EFFECTS OF NUTRIENT AMENDMENTS ON WATER USE AND WATER USE EFFICIENCY IN A NORTHEASTERN FOREST ECOSYSTEM

BY

ADAN HERNANDEZ HERNANDEZ

BS, Institute Technology of Oaxaca Valley, 2011

THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

the Requirements for the Degree of

Master of

Science

in

Natural Resources: Forestry

May, 2014

The thesis has been examined and approved.

Thesis Director, Heidi Asbjornsen,
Associate Professor of Natural Resources

Virginia Hernández Santana,
Postdoctoral Research Associate,
Institute for Natural Resources and
Agrobiology of Sevilla

Matthew, A. Vadeboncoeur,
Postdoctoral Research Associate,
University of New Hampshire

Mark J. Ducey
Professor of Forest Biometrics
and Management

Date

DEDICATION

I dedicate this achievement to my wife: Lucero, and my children: Eduardo, Lucero, and Alan.

ACKNOWLEDGEMENTS

My experience as a graduate student would have not been possible without the financial support provided by both the U.S. Agency for International Development (USAID) and the University of New Hampshire (UNH). I would like to express my gratitude to these institutions for providing me with this opportunity.

I would also like to extend special thanks to Heidi Asbjornsen for providing me with this great opportunity, her support, and her commitment throughout my career. To Virginia Hernandez Santana, Matthew A. Vadeboncoeur, and Mark J. Ducey for all the assistance they have provided and for sharing their knowledge with me.

Thanks to Ruth D. Yanai and Matthew A. Vadeboncoeur for allowing me conduct my fieldwork on two of their MELNHE experimental sites, located at the Bartlett Experimental Forest, in Bartlett, New Hampshire, USA.

Many thanks to Connor Breton, Katherine Sinacore, Cameron McIntire, Michelle Day, Susana Alvarado-Barrientos, Katie Jennings, Lauren Buzinski, Paul Pellissier, Rand Snyder, Zoe Aldag, José Antonio Gutierrez Lopez, Adam Wild and Craig See for all of their support and friendship.

TABLE OF CONTENTS

DEDICATION ..... **III**

ACKNOWLEDGEMENTS ..... **IV**

LIST OF TABLES ..... **VII**

LIST OF FIGURES ..... **VIII**

ABSTRACT ..... **IX**

1. INTRODUCTION **1**
- 1. 1 Research Question 9
- 1.2 Hypotheses 9

2. METHODS **10**
- 2.1 Study site 10
- 2.2 Sampling design 11
- 2.3 Field data collection 12
- 2.3.1 Sap velocity measurements 12
- 2.3.2 Sapwood area (A_s) 13
- 2.3.3 Whole tree sap flow estimation 14
- 2.3.4 Scaling transpiration to the stands 16
- 2.4 Instantaneous water-use efficiency at the leaf level 16
- 2.5 Meteorological data collection. 18
- 2.6 Soil moisture 18
- 2.7 Statistical analysis 18
- 2.7.1 Sap velocity analysis 18
- 2.7.2 Gas exchange analysis 19

3. RESULTS **20**
- 3.1 Sap velocity 20
- 3.1.1 Treatment effects 20
- 3.1.1.1 Mature stand 20
- 3.1.1.2 Young stand 20
- 3.1.2 Tree species differences 21
- 3.1.2.1 Mature stand 21
- 3.1.2.2 Young stand 21
- 3.1.3 Tree species comparison across stands 21
- 3.1.3.1 *Fagus grandifolia* 21
- 3.1.3.2 Genus: *Acer* 22
- 3.1.3.3 Genus: *Fagus* 22
- 3.2 Scaling transpiration to the stand level 22
- 3.2.1 Mature stand 22
- 3.2.2 Young stand 23
- 3.3 Gas exchange 23
- 3.3.1 Treatment effect 23

3.3.1.1 Leaf transpiration rate 23
3.3.1.2 Leaf photosynthetic rate 24
3.3.1.3 Instantaneous water use efficiency 24
3.3.2 Tree species differences 25
3.3.2.1 Young stand 25
3.4 Foliar nitrogen concentration in the young stand 25
3.4.1 Treatment effect 25
3.4.2 Tree species differences 26

4. DISCUSSION 26
4.1 Overview: comparing findings and previous hypotheses. 26
4.2 Treatment effect differences 28
4.3 Tree species differences in the young stand 32
4.4 Stand transpiration between the mature and young stand 36

5. CONCLUSION 40

TABLES 43

FIGURES 51

LIST OF TABLES

Table 1 Stand characteristic and location. 43
Table 2 Sap velocity calculation from Burgess *et al*. (2001). 44
Table 3 Sapwood water content and wood density determined by tree species. 45
Table 4 Allometric equation between DBH and A_s. 45
Table 5 Sample trees for V_s calculation in C2 and C8 stands. 46
Table 6 Mean DBH of tree sampled for V_s in C8 and C2 stands. 46
Table 7 Days having maximum VPD ≥1 kPa for V_s calculation in both stands. .. 46
Table 8 Mean V_s among trees species and treatments for both stands. 47
Table 9 Means of A, E, iWUE among tree species and among treatments in the C2 stand, obtained from one-way ANOVA. 48
Table 10 Sap velocity means for days having VPD ≥1 kPa between 12:00 to 13:45 hours. Only dates marked with a symbol (√) in C2 from table 7 were included for the calculation. 49
Table 11 Total sap flow for the C8 calculated upon V_s in days having VPD ≥ 1 kPa between 12:00 to 13:45 hrs. 49
Table 12 Total sap flow for the C2 calculated upon V_s in days having VPD ≥ 1 kPa between 12:00 to 13:45 hrs. 50
Table 13 Foliar N(%) concentration in trees growing in C2 and C8 stands. 50

LIST OF FIGURES

Figure 1 Map of the Bartlett Experimental Forest (BEF), located in NH (USA)...51

Figure 2 Map of the study site locations and treatment plot layouts. 52

Figure 3 MELNHE General Plot Layout. 53

Figure 4 Mean sap velocity compared among species and treatments in the old stand (C8). Data show days having maximum VPD ≥1 kPa, between 12:00 to 13:45 hours during daylights saving time. Error bars denote a standard error of the mean. 54

Figure 5 Mean sap velocity compared among species and treatments in the young stand (C2). Data show days having maximum VPD ≥1 kPa, between12:00 to 13:45 hours during daylights saving time. *Capital letters* denote differences among tree species. L*ower case letters* indicate the differences among treatments. Error bars denote standard error of the mean. 54

Figure 6 Mean leaf transpiration rates compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean. 55

Figure 7 Mean leaf photosynthetic rates compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean. 55

Figure 8 Mean leaf conductance compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean. 56

Figure 9 Mean instantaneous water use efficiency compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. L*ower case letters* indicate differences among treatments. Error bars denote standard error of the mean. 56

Figure 10 Mean foliar nitrogen concentration compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. L*ower case letters* indicate differences among treatments. Error bars denote standard error of the mean. 57

Figure 11 Basal area (BA) and sapwood area (A_s) are to the left axis, and stand transpiration (T) is to the right axis. Grey bar represents trees having DBH > 10 cm, while empty bar represent trees having DBH between 2-10 cm. 57

Figure 12 Transpiration rate as a function of conductance of the three dominant tree species in the C2 in the BEF, Bartlett, NH. 58

Figure 13 Photosynthesis rate as a function of conductance of the three dominant tree species in the C2 in the BEF, Bartlett, NH. 58

ABSTRACT

EFFECTS OF NUTRIENT AMENDMENTS ON WATER USE AND WATER USE EFFICIENCY IN A NORTHEASTERN FOREST ECOSYSTEM

by

Adan Hernandez Hernandez

University of New Hampshire, May, 2014

A nutrient addition study was carried out in a mature and young stands dominated by *Fagus grandifolia, Acer saccharum, Betula alleghaniensis, Acer rubrum* and *Betula papyrifera* at the Bartlett Experimental Forest, New Hampshire, USA. Individual tree WU was assessed through sap velocity (V_s) measurements using the Heat Ratio Method (HRM) (Burgess *et al*., 2001). Moreover, leaf photosynthetic rate (A) and transpiration rate (E) were measured using a Portable Photosynthesis System 6400XT, and instantaneous water-use efficiency (iWUE) calculated as A/E.

Only *A. rubrum* trees growing in the nitrogen plot had greater V_s compared to the C plot ($p < 0.05$). *Acer rubrum* trees also had lower V_s, A, E and foliar N concentration ($p < 0.05$) compared to *B. papyrifera* and *F. grandifolia* in the young stand. Mature stand trees did not differ among treatments and foliar N concentration did not increase for any tree species after two years of fertilization.

