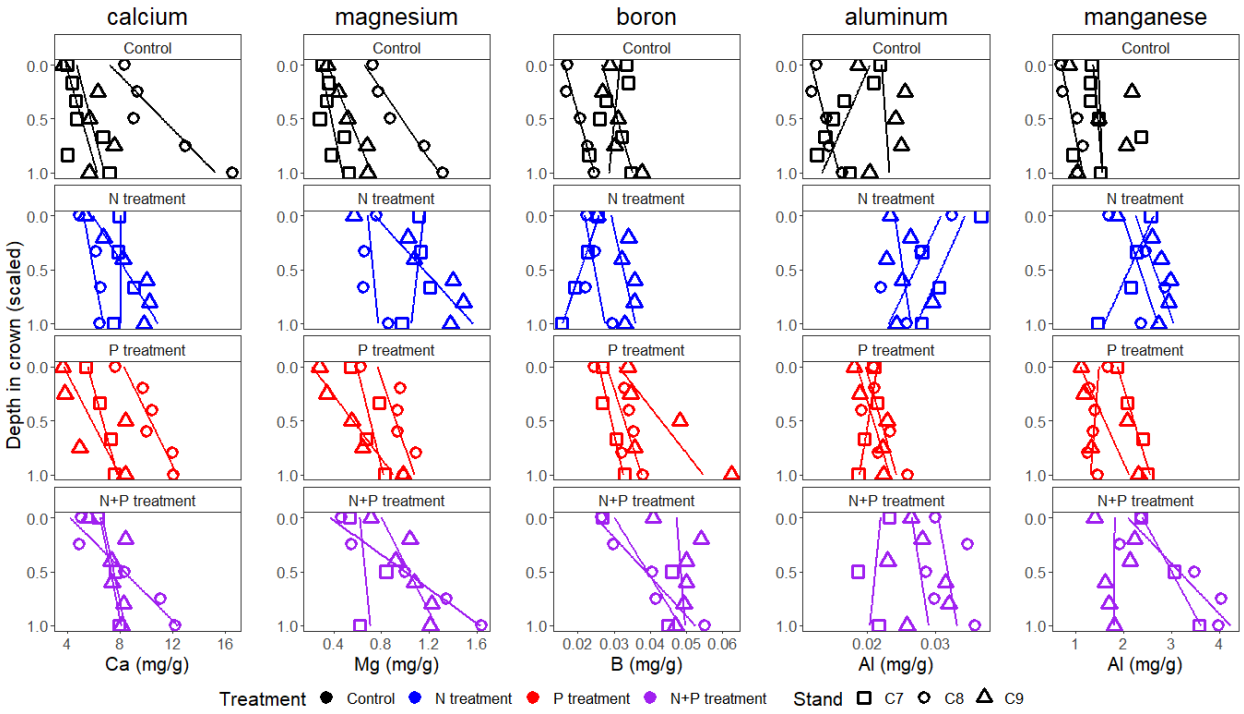


Figure 6. The concentration of Ca, Mg, B, Al, and Mn in sugar maple leaves as a function of depth in the crown. Aluminum and Mn are toxins and these concentrations were highest in the top of the crowns of trees that received N addition ($p \leq 0.05$). Trees that received P had significantly steeper increases in leaf Al and B ($p = 0.05$).

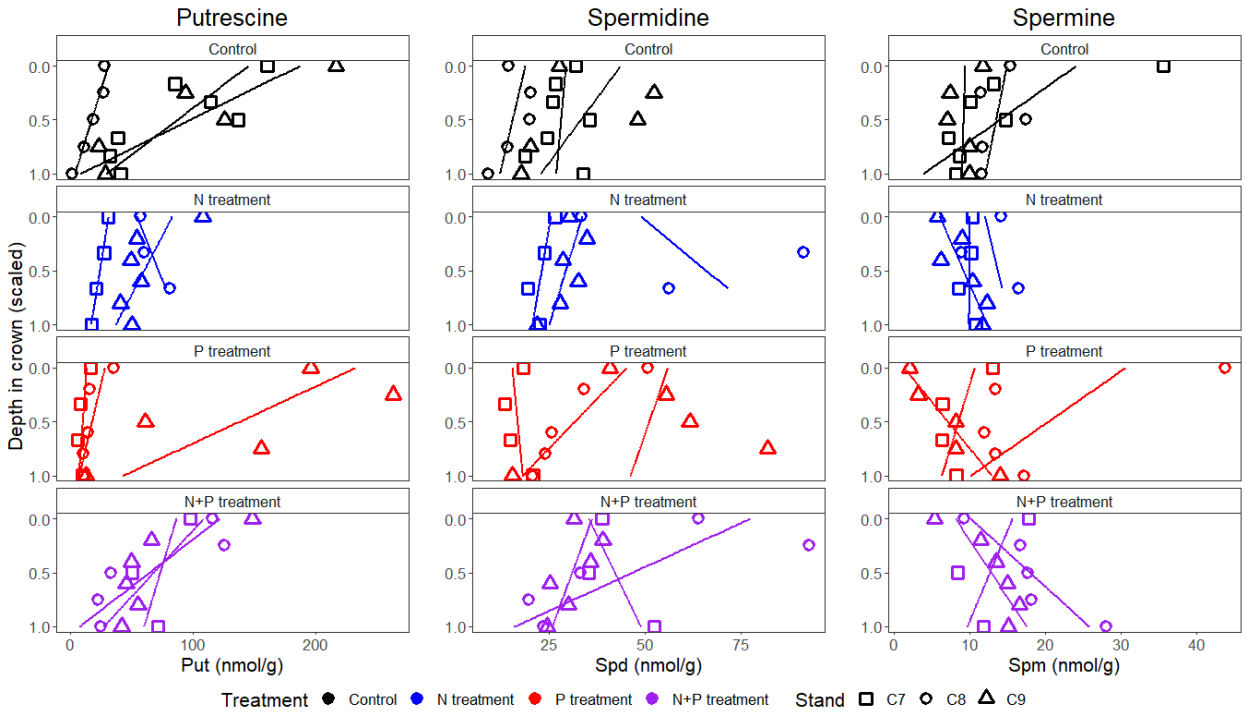


Figure 7. Polyamine concentrations as a function of depth in the crown for sugar maples in the Bartlett Experimental Forest, NH. Putrescine decreased with depth in the crown ($p = 0.01$). Spermidine was higher in trees that received P ($p = 0.02$). Spermine and spermidine did not have a strong relationship with depth in the crown.

Figure 8. Annual twig length as a function of depth in the crown for 12 mature sugar maple trees over seven years. Annual twig length increased over the 7 year period (p = 0.03).