1. INTRODUCTION

Forest ecosystems provide countless services that are vital to sustaining the well-being and livelihoods of human societies, including provisioning services, regulative services, supporting services and cultural services (de Groot *et al.*, 2002). Of these services, hydrologic services are particularly important given that increasing water scarcity and declining water quality are among the most critical global environmental problems facing society today. Forests play an important role in sustaining hydrologic services through their impacts on enhancing soil water infiltration and storage, reducing runoff (and associated nutrient and sediment loss), regulating stream flow, and flood mitigation (Ilstedt *et al.*, 2007; Wine and Zou, 2012). They also provide climate regulation services through the uptake and storage of atmospheric CO_2 (Dietze and Moorcroft, 2011).

In New England, the most common forest type is northern deciduous hardwood forest, which is dominated by *Acer saccharum* (sugar maple), *Fagus grandifolia* (American beech), and *Betula alleghaniensis* (yellow birch), while gradients of elevation and latitude play an important role in their regional distribution (Bormann *et al.*, 1970). These forests have undergone significant change over the past several centuries. Virtually all of the forested landscapes in the region were subjected to some kind of disturbance in the past (Perlin, 1991), such as heavy forest cutting, damages by insects, and heavy snow, ice, wind storms (Martin and Bailey, 1999). Consequently, the tree biomass and species present today are a reflection of the stage of recovery from past disturbance (Brown, 1997). Differences in forest species composition, stand age, and past land use and disturbance history can all interact to influence how forests impact hydrologic processes as well as water-nutrient interactions (Ollinger *et al.*, 1998).

In terrestrial ecosystems, plant growth, reproduction, plant species interactions, community composition, and plant diversity are greatly influenced by nitrogen (N) and phosphorus (P) availability (Gusewell, 2004), and a limitation in either P or N can substantially alter these characteristics (Roem and Berendse, 2000). Further, because of slow processes by which these nutrients are transformed from their ultimate source (the atmosphere for N; rock weathering for P) to forms that are biologically available to plants, they are often considered to be the most limiting nutrients in terrestrial ecosystems. Moreover, the high mobility of N causes it to be easily lost through leaching or volatilization and denitrification to the atmosphere, which can further reduce its availability for plant uptake (Vitousek and Howarth, 1991).

Nitrogen is generally considered to be the limiting element that governs net primary production (NPP) in the humid temperature northern hardwood forests (Aber *et al.*, 1989). This hypothesis was supported by a N fertilization study in the Great Mountain Forest located in Connecticut USA, which found that *Acer rubrum* (red maple) and *Fraxinus americana* (white ash) responded to the N treatment by increasing their basal area increment leading to the conclusion that the forest was strongly N limited (Finzi, 2009). More recently a meta-analysis conducted by Vadeboncoeur (2010) confirmed the existence of a strong positive correlation between biomass production and N fertilization; however, this correlation was reduced at elevated rates of N deposition, presumably due to N saturation.

Since the 1940 atmospheric emissions of N have rapidly increased as a result of industrial production in developed countries (Vitousek *et al.*, 1997). In particular, this has resulted in increased atmospheric N inputs to global forests (Nadelhoffer *et al.*, 1999). Previous research suggests that atmospheric deposition can have significant impacts on forest dynamics, including stimulating decomposition (Boxman *et al.*, 1995), reducing plant diversity (Bobbink *et al.*, 1988) and increasing mortality rates (Schulze, 1989). Likens *et al.* (1996) observed a strong relationship between acid deposition and leaching of base cations from soils in hardwood forests based on long-term data on watershed mass balances at the Hubbard Brook Experimental Forest (HBEF), New Hampshire. More recently, several studies emphasized that temperate forests may continue to undergo significant changes in the face of elevated N deposition, which may act synergistically with other drivers such as increasing atmospheric CO_2 concentrations and climate change (Huntington *et al.*, 2009; Mohan *et al.*, 2009).

In addition to N limitation, more recent theories point to the possibility of colimitation by both N and P (e.g., Vadeboncoeur, 2010). For example, Vitousek *et al.* (2010) found that certain ecosystems with high N deposition shifted to a situation of P limitation, and suggested that this may be because P is derived mainly from rock weathering which may be even less available than N in many ecosystems. Harpole *et al.* (2011) conducted a meta-analysis of 641 nutrient manipulation experiments across diverse ecosystems and found that 28% of the sites exhibited evidence of being co-limited by N and P, where both nutrients had to be applied in order to elicit a growth response.

Plant transpiration may be also be closely regulated by nutrient availability (Cramer *et al.*, 2008; Matimati *et al.*, 2013). For example, Cramer *et al.* (2008) conducted a nutrient-water interaction study in *Ehrharta calycina,* comparing between mass-flow plants (i.e., nutrient uptake occurs when nutrients are carried to the surface of the root by movement of water in the soil) and interception plants (i.e., nutrient uptake occurs when nutrients are deposited on the roots of plants). This investigation found that mass-flow plants had significantly lower height growth, higher transpiration and photosynthesis rates and higher tissue P, Ca and Na compared to interception plants. These differences were attributed to nutrient limitation, leading to the conclusion that plants growing in temperate regions under non-limiting water availability could depend on transpiration to promote mass-flow of nutrients to the plants (Cramer *et al.*, 2008). Nevertheless, nutrient-water interactions have not been widely studied in temperate forests.

Therefore, N and P play an important role in plant functions and productivity. Studies have found that temperate forests in the northeastern U.S are generally limited by N (Aber *et al.*, 1989; Finzi, 2009; Vadeboncoeur, 2010), and may be co-limited by N and P (Vadeboncoeur, 2010). Globally, fertilization studies have been carried out to assess the response of tree water use to enhanced nutrient availability as a means of testing hypotheses related to nutrient (co-) limitation. For example, a nutrient addition study conducted by Bucci et al. (2006) was conducted in an ecological reserve in Brasilia (Brazil). Experimental treatments included additions of P (100 kg ha^{-1} $year^{-1}$), N (100 kg ha^{-1} $year^{-1}$), NP (100 N kg ha^{-1} year $^{-1}$+100 P kg ha^{-1} $year^{-1}$), and no addition (control). After 63 months of fertilization, total leaf area increment and sap flow per individual tree were significantly greater across the five dominant woody species growing in the N and NP plots compared to the control plots (Bucci *et al.*, 2006). The short and long-term effect of fertilization on water use (WU) was evaluated in a study conducted in a eucalyptus forest (Saligna Experimental Forest near Hilo in Hawaii) by Hubbard *et al.* (2004), who reported that total WU in fertilized plots increased from 265 to 487 mm yr^{-1} ($p<0.01$), which was 33% higher than control plots, over a five month period immediately following treatment. The short-term responses to fertilization included significant increases in LAI from 3.5 to 6.0, and lower WU per unit carbon gain by the fertilized trees (361 to 751 kg H_2OkgC^{-1} compared to the C plot), indicating an increase in their water use efficiency (WUE), although no changes were observed in canopy conductance (G_s) (Hubbard *et al.*, 2004). However, in the temperate northern hardwood forest, a short term study of sap velocity (V_s) response to nutrient additions among the three dominant species (*F. grandifolia, A. saccharum* and *B. alleghaniensis*) immediately following fertilizer additions (N, P, and NP) at the HBEF revealed no significant differences in either WU (Hernandez-Santana and Asbjornsen, in prep.); although the data collection period was shorter than in the study conducted by Hubbard *et al.* (2004) (91 vs. 150 days, respectively) and more long-term data are needed to better understand tree WU in response to nutrient limitation in the northern hardwood forest.

Most previous studies that have compared WU between forest stands of different ages have reported a strong effect of tree age on stand transpiration (T). Leaf area index generally increases with stand age, but LAI tends to decrease at some point during forest succession with the amount of time varying depending on forest type and climate (Ryan *et al.*, 2004). A study conducted by Moore *et al.* (2004) in 40- and 450-year-old riparian forests at the H. J. Andrews Experimental Forest, located in the western Cascades of central Oregon USA, found that the mean sap flux density was 2.3 times higher in a young compared to an old Douglas-fir (*Pseudotsuga menziesii*) forest. Vertessy *et al.* (2001) conducted an study in the Central Highlands of Victoria (Australia) in which stands of *Eucalyptus regnans*, ranging from 5 to 240 years old were monitored. They concluded that V_s did not significantly change amongst the 19 differently aged stands. However, sap wood area index (SAI; total sapwood area divided by the measurement area per stand) and LAI varied considerably with stand age. Their results showed that on average a 9-year-old stand had a SAI of 5.8 m^2-ha^{-1}, a 16-year-old stand had a SAI of 9.5 m^2-ha^{-1}, and a 50-year-old stand and a SAI of 6.8 of m^2-ha^{-1}, suggesting strong differences in stand WU among stands of different ages. A similar tendency was observed for LAI, which increased rapidly to 5.4 at age 7 and then steady decreased to 3.6 by age 240 years. Buckley *et al.* (2012) found similar results for subalpine *Eucalyptus delegatensis* when comparing young stands of post-fire regrowth with mature stands. The young regrowth had 72% greater SAI, 35% greater LAI, and WU was two times greater than the older mature stands. These studies conducted in pure stands illustrate the importance of understanding how young and mature forest stands respond in terms of their WU patterns, and highlight the pattern that at certain point during forest succession tree density starts to decline, which in turn results in decreasing LAI and SAI, and by association total stand T.