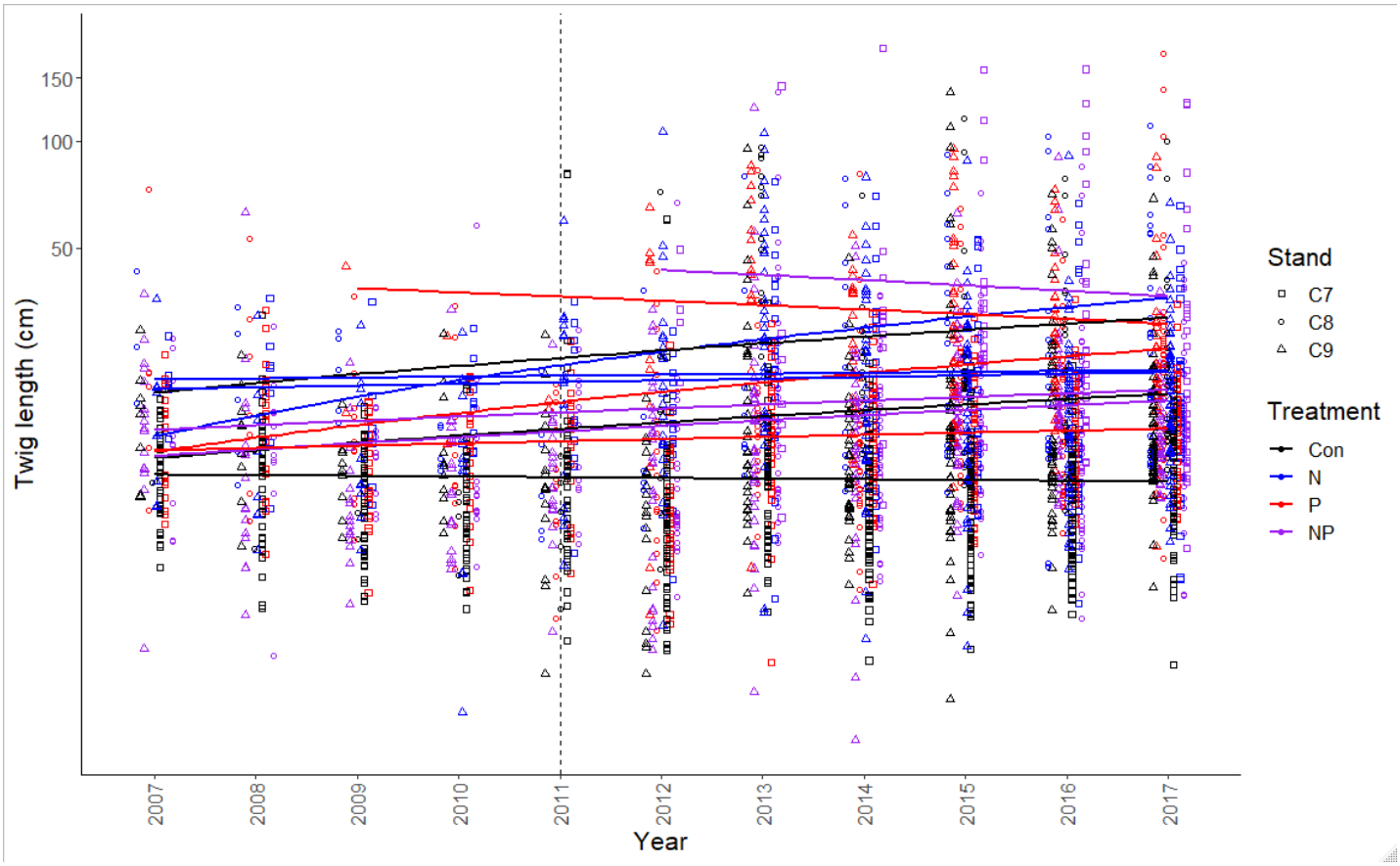
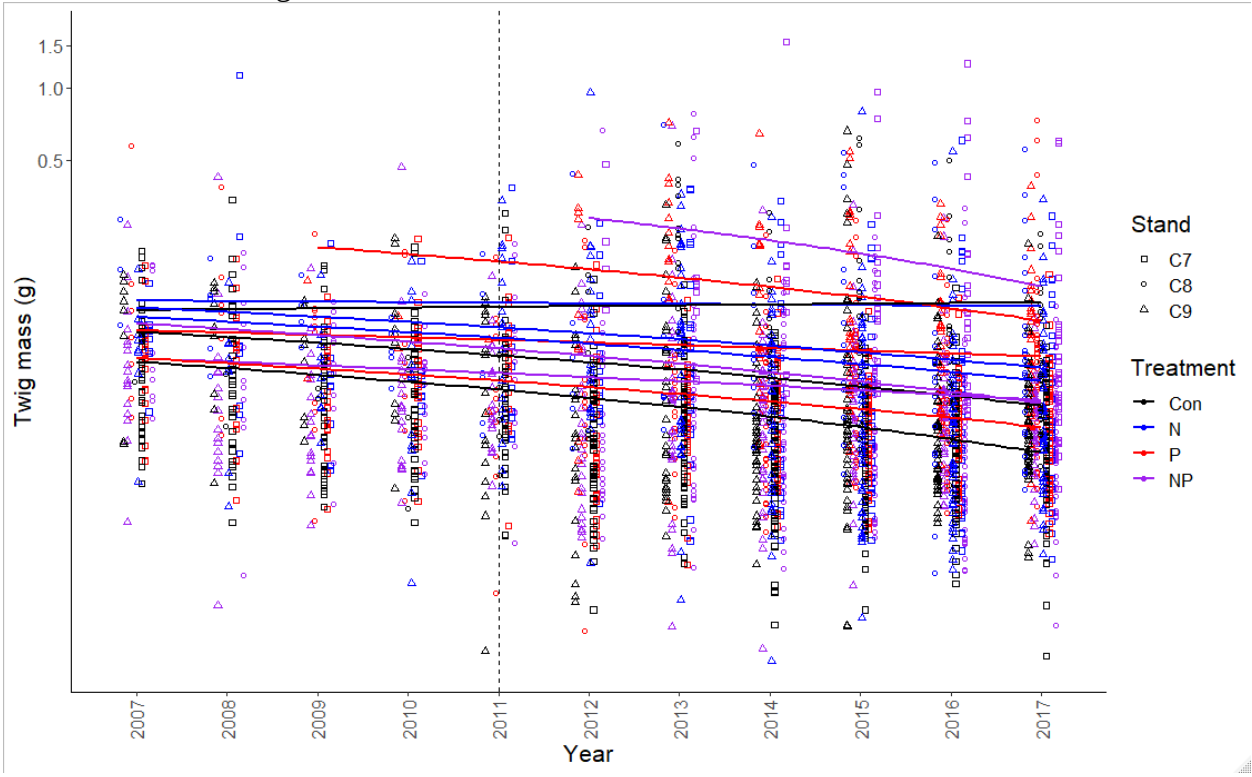


Figure 9. Annual twig mass increments as a function of depth in the crown over 7 years. Annual twig mass was lowest in youngest twigs ($p < 0.01$) due to secondary growth leading to diameter increases in older twigs.



Appendix

Appendix 1. Standard reference material was used to assess the recovery of analyte concentrations during microwave digestion. Panel A uses standard reference material NIST 1515 apple tissue. Panel B shows values for samples that were run with a duplicate sample during digestion.

Panel A.

	Sample	Al (%)	B (%)	Ca (%)	Fe (%)	K (%)	Mg (%)	Mn (%)	P (%)	S (%)	Sr (%)	Zn (%)
% Recovery for each	app1_A	78	99	103	90	96	94	103	94	93	99	99
	app2_A	77	93	100	86	93	92	99	89	87	97	95
	app1_B	78	108	98	85	94	87	103	95	88	101	90
	app2_B	72	114	97	83	89	84	100	93	85	99	88
	app1_C	75	96	100	90	86	89	102	92	84	91	93
	app2_C	78	98	104	91	90	96	107	93	85	96	92
All recoveries within		28	14	3	17	15	16	7	11	16	9	12
Average absolute recovery		24	6	2	12	9	10	3	7	13	3	7

Panel B.

	Sample	Al (%)	B (%)	Ca (%)	Fe (%)	K (%)	Mg (%)	Mn (%)	P (%)	S (%)	Sr (%)	Zn (%)
% difference for each	recov_101	109	97	105	100	105	107	103	103	105	108	108
	recov_132	107	104	102	108	105	104	104	106	107	102	102
	recov_139	100	109	103	100	109	97	103	104	107	114	94
All differences within		9	13	5	8	9	6	4	6	7	14	7
Average absolute recovery		5	5	3	3	7	4	3	4	6	8	5

Appendix 2. CN analysis used standard reference material NIST APP1575A, APP1547, and APP1515. Asterisks represent values that are not available for standard reference material, but come from 349 NIST1515, 315 NIST1547, and 318 NIST1575A laboratory measurements of C and N concentrations for reference material in the Durham lab.

	Apple tissue	N (%)	C (%)
% Recovery	NIST1575A	91.1	101.4
	NIST1575A	90.2	101.0
	NIST1547	92.9	101.8
	NIST1547	94.9	101.3
	NIST1515	93.8	101.1
	L14A	100.0	107.1
	L14A	94.7	105.9
All recoveries within		10.0	9.0
Average absolute recovery		6.0	3.0

Appendix 3. P values for the Shapiro Wilks test used to assess the normality of the distribution of the N by P ANOVA model residuals. We were not able to reject the null hypothesis that the data are normally distributed at an α of 0.05.

Response Variable	ANOVA on intercept	ANOVA on slopes
Aluminum	0.53	0.87
Alanine	0.99	0.98
Leaf area	0.53	0.35
Arginine	0.37	0.13
Asparagine	0.99	0.48
Boron	0.27	1.00
Carbon	0.62	0.97
Calcium	0.50	0.24
carotenoids	0.71	0.75
Chl-a	0.64	0.36
Chl-b	0.43	0.87
Chl-a:b ratio	0.44	0.92
Iron	0.60	0.23
GABA	0.45	1.00
Glutamate	0.06	0.50
Isoleucine	0.76	0.89
Potassium	0.97	0.38
Leucine	0.82	0.88
Lysine	0.19	0.22
Leaf mass	0.39	0.85
Magnesium	0.04	0.75
Manganese	0.92	0.07
Nitrogen	0.67	0.43
N:P ratio	0.54	0.25
Phosphorus	0.83	0.34
Proline	0.33	0.29
Protein	0.94	0.43
Putrescine	0.39	0.81
Sulphur	0.69	0.16
Soluble aluminum	0.97	0.81
Soluble calcium	0.63	0.81
Soluble potassium	0.98	0.54
Soluble magnesium	0.66	0.99
Soluble manganese	0.93	0.62
Soluble phosphorus	0.44	0.75
Soluble Zinc	0.13	0.30
Specific leaf area	0.64	0.74

Appendix 4. P values for the entire set of physical measurements and concentrations of photosynthetic pigments, amino acids, polyamines, and elements per unit mass. The t-test tests the 12 trees slope value against 0. The slope, intercept, and average values for each of the 12 trees were analyzed separately in three N x P factorial ANOVA with the three forest stands used as a blocking factor.

		t-test		Slope			Intercept			Average		
type	resp.var	y.mean	p-value	N	P	N*P	N	P	N*P	N	P	N*P
Physical	area.cm2	1.93	0.00	0.83	0.09	0.65	0.39	0.39	0.23	0.00	0.72	0.14
Physical	mass.g	-1.16	0.04	0.62	0.05	0.52	0.44	0.54	0.63	0.28	0.39	0.31
Physical	SLA	1.93	0.00	0.95	0.18	0.85	0.14	0.50	0.22	0.44	0.54	0.63
Photosynthetic pign	carot	1.69	0.00	0.11	0.29	0.31	0.02	0.34	0.82	0.25	0.87	0.88
Photosynthetic pign	Chl.A	2.19	0.00	0.70	0.27	0.44	0.04	0.30	0.98	0.17	0.51	0.33
Photosynthetic pign	Chl.B	2.29	0.00	0.92	0.43	0.66	0.07	0.27	1.00	0.57	0.67	0.66
Photosynthetic pign	Chl.R	-2.44	0.00	0.69	0.41	0.69	0.91	0.22	0.83	0.74	0.44	0.95
Photosynthetic pign	total.chl	2.22	0.00	0.82	0.31	0.50	0.05	0.29	0.98	0.32	0.99	0.90
Metabolites	Ala	0.39	0.41	0.11	0.41	0.81	0.00	0.34	0.84	0.04	0.88	0.41
Metabolites	Arg	-0.94	0.09	0.20	0.44	0.43	0.23	0.41	0.47	0.44	0.96	0.43
Metabolites	Asp	0.58	0.15	0.84	0.27	0.86	0.47	0.28	0.35	0.48	0.26	0.52
Metabolites	GABA	-0.75	0.10	0.38	0.43	0.33	0.07	0.38	0.88	0.01	0.71	0.02
Metabolites	Glu	1.65	0.00	0.13	0.88	0.94	0.02	0.44	0.57	0.37	0.33	0.90
Metabolites	Ile	-0.99	0.01	0.15	0.32	0.59	0.14	0.35	0.91	0.03	0.63	0.12
Metabolites	Leu	-1.10	0.03	0.33	0.96	0.47	0.43	0.66	0.76	0.21	0.39	0.06
Metabolites	Lys	0.39	0.59	0.11	0.59	0.64	0.10	0.58	0.58	0.24	0.94	0.13
Metabolites	Pro	-2.26	0.01	0.43	0.26	0.45	0.44	0.26	0.48	0.11	0.08	0.07
Metabolites	protein	-2.46	0.00	0.54	0.87	0.34	0.71	0.41	0.41	0.62	0.53	0.40
Metabolites	Val	-0.85	0.08	0.10	0.38	0.50	0.04	0.29	0.78	0.88	0.77	0.25
Polyamine	Put	-2.00	0.01	0.18	0.65	0.17	0.51	0.79	0.32	0.34	0.53	0.71
Polyamine	Spd	-0.72	0.08	0.78	0.39	0.43	0.43	0.30	0.79	0.23	0.02	0.41
Polyamine	Spm	0.46	0.70	0.11	0.59	0.98	0.31	1.00	0.67	0.10	0.09	0.08
Elements	Al	0.12	0.78	0.30	0.05	0.42	0.05	0.50	0.54	0.51	0.81	0.99
Elements	B	1.51	0.01	0.98	0.04	0.44	0.84	0.38	0.49	0.17	0.47	0.38
Elements	C	-2.70	0.00	0.10	0.28	0.22	0.49	0.45	0.12	0.15	0.61	0.56
Elements	Ca	1.64	0.00	0.59	0.68	0.40	0.89	0.67	0.64	0.50	0.56	0.34
Elements	Fe	0.54	0.24	0.92	0.08	0.56	0.07	0.19	0.99	0.01	0.02	0.20
Elements	K	0.81	0.07	0.90	0.66	0.86	0.12	0.38	0.83	0.03	0.08	0.51
Elements	Mg	1.75	0.02	0.82	0.40	0.44	0.16	0.42	0.22	0.07	0.10	0.55
Elements	Mn	0.77	0.12	0.58	0.25	0.49	0.01	0.74	0.29	0.27	0.89	0.33
Elements	N	1.39	0.00	0.92	0.14	0.78	0.01	0.96	0.36	0.23	0.46	0.56
Elements	N_P	-0.41	0.46	0.54	0.40	0.39	0.00	0.00	0.02	0.83	0.36	0.20
Elements	P	1.14	0.07	0.48	0.03	0.31	0.16	0.03	0.12	0.50	0.42	0.34
Elements	S	1.13	0.04	0.41	0.68	0.53	0.03	0.90	0.73	0.38	0.20	0.61
Elements	Sr	1.48	0.00	0.25	0.65	0.42	0.97	0.60	0.66	0.16	0.60	0.77
Elements	Zn	2.09	0.00	0.27	0.34	0.25	0.14	0.43	0.34	0.03	0.47	0.11
Elements (soluble)	s.Al	0.26	0.66	0.33	0.77	0.88	0.01	0.69	0.12	0.10	0.78	0.48
Elements (soluble)	s.Ca	0.99	0.05	0.36	0.35	0.92	0.64	0.97	0.53	0.19	0.34	0.93
Elements (soluble)	s.K	-0.76	0.19	0.98	0.21	0.70	0.04	0.12	0.30	0.06	0.69	0.39
Elements (soluble)	s.Mg	1.41	0.00	0.32	0.54	0.18	0.12	0.58	0.24	0.25	0.62	0.89
Elements (soluble)	s.Mn	0.41	0.31	0.52	0.79	0.92	0.06	0.72	0.33	0.11	0.62	0.71
Elements (soluble)	s.P	0.07	0.93	0.87	0.78	0.98	0.17	0.04	0.12	0.12	0.60	0.19
Elements (soluble)	s.Zn	0.90	0.15	0.48	0.85	0.62	0.50	0.91	0.65	0.28	0.30	0.08