Aboveground biomass and WUE usually increase after nitrogen addition (de Vries *et al.*, 2009; Liu *et al.*, 2013). For example, Liu *et al.* (2013) examined the effects of water and N supply on one-year-old seedlings of *Robinia pseudoacacia* under three soil water contents (non-limiting, medium, and severe drought) as well as to low and high N levels. Four months after treatments, biomass production, instantaneous water use efficiency (iWUE) and photosynthetic capacity increased under non-limiting water conditions under low and high N levels. However, under drought conditions both photosynthetic capacity and iWUE remained unchanged. Ripullone *et al.* (2004) investigated effects of three N supply rates (low, intermediate and high) on *Pseudotsuga menziesii* (Mirb.) and on *Populus* X *euroamericana*. Both species increased in biomass production, and short and long-term WUE with increasing N supply. Nitrogen addition did not influence either leaf transpiration (E) or stomatal conductance (g_s). The increased in WUE was due to the effects of N addition on enhancing photosynthetic rates (A). Finally, Matimati *et al.* (2013) conducted a nutrient-uptake experiment on *Phaseolus vulgaris*. Two N addition treatments were established: (1) interception and (2) mass-flow. This research found that mass-flow plants growing closer to the N source had significantly greater g_s and E, but lower WUE compared to the interception plants. Plants growing in both treatments had the same foliar N concentrations and A. Also, it was observed that A, g_s, E, foliar N and total biomass decreased, while WUE increased as distance to N source increased (0 mm to 20 mm) in mass-flow plants. According to previous studies, N availability has a positive influence on A resulting in an increased in both aboveground biomass and WUE. However, WUE may differ due to the changes in g_s as a function of nutrient availability (Matimati *et al.*, 2013).

Despite growing interest and substantial previous work focusing on the effects of nutrient availability on the dynamics of New England forests, relatively little information is available regarding the effects of nutrient limitation and nutrient amendments on tree WU and WUE. Examining tree water relations in response to the addition of nutrients is important in providing a better understanding of the impact of altered nutrient availability in temperate forest systems on hydrologic services. My research expands upon previous work conducted by Hernandez-Santana and Asbjornsen (in prep.) by assessing the effects of nutrient amendment treatments on the response of the dominant tree species in two forests of different ages, in terms of their WU and WUE, two years following treatment implementation. Additionally, I conducted intensive measurements of leaf gas exchange and an assessment of leaf-level instantaneous water-use efficiency (iWUE). The overarching objective of my research is to evaluate the response of tree WU and iWUE in *F. grandifolia*, *A. saccharum* and *B. alleghaniensis* to fertilization by N, P, and NP in a mature forest stand (>100 year-old) and *F. grandifolia*, *A. rubrum*, *Betula papyrifera* (white birch) for a young forest stand (25-year old) in the northern deciduous hardwood forest at both the individual species and stand levels.

1. 1 Research Question

The main question that I will address is: What are the effects of nutrient amendments on individual tree WU and iWUE of the dominant species (*B. alleghaniensis, F. grandifolia, A. saccharum, A. rubrum* and *B. papyrifera*) and on stand transpiration in young and mature forests in Bartlett Experimental Forest, New Hampshire?

1.2 Hypotheses

1. Fertilized plots will have higher individual tree WU and T due to increased LAI and/or sapwood area (A_s), relative to the control plot.
2. Trees in the NP, N and P plots in both the young and mature stands will have higher iWUE compared to the control plots due to greater foliar nutrient concentration, which enhances photosynthesis capacity and, in turn, iWUE.
3. The magnitude of the response of trees in the young stand to the fertilization treatments will be greater relative to the mature stand, because younger trees generally have faster growth rates and responses to resource

availability (e.g., by increasing dbh, height, leaf production, etc.) in order to compete with other trees, whereas older trees have already reached their maximum height and may allocate resources to other functions.

2. METHODS

2.1 Study site

The Bartlett Experimental Forest (BEF) encompasses 1,052 ha, over which the elevation ranges between 207 m a.s.l. to about 915 m a.s.l. The climate is humid continental, with warm summers (>32 °C) and cold winters (reaching -35 °C). Average annual precipitation is 1,270 mm and is well distributed throughout the year. During the winter, individual storm events can drop more than 600 mm of snow (http://nrs.fs.fed.us/ef/locations/nh/bartlett/). The dominant soil type is frigid Haplorthods developed on glacial till originated from granite and metamorphic bedrock (Vadeboncoeur *et al.*, 2012). The forest is comprised primarily of old-growth northern hardwood species dominated by *F. grandifolia, B. alleghaniensis, A. saccharum,* and *Tsuga canadensis* (eastern hemlock) (Leak, 1991). Currently, some parts of the BEF are actively managed by the US Forest Service, which provides an opportunity to compare forests of different ages in close proximity. *Prunus pensylvanica* (pin cherry), *B. papyrifera*, *A. rubrum*, *F. grandifolia* and several other tree species are found in young stands. *Picea rubens* stands dominate on the steeper slopes at the higher elevations and *Pinus strobus* (white pine) commonly grows on the lowest elevations.

Currently, the “Multiple Element Limitation in Northern Hardwood Ecosystems” (MELNHE) project is being conducted at the BEF. The primary aim of MELNHE is to better understand to what extent forest productivity is co-limited by multiple nutrients, specifically N and P. With this in mind, multiple sites were established in both mature and young stands, that each stand having four treatments plots (N, P, NP and Control plot). Each plot measured 50 m x 50 m (Figure 3), and received one of four treatments: N amendment (30 kg N/ha/yr as NH_4NO_3; hereafter ‘N plot’), P amendment (10 kg P/ha/yr as NaH_2PO_4; ‘P plot’), N+P amendment (‘NP plot’), and control (‘C plot’) (http://www.esf.edu/melnhe/). Two applications of nutrient amendments were made each year, starting in spring 2011 (Fisk *et al.*, 2013) and a total of six applications have been applied to date. My thesis research was conducted from July 5th to August 15th in 2013 within two of the MELNHE study sites; one classified as a mature stand (C8, >130-years old) and the other as a young stand (C2, 25-years old), and including all four treatment plots (i.e., N, P, NP and C).

2.2 Sampling design

I focused my data collection on the three dominant tree species in the young and mature stands (Table 5 for details). In the mature stand the species were *A. saccharum, F. grandifolia* and *B. alleghaniensis*, whereas in the young stand the species were *A. rubrum, B. papyrifera,* and *F. grandifolia*. A total of 36 trees were chosen across treatments per stand, approximately 3 trees per species within each plot, (Table 5). All sample trees were selected using the following criteria: (1) the tree is located within both the buffer and fertilized area, (2) it appears healthy, and (3) it has a well-distributed upper canopy. Although the majority of the *F. grandifolia* trees located within the mature stand showed signs of beech bark disease, those with fewer wounds were preferentially selected for the study, in addition to the selection criteria above. In contrast, the sampled *F. grandifolia* at C2 showed no obvious signs of the disease.

2.3 Field data collection

2.3.1 Sap velocity measurements

On each of the selected sample trees V_s was measured using the Heat Ratio Method (HRM) (Burgess *et al.*, 2001). This method uses three probes: a heater and two temperatures probes. The probes were arranged vertically with the heater located between the temperature probes forming a heat-pulse set (hereafter ‘sap flow sensor’). Each temperature probe consisted of three thermocouples located at depths of 1, 2.3 and 3.5 cm from the bark in order to measure three different depths across the radial sapwood profile (Gebauer *et al.*, 2008). This set of sap flow sensors was connected to one end of a 10 m shielded low voltage computer cable and placed in the field as described below. First, I measured the diameter at breast high (DBH; 1.38 m above the ground on the uphill side of the tree). Second, tree bark was removed carefully. Third, a steel drilling square guide was placed at DBH height in such a way that holes could be drilled parallel to the plant stem-root axis (Burgess *et al.*, 2001). A 1.3 mm diameter drill bit was used to make a series of three holes 0.6 cm apart and 3.8

cm deep into the xylem tissue (Bleby *et al.*, 2004). Petroleum jelly was used to ease probe insertion and maintain thermal contact between the probe and wood tissue (Burgess *et al.*, 2001). Each probe set was protected with aluminum insulation to minimize irradiation and ambient thermal gradients. Each probe set was connected via a 10 m shielded low voltage computer cable to a 64-channel multiplexer (Model AM 16/32B, Campbell Scientific Inc., Logan, UT, USA). This, in turn, was connected to a data logger (Model CR1000, Campbell Scientific Inc.) to store the temperature data. Temperatures were averaged and stored every 15 min using software developed by Maria Susana Alvarado-Barrientos (personal communication), and V_s was calculated according to Burgess *et al.* (2001) (Tables 2 and 3 for details).