Appendix 5. The p-values for an N x P ANOVA comparing the slope, intercept, and average values for each tree and leaf characteristic. Concentrations per unit mass are shown on the left, whereas concentrations per unit area are shown on the right.

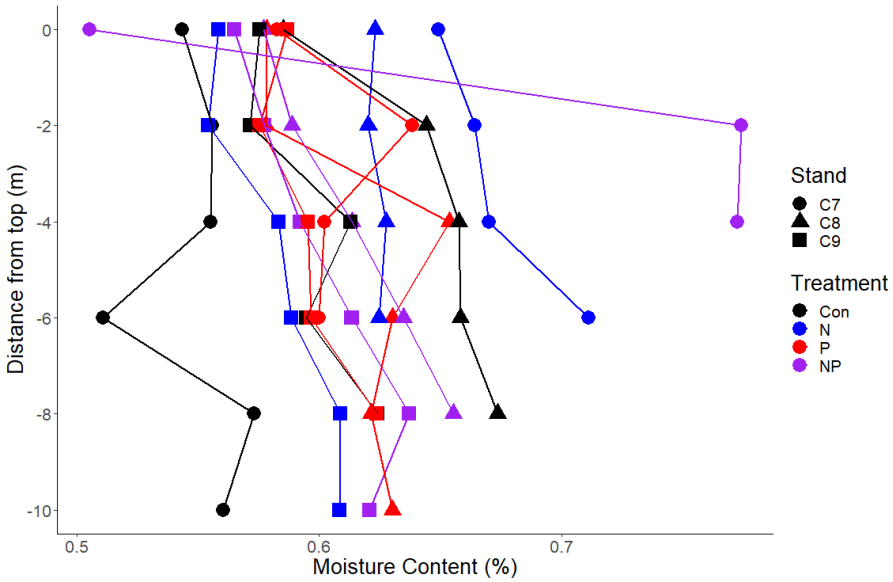
	MASS					AREA			
resp.var	type	Stand	N	P	N*P	Stand	N	P	N*P
Al	average	0.42	0.51	0.81	0.99	0.96	0.34	0.58	0.78
Ala	average	0.96	0.04	0.88	0.41	0.23	0.31	0.50	0.52
area.cm2	average	0.04	0.00	0.72	0.14	0.31	0.33	0.69	0.87
area2	average	0.85	0.59	0.96	0.15	0.96	0.79	0.67	0.78
Arg	average	0.21	0.44	0.96	0.43	0.03	0.13	0.33	0.32
Asp	average	0.53	0.48	0.26	0.52	0.02	0.02	0.49	0.12
B	average	0.83	0.17	0.47	0.38	0.22	0.01	0.31	0.36
C	average	0.08	0.15	0.61	0.56	0.85	0.38	0.99	0.46
Ca	average	0.55	0.50	0.56	0.34	0.85	0.31	0.97	0.21
carot	average	0.26	0.25	0.87	0.88	0.97	0.15	0.63	0.22
Chl.A	average	0.95	0.17	0.51	0.33	0.07	0.05	0.51	0.60
Chl.B	average	0.24	0.57	0.67	0.66	0.38	0.41	0.55	0.69
Chl.R	average	0.36	0.74	0.44	0.95	0.53	0.61	0.34	0.25
dry2	average	0.91	0.04	0.08	0.52	0.90	0.88	0.55	0.42
Fe	average	0.10	0.01	0.02	0.20	0.48	0.26	0.57	0.64
GABA	average	0.64	0.01	0.71	0.02	0.24	0.53	0.48	0.48
Glu	average	0.61	0.37	0.33	0.90	0.37	0.30	0.74	0.41
Ile	average	0.95	0.03	0.63	0.12	0.54	0.54	0.26	0.58
K	average	0.85	0.03	0.08	0.51	0.10	0.08	0.01	0.09
Leu	average	0.57	0.21	0.39	0.06	0.45	0.03	0.33	0.81
Lys	average	0.40	0.24	0.94	0.13	0.34	0.02	0.34	0.83
mass.g	average	0.67	0.28	0.39	0.31	0.53	0.19	0.76	0.38
Mg	average	0.97	0.07	0.10	0.55	0.99	0.04	0.63	0.47
Mn	average	0.76	0.27	0.89	0.33	0.19	0.36	0.71	0.57
N	average	0.53	0.23	0.46	0.56	0.41	0.47	0.13	0.76
N_P	average	0.06	0.83	0.36	0.20	0.46	0.39	0.82	0.55
P	average	0.79	0.50	0.42	0.34	0.31	0.05	0.88	0.33
Pro	average	0.23	0.11	0.08	0.07	0.98	0.06	0.87	0.23
protein	average	0.34	0.62	0.53	0.40	0.68	0.05	0.31	0.77
Put	average	0.29	0.34	0.53	0.71	0.15	0.94	0.18	0.59
S	average	0.98	0.38	0.20	0.61	0.93	0.56	0.77	0.83
s.Al	average	0.44	0.10	0.78	0.48	0.02	0.79	0.32	0.16
s.Ca	average	0.27	0.19	0.34	0.93	0.32	0.51	1.00	0.22
s.K	average	0.48	0.06	0.69	0.39	0.28	0.67	0.95	0.58
s.Mg	average	0.28	0.25	0.62	0.89	0.81	0.20	0.76	0.31
s.Mn	average	0.53	0.11	0.62	0.71	0.26	0.24	0.80	0.12
s.P	average	0.19	0.12	0.60	0.19	0.46	0.12	0.86	0.78
s.Zn	average	0.64	0.28	0.30	0.08	0.11	0.10	0.03	0.07
SLA	average	0.28	0.44	0.54	0.63	0.16	0.60	0.59	0.69

SLA2	average	0.96	0.68	0.88	0.47	0.63	0.58	0.57	0.66
Spd	average	0.04	0.23	0.02	0.41	0.02	0.22	0.02	0.42
Spm	average	0.22	0.10	0.09	0.08	0.90	0.62	0.75	0.40
Sr	average	0.35	0.16	0.60	0.77	0.06	0.12	0.18	0.17
STL	average	0.72	0.03	0.38	0.10	0.49	0.28	0.40	0.59
total.chl	average	0.55	0.32	0.99	0.90	0.43	0.02	0.61	0.08
twig.length	average	0.07	0.07	0.27	0.95	0.57	0.28	0.52	0.36
twig.mass	average	0.76	0.69	0.37	0.21	0.64	0.15	0.59	0.73
Val	average	0.76	0.88	0.77	0.25	0.42	0.36	0.36	0.36
wet2	average	0.94	0.48	0.95	0.39	0.44	0.18	0.20	0.91
Zn	average	0.11	0.03	0.47	0.11	0.71	0.38	0.94	0.17
Al	Intercept	0.61	0.05	0.50	0.54	0.67	0.28	0.39	0.31
Ala	Intercept	0.04	0.00	0.34	0.84	0.04	0.00	0.72	0.14
area.cm2	Intercept	0.81	0.39	0.39	0.23	0.28	0.44	0.54	0.63
area2	Intercept	0.04	0.41	0.95	0.65	0.08	0.15	0.61	0.56
Arg	Intercept	0.52	0.23	0.41	0.47	0.53	0.23	0.46	0.56
Asp	Intercept	0.05	0.47	0.28	0.35	0.06	0.83	0.36	0.20
B	Intercept	0.19	0.84	0.38	0.49	0.42	0.51	0.81	0.99
C	Intercept	0.23	0.49	0.45	0.12	0.83	0.17	0.47	0.38
Ca	Intercept	0.50	0.89	0.67	0.64	0.55	0.50	0.56	0.34
carot	Intercept	0.55	0.02	0.34	0.82	0.10	0.01	0.02	0.20
Chl.A	Intercept	0.90	0.04	0.30	0.98	0.85	0.03	0.08	0.51
Chl.B	Intercept	0.90	0.07	0.27	1.00	0.97	0.07	0.10	0.55
Chl.R	Intercept	0.63	0.91	0.22	0.83	0.76	0.27	0.89	0.33
dry2	Intercept	0.91	0.95	0.63	0.59	0.79	0.50	0.42	0.34
Fe	Intercept	0.92	0.07	0.19	0.99	0.98	0.38	0.20	0.61
GABA	Intercept	0.32	0.07	0.38	0.88	0.35	0.16	0.60	0.77
Glu	Intercept	0.22	0.02	0.44	0.57	0.11	0.03	0.47	0.11
Ile	Intercept	0.27	0.14	0.35	0.91	0.27	0.19	0.34	0.93
K	Intercept	0.24	0.12	0.38	0.83	0.48	0.06	0.69	0.39
Leu	Intercept	0.40	0.43	0.66	0.76	0.28	0.25	0.62	0.89
Lys	Intercept	0.51	0.10	0.58	0.58	0.53	0.11	0.62	0.71
mass.g	Intercept	0.28	0.44	0.54	0.63	0.44	0.10	0.78	0.48
Mg	Intercept	0.56	0.16	0.42	0.22	0.64	0.28	0.30	0.08
Mn	Intercept	0.05	0.01	0.74	0.29	0.19	0.12	0.60	0.19
N	Intercept	0.04	0.01	0.96	0.36	0.29	0.34	0.53	0.71
N_P	Intercept	0.00	0.00	0.00	0.02	0.04	0.23	0.02	0.41
P	Intercept	0.10	0.16	0.03	0.12	0.22	0.10	0.09	0.08
Pro	Intercept	0.52	0.44	0.26	0.48	0.53	0.48	0.26	0.52
protein	Intercept	0.39	0.71	0.41	0.41	0.61	0.37	0.33	0.90
Put	Intercept	0.15	0.51	0.79	0.32	0.21	0.44	0.96	0.43
S	Intercept	0.69	0.03	0.90	0.73	0.96	0.04	0.88	0.41
s.Al	Intercept	0.81	0.01	0.69	0.12	0.64	0.01	0.71	0.02
s.Ca	Intercept	0.91	0.64	0.97	0.53	0.76	0.88	0.77	0.25