2.3.2 Sapwood area (A_s)

Sapwood is the active living tissue located between the bark and heartwood in a woody plants. Twenty representative (non-sample) trees of each dominant species in the young stand were surveyed in September 2012 to determine A_s. For each tree, I recorded the DBH and extracted at least one increment tree core and sapwood depth was determined on each core by measuring the translucent section between the bark and heartwood using a Vernier caliper. Species-specific allometric equations were developed to describe the relationship between sapwood depth and tree DBH data for the stand. Each tree species A_s was best described by a power allometric equation, whose form is $f(Y)= a X^{b}$, described by Meinzer *et al.* (2005). This model has showed a strong positive relationship between A_s and the DBH (Table 4). Allometric equations relating sapwood depth and DBH for the three dominant tree species in the mature stand were obtained from Hernandez-Santana and Asbjornsen (in prep.).

2.3.3 Whole tree sap flow estimation

In addition to the dominant tree species, *B. alleghaniensis, F. grandifolia, A. saccharum, A. rubrum* and *B. papyrifera*, *Tsuga canadensis*, *Fraxinus americana*, *Tilia Americana* (basswood) and *Acer pennsylvanicum* (striped maple) were also present in these stands.

Whole tree sap flow (Q_s, cm^3 h^{-1}) was determined on each sample tree in both stands by multiplying the effective A_s determined using the weighted-average method described in Hatton *et al.* (1990), and was multiplied by the respective tree species mean of V_s for days having maximum VPD ≥ 1 kPa between 12:00 to 13:45 hours (Table 10).

Whole tree sap flow was calculated as follows: First, A_s was determined for all trees including other tree species using available allometric equations (Table 4). However, allometric equations were not readily available for *T. canadensis, F. americana, T. americana,* and *A. pennsylvanicum*. The allometric equation for *A. saccharum* was used to estimate A_s for *T. canadensis, F. americana* and *T. americana,* since their DBH sizes were within a similar range as *A. saccharum* for trees with a DBH >10 cm. For *T. canadensis* between 2-10 cm of DBH sizes, the allometric equation develop for *F. grandifolia* in the mature stand was used. A similar approach was used to estimate A_s of species in the young stand that were not measured with sap flow (Table 4). Specifically, A_s for *A. pennsylvanicum*, *T. canadensis* and *F. americana* was estimated using the equation developed for *A. rubrum* given the similarity among their DBH sizes.

Second, the weighted-average method was applied on each individual tree's A_s to define three effective areas (e.g., outer, middle and inner effective area; an effective area is assumed to have the same V_s) for all trees with DBH > 10 cm. Therefore, effective areas were determined and multiplied by the mean V_s per thermocouple (Table 10a) to determine the total sap flow of each effective area. Thus, Q_s was obtained by summing all three effective area's sap flow.

Whole tree sap flow was also determined for trees categorized as "other" (or non-dominant) tree species having DBH > 10 cm for both stands, by multiplying their effective areas by the mean of each V_s (Table 10a* and 10b*).

Whole tree sap flow was determined for trees with DBH between 2-10 cm in both stands differently than the calculations used to determine Q_s in trees having greater than 10 cm in DBH. It was not possible to estimated effective area due to stem size. Thus, Q_s was calculated by multiplying each tree's total A_s by the corresponding V_s (Table 10a** or 10b**).

Whole tree sap flow was determined for trees classified as "other" tree species having DBH between 2-10 cm in a similar manner and their A_s multiplied with V_s having marked with tree asterisk (Table10a*** or 10b***).

2.3.4 Scaling transpiration to the stands

Stand transpiration (T, mm h^{-1}) was calculated for both the mature and young stands using inventory data collected in 2011 (unpubl. results, M. Vadeboncoeur). In the inventory database, trees were classified in two groups for both stands: (1) stems > 10 cm in DBH measuring in 3600 m^2 of total surface area after summing data across all four treatments, and (2) stems between 2-10 cm DBH measuring in a 500 m^2 after summing data across all four treatments.

Stand transpiration was calculated on a per hectare basis per group as follows: individual Q_s were summed per group, with each group having a different dimension (i.e., 3600 or 500 m^2). Therefore, both the Q_s and group surface area were used to estimate T through a cross-multiplication (Tables 11 and 12).

2.4 Instantaneous water-use efficiency at the leaf level

Gas exchange measurements were conducted on sample trees in C2 and C8 stands (n = 72) using a Portable Photosynthesis System (model LI-6400/LI-6400 XT), which is an open system that integrates a chamber, a standard 6 cm^2 leaf cuvette and a 6400-02 or -02B LED light source. All measurements were taken on relatively cloud-free days, between 10:00 and 14:00 hours (maximum values are expected at this time), on the following days of 2013: August 5th (C and P, C2 plots), August 6th (NP and N, C2 plots) and August 7th (C8 plots). Two small branches were obtained per tree and one leaf was sampled per branch (n = 144 samples, both stands). Branches in C2 trees were reached using a 20-ft aluminum 225-Ib type II extension ladder and wooden pole pruner. In C8, due to tree heights > 20 m, a shotgun sampling method was used in which small branches were shot down using # 4, 1-1/4 oz steel shot. Upon retrieval, branches were immediately submerged in water in accordance with Ambrose *et al.* (2009), and transported to the portable gas analyzer. Stems were re-cut while under water, and a leaf with a surface area greater than 6 cm^2 (the area of the LI-6400 xt cuvette) free of holes was selected. When possible leaves located further from the cut were selected in order to increase the probability that it was fully exposed to sunlight and reduce the probability of embolisms. The selected leaf was then clamped into the LI-6400 XT cuvette, and leaf CO_2, H_2O, and flow values were allowed to stabilize prior to taking A and g_s measurements. For each leaf, each measurement was repeated ten times in order to capture variability. Sampling times averaged 9 minutes per leaf for C2 and 5 minutes for C8. Temperature, CO_2 concentration, flow rate and saturation photosynthetic active radiation (PAR) were maintained at 26 ºC, 400 µmol/mol, 500 µmol s^{-1}, 1800 µmol m^{-2} s^{-1} respectively (Moreno-Sotomayor *et al.*, 2002; Elvir *et al.*, 2006; Ambrose *et al.*, 2009). The relative humidity (%) inside the chamber was maintained at 60% ± 10%, allowing the leaf to take back the humidity lost at the boundary layer during the measurement process (http://www.licor.com/env/newsline/2011/09/when-ambient-h2o-is-not-leaf-chamber-h2o/). Instantaneous water-use efficiency at the leaf level was calculated as the ratio A, µmol m^{-2} s^{-1} and E, mmol H_2O m^{-2} s^{-1}:

$iWUE = \frac{A}{E}$ expressed in µmol $mmol^{-1}$

2.5 Meteorological data collection.

Humidity and air temperature were determined at canopy height with Hobo Pro v2 sensors (U23-001) (Onset Computer Corporation, MA, US), installed in each of the treatments plots in each stand, and measurements recorded during the entire data collection period. Precipitation (TR-525, Texas Electronics, Inc., TX, USA) and incoming shortwave solar radiation (CM3, Kipp & Zonen) were obtained from a nearby eddy flux tower, (http://ameriflux.ornl.gov/), above the canopy). Vapor pressure deficit (VPD) was calculated from the relative humidity and air temperature data (http://cronklab.wikidot.com/calculation-of-vapour-pressure-deficit).

2.6 Soil moisture

Soil moisture (θ_{soil}) was monitored in both stands at 4 depths with 10HS/EM50 (5, 15, 25 and 50 cm) sensors of 10 cm in length (ECH2O probes Decagon, Inc., WA, USA) during the entire data collection period.

2.7 Statistical analysis

2.7.1 Sap velocity analysis

Sap velocity data were available on a daily basis at 15-minute intervals for each individual thermocouple in each sample tree throughout the entire fieldwork period. Sap velocity data from only the middle thermocouple were employed for calculation in order to assess the effect of nutrient amendments on individual tree V_s, analyzing only days having maximum VPD ≥1 kPa between the hours of

12:00 and 13:45 (Table 10). In order to focus on conditions likely to produce the highest daily transpiration rates I only included days having maximum VPD ≥1 kPa.

Statistical analyses were performed using JMP, version 9.0 (2010 SAS Institute Inc). Mean differences among treatments and tree species in V_s were determined using a mixed model under the Fit Model launch window; Fitting Personality becomes Standard Least Squares; emphasis under Minimal Report, and restricted maximum likelihood (REML) for fitting models with random effects:

Response variable = Sap velocity.

Random effects = Day of year and Tree sampled (n).

Fixed effects = Tree specie and Treatments.

Tukey-Kramer HSD Test was performed under the effect details for means comparisons among treatments and tree species.

2.7.2 Gas exchange analysis

Treatment and tree species mean differences were tested using one-way ANOVA under the Fit Y by X platform in JMP.

Response variables: Leaf transpiration rates, leaf photosynthetic rates, and instantaneous water use efficiency.

Nominal in the modeling type: Tree specie and Treatments.

Additionally, an all pairs Tukey-Kramer HSD Test was performed for means comparisons among treatments and among tree species.

3. RESULTS

3.1 Sap velocity

All sap velocity values presented in this section were derived using V_s data for the middle sensor for days having maximum VPD ≥ 1 kPa between 12:00 to 13:45 hours presented in table 10a and 10b.

3.1.1 Treatment effects

3.1.1.1 Mature stand

Sap velocity was determined for *F. grandifolia, B. alleghaniensis* and *A. saccharum* in the mature stand. Based on their diameter structure, all sampled trees were observed to be relatively similar (mean DBH = 40.68 ± 2 cm; see table 8a). However, *F. grandifolia* had a higher average DBH. *Fagus grandifolia* trees in the P, NP and N treatment plots had greater V_s compared to the C plot, but these differences were not statistically significant ($p > 0.05$) (Table 6a and 8a; figure 4). A similar pattern was observed for *B. alleghaniensis* growing in NP, N and P, which had slightly greater V_s compared to the C plot, but they were not statistically different either ($p > 0.05$), and no treatment effect was detected in *A. saccharum* (Table 8a; Figure 4).

3.1.1.2 Young stand

Sapwood area and V_s were estimated for *F. grandifolia*, *B. papyrifera* and *A. rubrum* in the young stand. Sample trees' DBH distribution across treatments and among tree species were similar (mean DBH = 11.97 cm; table 6b and 8b). *Acer rubrum* had the highest A_s, followed by *B. papyrifera*, while *F. grandifolia* had the lowest (Table 8b). Sap velocity differed significantly among treatments for *A. rubrum*, with trees growing in the N plot having a higher V_s compared to those in C plots ($p < 0.05$). No significant treatment effects were found in V_s for *B. papyrifera* or *F. grandifolia* (Table 8b; Figure 5).

3.1.2 Tree species differences

3.1.2.1 Mature stand

Fagus grandifolia had a consistently greater V_s across treatments compared to *B. alleghaniensis* and *A. saccharum* with the exception of the C plot, where *A. saccharum* had the highest V_s. However, among tree species no statistical differences were observed in their V_s ($p > 0.05$), (Table 8a; Figure 4).

3.1.2.2 Young stand

Comparing V_s means among tree species, I observed significant differences between *A. rubrum* and *B. papyrifera* ($p < 0.035$*), where *A. rubrum* had the lowest V_s. No statistical differences were seen between *B. papyrifera* and *F. grandifolia* V_s ($p > 0.05$) (Table 8b; Figure 5).

3.1.3 Tree species comparison across stands

3.1.3.1 *Fagus grandifolia*

Fagus grandifolia trees growing in the mature and young stands did not differ significantly from each other in V_s.

3.1.3.2 Genus: *Acer*

For tree species belonging to the genus *Acer* sampled in the young and mature stands, both *A. rubrum* and *A. saccharum* had the lowest rates of V_s when compared with other tree species monitored within their respective stands. When comparing between stands, V_s did not differ significantly from each other (Table 10a and 10b).

3.1.3.3 Genus: *Betula*

For trees belonging to the genus *Betula* sampled in the young and mature stands, both *B. papyrifera* and *B. alleganiensis* had higher V_s rates when compared with other tree species monitored within their respective stands. When comparing between stands, V_s was significantly higher for *B. papyrifera* growing in the young stand compared to the mature stand (Table 10a and 10b).

3.2 Scaling transpiration to the stand level

3.2.1 Mature stand

Total transpiration in the mature stand was 0.726 mm h^{-1}; whereas 94% of the transpiration was contributed by stems > 10 cm in DBH's and the remaining 6% by stems between 2-10 cm in DBH (Table 11).

3.2.2 Young stand

Total transpiration estimated for the young stand was 0.554 mm h^{-1}; whereas 85% was contributed by trees having DBH between 2-10 cm and 15% by trees > 10 cm (Table 12).

3.3 Gas exchange

Only gas exchange results for C2 are presented in this section because the data collected from C8 were determined to be erroneous due to incorrect settings on the equipment.

3.3.1 Treatment effect

3.3.1.1 Leaf transpiration rate

Betula papyrifera trees growing in the C plot had the highest E followed by trees growing in the P and N plots, while trees in the NP plot had the lowest E. No statistical differences in E were observed between treatments ($p > 0.05$). *Fagus grandifolia* trees growing in the NP plot had the highest E, followed by trees in the N and C plots, while trees in the P plot had the lowest E, but no statistical differences among treatments were observed ($p > 0.05$). *Acer rubrum* trees in NP plot had the highest E followed by those in the C plot, and trees measured in the N and P plot had the lowest E, but once again these differences were not significant ($p > 0.05$) (Table 9b; Figure 6).

3.3.1.2 Leaf photosynthetic rate

Betula papyrifera trees growing in the C and P plot had a similar and higher A compared to the other treatments, followed by trees growing in the N plot, while trees in NP plot had the lowest A; no statistical differences were observed between treatments ($p > 0.05$). For *F. grandifolia*, trees measured in the NP and N plots had greatest A, whereas A for trees growing in the P plot were lowest, all in comparison to the C plot. No statistical differences were observed between treatments ($p > 0.05$). Lastly, *A. rubrum* growing in the NP plot had the highest A, followed by trees in C plot. Trees growing in the P and N plots had similar A, which was lower than the C plot. Again, no statistical differences were observed between treatments ($p > 0.05$), (Table 9a; Figure 7).

3.3.1.3 Instantaneous water use efficiency

Betula papyrifera trees growing in P, NP and N plots had higher iWUE compared with the C plot, although no statistical differences were observed between treatments ($p > 0.05$). *Fagus grandifolia* trees had similar iWUE across treatments. Notably, I have observed a statistical mean differences in iWUE in *A. rubrum*, where trees growing in the N plot had lower iWUE compared to trees in the C, P and NP plots ($p < 0.05$) (Table 9d; Figure 9).

3.3.2 Tree species differences

3.3.2.1 Young stand

Fagus grandifolia and *B. papyrifera* responded similarly in terms of E, A, and g_s, compared with *A. rubrum* that had the lowest E, A and g_s but higher iWUE. For all three variables, *A. rubrum* was significantly different compared to both *F. grandifolia* and *B. papyrifera* ($p < 0.05$) (Table 9; Figures 6, 7 and 9).

3.4 Foliar nitrogen concentration in the young stand

3.4.1 Treatment effect

Prior to treatment initiation, *F. grandifolia* trees growing in the P plot had significant lower foliar N concentration compared to the C plot (0.037*) (data not shown; unpubl. results collected and analyzed by Quinn Thomas, Bali Quintero, Dusty Wood, Bill O'Neill, Ruth Yanai, and Craig See). Two years after fertilization, I observed that *F. grandifolia* growing in the P plot still had significantly lower foliar N concentration compared to the NP plot ($p < 0.019$*) and similar to trees growing in C, NP and N plots (Figure 10). Therefore, foliar N concentration in *F. grandifolia* trees did not significantly differed across treatments. For *B. papyrifera*, foliar N concentration did not differed across treatments ($p > 0.05$). Lastly, the mean foliar N concentration was significantly different among treatments for *A. rubrum*, with trees in the P plot having the lowest N concentration compared to the other treatment plots ($p < 0.024$*) (Figure 10).

3.4.2 Tree species differences

Foliar N concentration of *A. rubrum* was significantly lower compared to *B. papyrifera* and *F. grandifolia* ($p < 0.0001$*), (Figure 10).