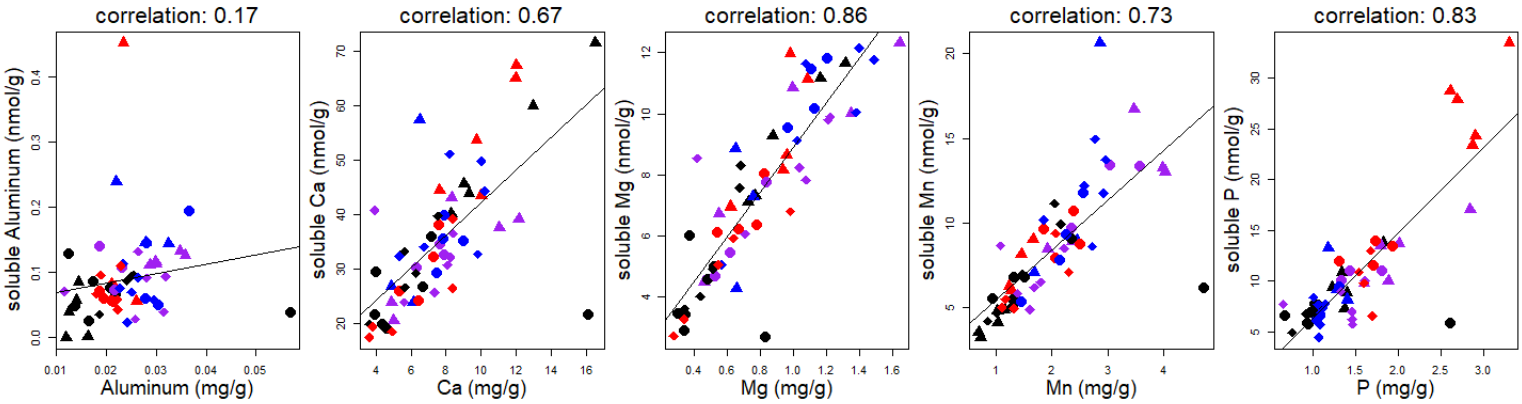
s.K	Intercept	0.60	0.04	0.12	0.30	0.95	0.03	0.63	0.12
s.Mg	Intercept	0.68	0.12	0.58	0.24	0.57	0.21	0.39	0.06
s.Mn	Intercept	0.29	0.06	0.72	0.33	0.40	0.24	0.94	0.13
s.P	Intercept	0.20	0.17	0.04	0.12	0.23	0.11	0.08	0.07
s.Zn	Intercept	0.65	0.50	0.91	0.65	0.55	0.32	0.99	0.90
SLA	Intercept	0.87	0.14	0.50	0.22	0.95	0.17	0.51	0.33
SLA2	Intercept	0.10	0.83	0.90	0.50	0.24	0.57	0.67	0.66
Spd	Intercept	0.25	0.43	0.30	0.79	0.36	0.74	0.44	0.95
Spm	Intercept	0.18	0.31	1.00	0.67	0.26	0.25	0.87	0.88
Sr	Intercept	0.43	0.97	0.60	0.66	0.34	0.62	0.53	0.40
STL	Intercept	0.63	0.99	0.63	0.71	0.94	0.48	0.95	0.39
total.chl	Intercept	0.91	0.05	0.29	0.98	0.91	0.04	0.08	0.52
twig.length	Intercept	0.99	0.86	0.85	0.56	0.85	0.59	0.96	0.15
twig.mass	Intercept	0.98	0.96	0.97	0.86	0.96	0.68	0.88	0.47
Val	Intercept	0.08	0.04	0.29	0.78	0.07	0.07	0.27	0.95
wet2	Intercept	0.86	0.86	0.56	0.41	0.76	0.69	0.37	0.21
Zn	Intercept	0.72	0.14	0.43	0.34	0.72	0.03	0.38	0.10
Al	Slope	0.03	0.30	0.05	0.42	0.51	0.32	0.07	0.29
Ala	Slope	0.77	0.11	0.41	0.81	0.21	0.00	0.51	0.20
area.cm2	Slope	0.24	0.83	0.09	0.65	0.01	0.62	0.05	0.52
area2	Slope	0.99	0.16	0.47	0.39	0.37	0.04	0.05	0.18
Arg	Slope	0.54	0.20	0.44	0.43	0.57	0.20	0.52	0.54
Asp	Slope	0.78	0.84	0.27	0.86	0.25	0.76	0.49	0.22
B	Slope	0.28	0.98	0.04	0.44	0.70	0.75	0.13	0.68
C	Slope	0.34	0.10	0.28	0.22	0.85	0.57	0.15	0.77
Ca	Slope	0.15	0.59	0.68	0.40	0.82	0.91	0.27	0.48
carot	Slope	0.16	0.11	0.29	0.31	0.90	0.11	0.04	0.31
Chl.A	Slope	0.09	0.70	0.27	0.44	0.49	0.28	0.06	0.36
Chl.B	Slope	0.15	0.92	0.43	0.66	0.51	0.51	0.10	0.48
Chl.R	Slope	0.91	0.69	0.41	0.69	0.78	0.92	0.29	0.41
dry2	Slope	0.40	0.89	0.46	0.55	0.61	0.90	0.21	0.50
Fe	Slope	0.15	0.92	0.08	0.56	0.72	0.63	0.05	0.45
GABA	Slope	0.89	0.38	0.43	0.33	0.55	0.13	0.64	0.60
Glu	Slope	0.11	0.13	0.88	0.94	0.12	0.00	0.19	0.07
Ile	Slope	0.46	0.15	0.32	0.59	0.32	0.13	0.35	0.83
K	Slope	0.94	0.90	0.66	0.86	0.56	0.48	0.70	0.52
Leu	Slope	0.75	0.33	0.96	0.47	0.44	0.26	0.71	0.64
Lys	Slope	0.40	0.11	0.59	0.64	0.49	0.10	0.69	0.73
mass.g	Slope	0.01	0.62	0.05	0.52	0.42	0.32	0.52	0.78
Mg	Slope	0.16	0.82	0.40	0.44	0.59	0.88	0.21	0.37
Mn	Slope	0.71	0.58	0.25	0.49	0.83	0.88	0.13	0.34
N	Slope	0.79	0.92	0.14	0.78	0.66	0.69	0.09	0.62
N_P	Slope	0.36	0.54	0.40	0.39	0.21	0.36	0.06	0.51
P	Slope	0.13	0.48	0.03	0.31	0.91	0.47	0.45	0.34

Pro	Slope	0.54	0.43	0.26	0.45	0.52	0.44	0.26	0.49
protein	Slope	0.50	0.54	0.87	0.34	0.93	0.77	0.19	0.93
Put	Slope	0.11	0.18	0.65	0.17	0.18	0.28	0.94	0.33
S	Slope	0.42	0.41	0.68	0.53	0.73	0.22	0.33	0.50
s.Al	Slope	0.40	0.33	0.77	0.88	0.54	0.04	0.52	0.28
s.Ca	Slope	0.03	0.36	0.35	0.92	0.76	0.58	0.61	0.57
s.K	Slope	0.27	0.98	0.21	0.70	0.64	0.61	0.97	0.54
s.Mg	Slope	0.02	0.32	0.54	0.18	0.52	0.32	0.16	0.17
s.Mn	Slope	0.37	0.52	0.79	0.92	0.40	0.78	0.73	0.59
s.P	Slope	0.16	0.87	0.78	0.98	0.83	0.42	0.98	0.24
s.Zn	Slope	0.95	0.48	0.85	0.62	0.94	0.80	0.84	0.62
SLA	Slope	0.55	0.95	0.18	0.85	0.55	0.08	0.88	0.41
SLA2	Slope	0.41	0.17	0.90	0.78	0.19	0.28	0.36	0.65
Spd	Slope	0.61	0.78	0.39	0.43	0.34	0.97	0.52	0.64
Spm	Slope	0.21	0.11	0.59	0.98	0.30	0.18	0.51	0.96
Sr	Slope	0.10	0.25	0.65	0.42	0.82	0.52	0.48	0.41
STL	Slope	0.29	0.32	0.42	0.97	0.38	0.54	0.52	0.80
total.chl	Slope	0.11	0.82	0.31	0.50	0.49	0.35	0.08	0.39
twig.length	Slope	0.80	0.52	0.84	0.62	0.87	0.38	0.66	0.31
twig.mass	Slope	0.80	0.45	0.72	0.69	0.83	0.37	0.56	0.45
Val	Slope	0.53	0.10	0.38	0.50	0.22	0.06	0.40	0.68
wet2	Slope	0.73	0.81	0.34	0.29	0.85	0.76	0.16	0.26
Zn	Slope	0.32	0.27	0.34	0.25	0.62	0.17	0.17	0.22