4. DISCUSSION

4.1 Overview: comparing findings and previous hypotheses.

The first hypothesis presented in the beginning of this research was that fertilized plots would have higher individual tree WU and stand level transpiration due to increased LAI and/or A_s, relative to the C plot. According to my results, only *A. rubrum* trees growing in the N plot had greater WU compared to the C plot. However, this hypothesis was not supported for any of the other species or treatments, either at the individual tree or stand level, mainly because V_s was not affected by nutrient addition in most of the sample trees growing in the mature and young stands (Figures 4 and 5).

Unfortunately, because LAI and A_s data were not available, it was not possible to ascertain whether these variables responded significantly to the treatments. (Note: A_s values were determined using allometric equations derived from trees growing outside of the treatment area in each stands, and therefore do not capture the treatment effects; Table 4). However, given the lack of a response in WU or T for most species and treatment combinations, it is possible to speculate that LAI and A_s did not increase significantly after two years of nutrient amendments.

The second hypothesis was that trees in the NP, N and P plots in both the young and mature stands would have higher iWUE compared to the C plots because enhanced nutrient availability would presumably increase foliar N concentration and, hence, A, allowing leaves to become more efficient in fixing more carbon per unit of water. The iWUE data were not available in the mature stand. However, this hypothesis was not supported for *B. papyrifera* and *F. grandifolia* growing in the young stand because iWUE remained unchanged compared to the C plot (Figure 9). In contrast, for *A. rubrum*, iWUE for trees growing in the N plot was lower relative to the C and P plot, which was contrary to what was predicted by this hypothesis. It is important to mention that the E and A data used to calculate iWUE were from a single gas exchange sampling time for the entire field season. Therefore, more intensive sampling is needed in order to confirm these patterns in iWUE among species.

The third and final hypothesis was that the magnitude of the response of trees growing in the young stand to the fertilization treatments would be greater relative to the mature stand, because younger trees generally have faster growth rates (including dbh, height, and foliage) and experience intense competition, whereas old trees have reached the maximum growth and may allocate resources for other part of the tree (e.g., maintenance respiration).

Also, this hypothesis predicted an increase in tree growth in response to the fertilization treatments, which as noted above, likely did not occur. Therefore, my results did not support this hypothesis either.

The remainder of the Discussion is divided into three main topics, each of which will be discussed in greater detail: treatment effect differences, tree species differences in the young stand, and stand transpiration between the mature and young stand.

4.2 Treatment effect differences

Two years after fertilization, V_s for *B. alleghaniensis*, *F. grandifolia* and *A. saccharum* in the treatment plots in the mature stand were not significantly different from trees in the C plot ($p > 0.05$) (Figure 4). In the young stand, V_s and gas exchange variables for *B. papyrifera* and *F. grandifolia* were not significantly different between trees growing in the treatment plots compared to the C plot ($p > 0.05$) (Figures, 5, 6, 7, 8 and 9); however, a significant treatment effect was observed for V_s and iWUE in *A. rubrum* ($p < 0.05$) (Figures 5 and 9). *Acer rubrum* trees growing in the N plot had significantly greater V_s and lower iWUE compared to the C plot ($p < 0.05$).

Interestingly, foliar N concentration did not increase in response to the nutrient amendment treatments for any of the sample tree species in both stands compared to the C plots. Surprisingly, both the *A. rubrum* in the young stand and *B. alleghaniensis* in the mature growing in P plots actually had significantly lower foliar N concentration, compared to C plots ($p < 0.05$) (Table 13 and Figure 10). This finding in foliar N concentration for *A. rubrum* did not help to explain the treatment effect on V_s observed in *A. rubrum* between the N and C plot.

The greater V_s observed in *A. rubrum* trees growing in the N plot may have resulted from an increase in LAI, either through an increase in the total number of leaves or in leaf size in response to greater N availability (Bucci *et al.*, 2006). Other nutrient addition studies have shown that both LAI and leaf size often increase after fertilization. For example, Cramer *et al.* (2000), conducted a study in two early successional northern hardwood stands (19-years old and 25-years old) in the White Mountain National Forest, central New Hampshire (USA) focused on *P. pensylvanica*, *B. papyrifera*, *B.alleghaniensis*, *A. rubrum*, *A. saccharum*, *F. grandifolia* and *A. pennsylvanicum* (i.e., N= 167, P = 58, K = 254, Ca = 310 and Mg = 37, all expressed in kg ha^{-1} yr^{-1}). After 7 years of nutrient addition treatments, LAI and canopy height had notably increased in the fertilized plots compared to the C plots. Another study was conducted by Hubbard *et al.* (2004) to assess short-term (5 mo) and long-term (5 years) effects of fertilization on WU in a non-water limited fast growing *Eucalyptus saligna* experimental forest near Hilo, HI. Fertilized plots received 310 kg N ha^{-1}, 130 kg P ha^{-1}, 260 kg K ha^{-1}, 125 kg Ca ha^{-1}, 58 kg S ha^{-1}, 23 kg Mg ha^{-1}, and 10 kg ha^{-1} micronutrients (5 % Mn, 5 % Zn, 5% Fe, 5% S, 1.5 % Cu, and 0.5 % B). Compared to the C plot, LAI increased following fertilization treatment in both short-and long-term studies (Hubbard *et al.*, 2004). Additionally, Bucci *et al.* (2006) conducted a nutrient manipulation study in five dominant woody savannah species (i.e., *Blepharocalyx salicifolius, Caryocar brasiliense, Ouratea hexasperma, Qualea parviflora,* and *Schefflera macrocarpa*), in Central Brazil (Cerrado) to test whether carbon allocation, water relations and hydraulic architecture responded to nutrient amendments on oligtrophic Cerrado soils. Four plots were established (N = 100 kg ha^{-1} yr^{-1}, P = 100 kg ha^{-1} yr^{-1}, NP = 100+100 kg ha^{-1} yr^{-1}, and C= no added fertilizer), and 63 months after the first nutrient additions total leaf area increment was found to increase in trees growing in the N and NP compared to the C plot.

It is clear from these studies that forest stand LAI commonly increases for a range of different tree species and forest ecosystems after nutrient addition. Of these studies mentioned above, in two of the studies (Cramer *et al.*, 2000; Bucci *et al.*, 2006), the response of LAI after fertilization was accompanied by an increase in foliar N concentration for most species, with the exception of *A. rubrum* and *F. grandifolia* growing in a younger stand (Cramer *et al.*, 2000). Similar findings were documented in a nutrient manipulation study conducted in a mature forest stand in a northern hardwood forest by Lovett *et al.* (2013) (C plot = no nutrients added and N plot = 50 kg N ha^{-1} y^{-1} in the form of NH_4NO_3). After 6 years of treatment, only *A. saccharum* and *B. alleghaniensis* had significantly higher foliar N concentration, while no response was observed for *F. grandifolia* in the N-treated plots. Previous N fertilization experiments conducted in the northern hardwood forest (Cramer *et al.*, 2000; Lovett *et al.*, 2013) have applied larger annual doses of fertilization compared to my treated plots. It appears that *A. rubrum* and *F. grandifolia* require more than 6 years before significant increases in foliar N concentration occurs with greater doses. Thus, trees in my plots may take longer to respond in their foliar N concentration given the annual relatively low doses applied.

Stem growth was not monitored as part of this study. However, previous nutrient addition studies have reported increases in above ground growth as a response to fertilization: Hubbard *et al.* (2004) reported a significant increase in aboveground woody growth after fertilization compared to the C plot. Moreover, Bucci *et al.* (2006) observed an increased in leaf area to sapwood area ratios (A_L:A_S) in the N and NP plots relative to the to the C plot.

According to previous nutrient addition investigations that also included tree WU measurements (Hubbard *et al.*, 2004; Bucci *et al.*, 2006), LAI typically increased in most of the studied tree species following fertilization, while LAI increases usually stimulated stem growth and tree transpiration rates. For example, Hubbard *et al.* (2004) observed an increase in tree WU for both the short-term study (from 265 to 487 mm yr^{-1}) and long-term study (from 302 to 401 mm yr^{-1}) compared to the C plots. The higher tree WU observed in the short-term study was driven by greater stand leaf area and sap flux per unit sapwood area, whereas higher tree WU in the long-term study was due to greater leaf area only. Also, Bucci *et al.* (2006) documented higher individual daily sap flow, greater LAI and A_L:A_S in the N and NP plot compared to the C plot.

Previous studies have found increased LAI after fertilization (Cramer *et al.*, 2000; Hubbard *et al.*, 2004; Bucci *et al.*, 2006), which largely determines tree transpiration rate and is also highly correlated with stem-wood growth (Forrester *et al.*, 2012). Further, it has been well documented that A_L/A_S increases with tree size (Schafer *et al.*, 2000; McDowell *et al.*, 2002; Forrester *et al.*, 2012).

The greater response observed in V_s of *A. rubrum* in the N plot may be explained by greater stem-growth, and in turn, A_s, which should lead to greater LAI compared to *A. rubrum* trees growing the C plot in the young stand.

My results are similar to those reported by Hernandez-Santana and Asbjornsen (in prep.), indicating no difference in V_s for *B. alleghaniensis, A. saccharum* and *F. grandifolia* growing in the same BEF stands and treatments at during summer in 2011.

To the best of my knowledge, no other studies have assessed changes in V_s in response to nutrient amendments in the northern hardwood forest region, and more work is needed. It is possible that the overall lack of a significant treatment response found in the present study may be a result of the relatively short time frame or low nutrient addition levels, and therefore measurements are needed over a longer time period to better understand these patterns.

4.3 Tree species differences in the young stand

Betula papyrifera (shade-intolerant) had significantly greater rates of V_s, gas exchange (e.g., E, A, and g_s) and foliar N content compared to *A. rubrum* (shade-tolerant) in the young stand (Figures 5, 6, 7, 8 10). The differences between species may be attributed to many characteristics, including (1) leaf-level properties, (2) site characteristics, and (3) canopy position.

With regard to leaf-level properties, it is believed that leaf morphology and structure, particularly the stomatal density and guard cell length contribute to the differences I found between *B. papyrifera* and *A. rubrum*. Stomata are small pores found on the epidermis of leaves formed by a pair of specialized cells called guard cells (Colin and Mark, 1996; Hetherington and Woodward, 2003). Stomatal density is the number of stomata per unit leaf area of a leaf surface, and varies considerably within leaves in the same plant (Colin and Mark, 1996). During gas exchange, stomatal pores are the portals where carbon dioxide enters the leaf and water is released (Colin and Mark, 1996). Guard cells control the opening and closing of the stomata, regulating gas exchange between a plant and its environment (Marcus *et al.*, 2001). Many studies have found that smaller guard cells are associated with higher stomatal densities (Abrams and Kubiske, 1990; Colin and Mark, 1996; Hetherington and Woodward, 2003; Rossatto *et al.*, 2009). Furthermore, research in central Wisonsin (USA) found that stomatal densities and guard cell lengths in open grown versus understory trees were significantly different (Abrams and Kubiske, 1990). In particular, this study found that *A. rubrum* tree leaves growing in an open location had a mean stomatal density of 378±32 mm^{-2} and guard cell length of 12.7±0.4 μm. For *B. papyrifera* trees, leaves growing in an open location had mean stomatal densities of 215±0.15 mm^{-2} and guard cell lengths of 30.0±1.1 μm. In conclusion, *B. papyrifera* leaves had lower stomatal densities and longer guard cells than *A. rubrum* leaves.

My findings most closely follow the trends found by Abrams and Kubiske (1990). For example, I found that *B. papyrifera* had greater V_s, E, A and g_s than *A. rubrum* (Figures 5, 6, 7, 8, 10). These findings can be attributed to many factors, including site characteristics and physiological tree attributes. In particular, *B. papyrifera* is known to have lower stomatal densities and longer guard cells that contribute to higher V_s rates, as found in the present study. Additionally, the greater stomatal density and shorter guard cell length found in the *A. rubrum*, could explain its lower rates of E and A. A comparative study of stomata anatomical characteristics, stomata conductance and transpiration of 10 congeneric species pairs of cerrado and gallery forest species in Central Brazil by Rossatto *et al.* (2009) reported that the area of the stomatal pore had the largest influence on gas exchange, whereby pore area and rate of g_s increased linearly for most of their species ($r^2 = 24$ %). Another study on *Rosa hydrida* by Giday *et al.* (2013) observed that stomata size (length multiplied by width) correlate linearly with E, ($r^2 = 49$ %). Also, Rossatto *et al.* (2009) and Giday *et al.* (2013) noted that stomata size correlates with g_s and E.

I observed that E and A increased linearly with g_s (Figures 12 and 13). *Betula papyrifera* had greater E and A as a function of g_s. In contrast, *A. rubrum* had the lowest E and A compared to *B. papyrifera* and *F. grandifolia*.

Additional information associated with gas exchange and foliar N content from previous works was found for *A. rubrum* and reported as follows: (1) A gas exchange study conducted in xeric sites in Pennsylvania (USA) by Kubiske and Abrams (1994) found that *A. rubrum* stomatal conductance of water vapor measured in a wet year was significantly greater compared to that measured during a dry year; however, similar photosynthetic rates were observed and maintained during wet and dry years. In this study, *A. rubrum* had the lowest A and g_s compared to other tree species measured in same xeric site. (2) Abrams

(1998) presented a summary on foliar N content from studies conducted in broad-leaved tree species under various light regimes in situ in eastern North America, including trees growing in an opening, gap, or the understory. In this study *A. rubrum* usually had lower foliar N content compared to other tree species on the same or different sites (mean N = 1.66 %). (3) Similarly, a study conducted by Cramer *et al.* (2000) in two young northern hardwood forests (19-years old and 25-years old stands) found relatively low foliar N concentration (1.5 g m^{-2} of nitrogen per leaf unit area) for *A. rubrum* growing in their C plots. (4) Finally, a study conducted by Reich *et al.* (1990) found that foliar N concentration for *A. rubrum* was also relatively low, at 1.73 %. In this study, *A. rubrum* had the lowest foliar N concentration compared to other tree species in the study (i.e. *Prunus serotina, Quercus ellipsoidalis, Rubus allegheniensis*). In my study, *A. rubrum* trees growing in the young plot had foliar N concentrations of 1.71% and had a lower N concentration than *B. papyrifera* or *F. grandifolia* (Figure 10). Thus, V_s, leaf gas exchange (e.g., E, A, g_s) and foliar N concentration measurements were significantly lower in *A. rubrum* trees, agreeing with Abrams (1998) who suggested that *A. rubrum* has low nutrient requirements in general and presents low A, which may be related to its modest leaf structural characteristics. These characteristics can likely be attributed to *A. rubrum*'s adaptation to xeric sites and high light environments (Abrams and Kubiske, 1990).

In brief, I observed significant physiological differences between *A. rubrum* and *B. papyrifera*. Most notably, *B. papyrifera* had significantly greater V_s, E, A, and g_s than *A. rubrum*. Here, I suggest that these differences are a result of leaf-level characteristics (i.e., stomatal densities and guard cell lengths), site characteristics (i.e., dry versus wet sites), and canopy position (i.e., open canopy or understory).

4.4 Stand transpiration between the mature and young stand

The mature stand had greater BA, A_s and T (60%, 59% and 56% respectively) compared to the young stand (Tables 11, 12 and figure 11). My findings are contrary to what has been reported in similar studies.

For example, Vertessy *et al.* (2001) conducted a stand WU study in pure *Eucaliptus regnans* stands*,* in which the total amount of water yield in the old-growth stands was almost twice as much as in 25-years old re-growth stands. The greater WU observed in the younger stand was driven by greater LAI and SAI rather than differences in V_s. Another study conducted by Buckley *et al.* (2012) observed a similar pattern in a post-fire regrowth (7-years old) and a mature stand (71-years old) of *E. delegatensis* R. Baker, in which the post-fire regrowth stand had 72% greater SAI, 35% greater LAI and consumed 2.2 times more water compared to the older stand. Moore *et al.* (2004) investigated the impact of tree age, species composition and sapwood basal area on stand-level transpiration in adjacent watershed at the H. J. Andrews Forest in the western Cascades of Oregon in a young and a mature stand (40 vs. 450-years old). The mature stand was dominated by *Pseudotsuga menziesii* and *Tsuga heterophylla*, whereas the young stand was predominantly comprised of *Pseudotsuga* *menziesii* and *Alnus rubra*. The main finding of this study was that T was higher in the young stand because of the greater sap flux density (sap flow per unit sapwood area) by aged class and greater total stand A_s.

In summary, the studies reviewed above have reported greater T in younger stands, where greater T was driven mainly by higher A_s instead of higher V_s. Therefore, greater tree density per surface area means greater A_s, and LAI. In contrast, in older stands, tree density usually decreases, reducing automatically A_s, LAI and in turn, T. These findings tend to be expected for pure stands rather than for mixed species forest.

The northern hardwood forest is characterized as a mixed-species forest due to its species composition with different abilities to survive (shade intolerant, intermediate, and tolerant tree species) throughout the complex stand dynamics process. Therefore, the greater T observed in the mature stand may be explained by the greater complexity in tree species compositions and shade tolerances.