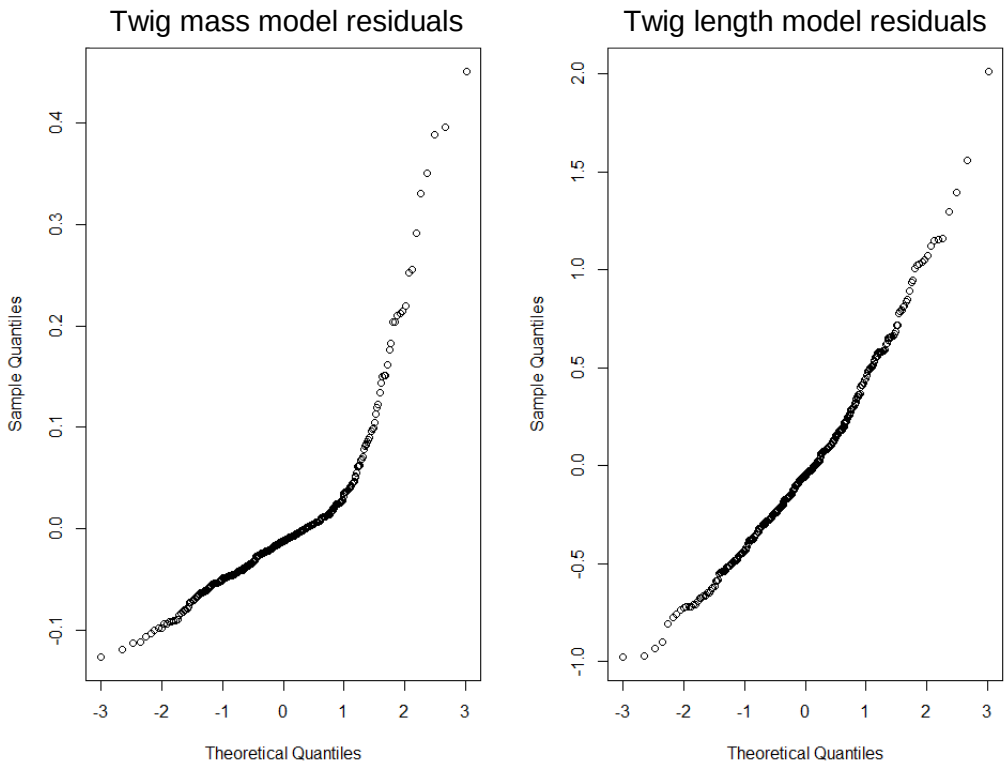
Appendix 6. Leaves were collected in the same 12 trees one year after the original collection to test for a treatment effect on leaf water content. There was no detectable effect of N or P addition on leaf water content.



Appendix 7. Correlations between total leaf element concentrations and dilute-acid soluble element concentrations.



Appendix 8. Log-transformed residuals for the generalized linear models for twig mass (left) and twig length (right) as a function of age.



Curriculum Vita

Alexander R. Young
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EDUCATION

Master of Science: Forest and Natural Resources Management August 2019
State University of New York College of Environmental Science and Forestry, Syracuse NY
Thesis title: "Sugar maple leaf characteristics respond to depth within the crown and to nitrogen and phosphorus addition"
Advisor: Dr. Ruth D. Yanai

Bachelor of Arts: Biology Major May 2015
Lewis & Clark College, Portland, Oregon
Biology focused study abroad program: Kenya and Tanzania
Advisor: Dr. Ken Clifton

PUBLICATIONS

Young, A., Miller, J., Villella, J., Carey, G., Miller, W. 2018. Epiphyte type and sampling height impact Microfauna communities in Douglas-fir trees. PeerJ6:e5699;DOI10.7717/peerj.5699

Young, A., Miller, W., Lowman, M. 2016. Tardigrades of the Canopy: *Milnesium swansoni* **sp. nov.** (Eutardigrada: Apochela: Milnesiidae) a new species from Kansas, U.S.A. *Zootaxa*. 4071(5).

Young, A., Clifton, K. 2015. Tardigrades inhabit lichen and moss in Smith Rocks State Park, Oregon. *Bulletin of the California Lichen Society 22(2).*

TECHNICAL SKILLS

- Programming: R, Python, SAS, Sigmaplot
- Geospatial Information Systems: ArcGIS, QGIS, Google Earth Engine
- Microsoft office: Access, Excel, Powerpoint, Word
- Canopy access: Tree Climbers International training

PROFESSIONAL EXPERIENCE

Dropcopter: Syracuse Tech Garden. Syracuse, NY January 2019
Commercial Drone Pilot

- Synthesized pollination trial data to demonstrate value to apple and cherry growers
- Received training to fly hex-copters to pollinate apple flowers in compliance with the FAA

Multiple Element Limitation in Northern Hardwood Ecosystems. Bartlett, NH Summer 2017, '18
Field Crew Co-Leader

- Coordinated and scheduled field operations for summer technicians and visiting researchers
- Executed stratified collection of sugar maple leaves to examine plasticity in leaf traits
- Used NEON AOP hyperspectral data to examine spectral indicators of N or P availability

SUNY College of Environmental Science and Forestry. Syracuse, NY Fall 2017, ’18
Graduate Teaching Assistant: Watershed Ecology & Management (’17), Introduction to Soils (’18)

- Designed quizzes to assess students’ engagement with course topics
- Instructed soil pit excavation to delineate soil horizons and root exploitable volume
- Graded written work for 81 students and graphed class scores to view grade distribution

SUNY College of Environmental Science and Forestry. Syracuse, NY Spring 2017, ’18
Graduate Research Assistant: Yanai Forest Ecology lab

- Guided leaf identification by high school students to record annual litter production
- Performed laboratory techniques to add to existing long-term datasets

Bartlett Tree Experts. Baltimore, MD Fall 2016
Production arborist & tree climber in training

- Pruned urban trees to improve tree growth and property aesthetics
- Abided by OSHA safety ordinances to promote site safety for general public and workers

United States Geological Survey. Sequoia National Park, CA Summer 2016
Biological Field Technician (GS-5, temporary)

- Assessed 34 sites for disease and digitally recorded detailed mortality reports
- Identified bark beetle galleries and fungal pathogens by removing bark
- Mapped and installed research plots with precise GPS points to align remote sensed data

California Academy of Sciences. San Francisco, CA Spring 2016
Accession Technician Specialist

- Identified, imaged, and accessioned 8,000 tardigrade specimens to digitize the collection
- Used morphometric analyses to view population dynamics of tardigrade communities

Siskiyou Biosurvey. Six Rivers, CA Fall 2015
Primary Investigator Canopy Microfauna

- Designed explorative investigation to view tardigrade, nematode, and rotifer ecology
- Identified epiphyte and micro-animal specimens using spot tests and morphometry

NSF REU “Tardigrades of the Canopy”. Baldwin City, KS Summer 2015
Canopy Research Assistant

- Taught, oversaw and ensured safety of 8 undergraduates’ tree climbing field work
- Analyzed vertical stratification of 18 tardigrade species across 104 trees, in 8 field sites

NSF REU “Tardigrades of the Canopy**”.** Baldwin City, KS Summer 2014
Canopy Research Intern

- Ascended into trees with rope, harness, and helmet to collect epiphyte habitat
- Created 2,152 slides of tardigrade specimens to archive community composition

Lewis & Clark College. Portland, OR Spring 2014
Teaching Assistant: Origins of life in the universe

- Assisted with 2 laboratory sections, graded lab reports and exams, held weekly office hours
- Supervised 24 undergraduates during experiential laboratory investigations

Lewis & Clark College. Portland, OR Fall 2014–Spring 2015
Biology Office Assistant

- Proctored student exams to facilitate compliance with student handbook guidelines
- Created flyers for visiting professors and 10 "biology talk" seminars

College Outdoors: Lewis & Clark College. Portland, OR Fall 2011–Spring 2014
Trip Leader + Gear Repair Specialist

- Guided students through technical environments to build familiarity with natural world
- Developed and taught ancient forest ecology clinics to promote forest conservation

Cylburn Arboretum. Baltimore, MD Summer 2012, Summer 2013
Senior Nature and Science Camp Councilor

- Guided 8-11 year old inner city Baltimore children to engage with natural environments
- Taught clean water curriculum to increase children's capacity to reduce, reuse, and recycle
- Led interactive water shed experiments using spray bottles and food dye simulating pollution

POSTERS

Young, A. Gabriel, M., Yanai. R. Detecting nutrient limitation from the sky using NEON AOP hyperspectral data. LTER All Scientists Meeting, Monterey, 2018

Yanai, R., Dillon, G., Drake, J., McConnel, T., **Young, A.,** Campbell, J., Green, M., Buckley, H., Case, B., Woollons, R. Measurement error in forest inventory (FIA) and error propagation in forest biomass models. Ecological Society of America, New Orleans LA, 2018.

Young, A., Ambrose, A., Baxter, W., Miller, W. Tardigrades in the Canopy: are there water bears at the top of giant sequoia? 14th Intl Syposium on Tardigrada, Copenhagen, Denmark Aug 1, 2018.

Dillon, G., **Young, A.**, Campbell, J., Green, M., Yanai, R. Tree measurement error in forest inventory and analysis (FIA) plots in the northern region. ESF Student research spotlight April 24th 2018.

Young, A., Yanai, R., Minocha, R., Long, S. Sugar Maple Canopy Response to Nutrient Treatment. Forest Ecosystem Monitoring Cooperative, Burlington VT. December 15th 2017.

Young, A., Yanai, R., Minocha, R., Long, S. Specific leaf area and amino acids respond to nutrient amendments and canopy depth. Rochester Academy of Sciences, Rochester, NY. Nov 11th 2017.

Young, A., Miller, J. Villella, J., Emanuels, A., Carey, G., Miller, W. Nest Guests: Water bears inhabit vole nests in Douglas-fir canopies. SUNY-ESF Student Spotlight, Syracuse, NY. April 24th 2017.

Young, A., Miller, J. Villella, J., Emanuels, A., Carey, G., Miller, W. Tardigrades in Red Tree Vole Nests. New York Society of American Foresters, Syracuse NY. January 26th 2017.

Young, A., Tripp, R., Lowman, M., Miller, W. Tardigrades in the Canopy. 13th International Symposium on Tardigrada, Modena, Italy. June 23-26th 2015.

Young, A., Digital herbarium: lichens and beetles of Lewis & Clark College. Lewis & Clark Speaker Series. April 25th 2015.

Young, A., Miller, W. In the canopy with Tardigrades and wheelchairs. Sigma Xi International Conference, Phoenix AZ. November 7-8th 2014.

Young, A., Chappell, B., Miller, W. Tardigrades of the canopy: Milnesium *sp. nov* C. California Academy of Sciences, August 5th 2014.

PRESENTATIONS

Young, A., Yanai, R., Drake, J., Minocha, R., Fernando, D. Sugar maple leaf characteristics respond to the vertical gradient and to N and P addition. New York Society of American Foresters conference. Syracuse, New York. January 24th, 2019.

Young, A., Kirkpatrick, S., Barkley, M., Yanai, R., Miller, W. Tardigrades response to N and P fertilization. 14th International Symposium on Tardigrada. Copenhagen, Denmark. July 30th – Oct 3rd 2018.

Young, A., Drake, J., Fernando, D., Minocha, R., Yanai, R. Sugar maple foliar traits respond to N, P, and the vertical gradient. Hubbard Brook Cooperators of Science, NH, July 12th 2018.

Young, A., Yanai, R. How foliage traits respond to the vertical gradient and nutrient amendment. Hubbard Brook Cooperators of Science, NH, July 7th 2017.

Young, A. Miller, J., Villella, J., Carey, C., Miller, W. Meiofauna zonation in a Douglas-fir forest canopy. Northwest Scientific Association. April 2nd 2017.

Young, A. Tardigrades are Extremophiles in your back yard. Friends School of Baltimore Speaker Series. March 14th 2016.

Young, A. Lichens, Mosses, and Water Bears, Oh My! The Northwest Academy. Portland, OR. October 16th, 2016.

Young, A. Clifton, K. Tardigrades of Smith Rock State Park, OR. Northwest Scientific Association. Pasco, WA. April 3rd 2015.

Young, A. A closer look at tardigrades: your local extremophiles. Leadership & Entrepreneurship Public Charter High school. April 11th 2014.

GRANTS & AWARDS

Albert L. Leaf Memorial Award: Dept. Forest and Natural Resource Management ($500)	Fall 2018
Edna Bailey Sussman Foundation "Detecting foliar nutrient from the sky" ($7,325)	Summer 2018
Graduate Student Travel Grant ($500)	Spring 2018
ESF Career Fellowship travel grant ($500)	Spring 2018
Cline Award: SUNY-ESF: Dept. Forest and Natural Resource Management ($1,800)	Fall 2017
Graduate Student Travel Grant: SUNY-ESF ($500)	Spring 2017
Kent Swanson Jr. Award: Lewis & Clark College Biology Department ($20,000)	2013 – 2015
Miller Science Award: Lewis & Clark College	2011 – 2015

CERTIFICATIONS

FAA Part 107: Commercial sUAS pilot	March 2019
Wilderness First Responder: SOLO	May 2016
Advanced Tree Research & Aerial Rescue: Tree Climbing Planet	May 2015
Basic Tree Climber: Tree Climbers International	June 2014
Open Water Diver: Scuba Schools International	April 2014

TREE CLIMBING VOLUNTEERING

Douglas-fir Swiss needle cast severity mapping August 2016
Oregon State University, Oregon Coast, OR

Giant sequoia drought response field campaign July 2016
UC Berkeley, Sequoia National Park, CA

Public tree climbing Summer 2014 – 2015
Tree Climbing Kansas City, Olathe, KS