The BA found in the mature stand was compared with a few other studies conducted in nearby research sites, specifically the Bowl Research Natural Area (RNA) and the Hubbard Brook Experimental Forest (HBEF). These sites are located in the White Mountains in the state of New Hampshire and are relatively close to my research sites. Both experimental sites have mature stands under long-term studies, dominated mainly by *F. grandifolia, B. alleghaniensis* and *A. saccharum*, and other soft and hardwood species (Leak, 1987; Martin and Bailey, 1999; Fahey *et al.*, 2005). The western part of the RNA watershed does not have evidence or record of when the last disturbances occurred (Martin and Bailey, 1999). Conversely, the HBEF was logged between 1909 and 1917, and was also damaged during the 1938 hurricane. However, one of the watersheds of this research site (watershed 6 or W6) experienced less overall disturbance, which was in turn designated as the reference site since 1965 as part of a long-term research project at HBEF (Likens *et al.*, 1994). These two experimental forests share similarities with my research sites in terms of tree species, climate, soils, etc. Martin and Bailey (1999) conducted an inventory in 1974 and 1994 in the West and East part of the RNA (no evidence of logging vs. ~ 106-years old). No significant differences in changes of BA were observed after 20 years in both stands. They concluded that it is expected for a large area of the northern hardwood forests in central New Hampshire to have a mean basal area of 35 m^2 ha^{-1} and aboveground living biomass of 225 Mg ha^{-1}. Also, the northern hardwood forest that have been subjected to heavy forest cutting are expected to be fully recovered (BA and biomass levels) in about 100 years in order to be reaching and be compared as a mature forests (Martin and Bailey, 1999). Conversely, Fahey *et al.* (2005) conducted a study at the HBEF and observed that total tree biomass (live trees ≥ 10 cm DBH) of the W6 increased from about 140 Mg ha^{-1} to a maximum of 230 Mg ha^{-1} from 1965 to ~1980. Total biomass accumulation remained stable until about 1995, then started to decline. At the same time, total tree biomass (dead trees ≥ 10 cm DBH) started increasing slightly from 1975, followed by a pronounced increase starting around 1995.

Similar mean BA values were reported from three mature hardwood forest in three New England states between 80-120 years old stands (Maine, 32.07 m^2 ha^{-1}: Vermont, 34.47 m^2 h^{-1}: and in New Hampshire, 25.43 m^2 h^{-1}), (Hoover *et al.*, 2012).

All studies mentioned above have reported mean BA similar or close to what has been found in the mature study site (35 m^2 ha^{-1}), especially the BA reported by Martin and Bailey (1999). Moreover, the aboveground living biomass reported from the RNA and the W6 was quite similar (Martin and Bailey, 1999; Fahey *et al.*, 2005).

A few literature reviews were conducted in order to compare the BA found in the young stand BA (23 m^2 ha^{-1}) and other findings conducted nearby in other young northern hardwood forest stand studies in or near the White Mountains. For example, Reiners (1992) conducted a study of species composition in a 20-years old stand after deforestation in the watershed 2 (W2) of the HBEF, and reported a BA of 18.7 m^2 ha^{-1}. Allison *et al.* (2003) investigated the effects of strip width of tree cutting on regeneration of northern hardwoods in northwestern Massachusetts after 20-years of cutting, and found a BA of 15.0 m^2 ha^{-1}. Additionally, a study conducted by Fahey *et al.* (1998) in a nutrient manipulation study within the White Mountain National Forest in three 22-year old even-aged of mixed species, and reported a mean BA of 27.76 m^2 ha^{-1}. Finally, a study was conducted by Liptzin and Ashton (1999) in two 28-year old mixed-species single-aged stands growing in two sites (xeric and mesic) originated after a clearcutting at the Great Mountain Forest in northwestern Connecticut. The basal area for the xeric site was 45.5 m^2 ha^{-1}, whereas for the mesic site it was 31.94 m^2 ha^{-1}.

Overall, the BA values reported in other young northern hardwood studies were quite variable and very different to what was observed in the young site (23 m^2 h^{-1}). However, the BA reported by Reiners (1992) from the HBEF was the only BA reasonably similar to my results, and this may be because HBEF and BEF are closer in terms of location and thus may share many of the same environmental and edaphic characteristics as well as tree species composition.

My current results also indicate that shade tolerant tree species such as *F. grandifolia* and *A. saccharum* of DBH ≥ 10 cm were the main contributors to the rates of T observed in the mature stand (Table 11). The young stand in the other hand, had lower BA, As and consequently lower T, which was mainly a result of a larger number of trees with DBH between 2-10 cm, particularly *F. grandifolia*, *A. rubrum* and *P. pensylvanica*.

5. CONCLUSION

No treatment effects were observed in V_s for the majority of the sampled trees species in the mature and young stand conducted in the northern hardwood forest two years after nutrient amendment additions started. No treatment effect differences were observed or differences among tree species in V_s for trees growing in the mature stand.

Sap velocity, E, A, g_s, iWUE and foliar N concentration (Figures 5, 6, 7, 8, 9. 10) remained unchanged for *B. papyrifera* and *F. grandifolia* after all treatments ($p > 0.05$). However, *A. rubrum* trees growing in the N plot had significantly greater V_s ($p < 0.05$) (Figure 5) and significantly lower iWUE ($p < 0.016$*) (Figure 9) compared to C plots in the young stand. Thus, the greatest V_s observed for *A. rubrum* is believed to be associated with tree growth as a response to N addition. Trees usually increase LAI after fertilization and in turn have a higher transpirational demand. Greater LAI also stimulates stem growth that consequently increases A_s for water to be transported. Consequently, increasing LAI and E in the N plot made *A. rubrum* become less efficient in controlling WU that is reflected in lower iWUE (greater water loss per unit carbon gain).

Also, *A. rubrum* had significantly lower V_s, E, A, g_s, foliar N concentration and greater iWUE compared to *B. papyrifera* and *F. grandifolia* in the young stand. These low gas exchange rates observed for *A. rubrum* may be explained by the morphology and structure of its leaves, especially the guard cell length, which controls the opening and closing of the stomata. *Acer rubrum* guard cell length was 12.7± 0.4μm shorter compared to the guard cell length reported for *B. papyrifera* was 30.0±1.1μm (Abrams, 1998).

Even though foliar N concentration did not increases for *A. rubrum* trees growing in the N plot, it responded with greater V_s, which could be a result of having low nutrient requirements (Abrams, 1998). By adding low nutrient doses, *A. rubrum* may have been able to respond in growth. This is contrary to what was observed for *B. papyrifera* and *F. grandifolia*, which remained unchanged in V_s, E, A, g_s and iWUE two years after treatment.

Thus, the significantly higher in V_s and significantly lower iWUE values observed in *A. rubrum* trees growing in the N plot had a certain similarity to what has been observed for *phaseolus vulgaris* under mass-flow nutrients experiment (Matimati *et al.*, 2013). *Phaseolus vulgaris* growing closer to the N source responded in greater E with low WUE in response to N uptake. The low WUE observed in this study was due to a broad range in g_s challenged with limited N availability. However, no treatment effect was observed in g_s, E or A for *A. rubrum* for further comparisons.

Overall, longer studies are necessary to fully understand how nutrient additions affect tree WU. Over the short term, I found interesting results by species that have the potential to become establish more pronounce over more years of nutrient additions. Further research should continue monitoring in WU for these dominant tree species.

TABLES

Table 1 Stand characteristic and location.

Stand	C2	C8
Cut	1988	1883
Location	44º 04´N, 71º 16’ W	44º 03’ N, 71º 18’ W
Elevation (m)	340	330
Slope (%)	15-30	5-35
Tree density (ha^{-1})		
DBH > 10 cm	319	496
DBH 2-10 cm	11820	960

From latest survey conducted in 2011 by Shinjini Goswami and Matthew Vadeboncoeur. The C2 is the young stand (25-year old) and C8 is the old stand (> 100-year-old).

Table 2 Sap velocity calculation from Burgess *et al*. (2001).

Equation	**Equation form**	**Variable or coefficient description**	**Equation # from the paper**
Heat pulse velocity (v_h)	$V_h = \frac{k}{x}\ln(v_1 / v_2)3600$	k= Thermal diffusivity of a green fresh wood used a nominal value from the paper. X= 0.6 cm as the distasnce between the heater probe to eisther temperature probes. V_1 = upstream increasing temperature V_2 = downstream increasing temperature	(2)
Heat pulse velocity correction (V_c)	$V_c = bV_h + cV_h^2 + dV_h^3$	b, c, d = wound correction coefficients provided from the paper, on table 1, (b = 2.4908, c = -0.0057, d = 0.0010).	(6)
Sap velocity (V_s)	$V_s = \frac{V_c \mathrm{P}_b (c_w + m_c c_s)}{\mathrm{P}_s c_s}$	P_b = basic density of wood (dry weight/green volume), see table 4. c_w = a specific heat capacity of the wood matrix (1200 J kg^{-1} °C). m_c = water content of sapwood, see table 4. C_s = specific heat capacity of the sap (water, 4182 J kg^{-1} °C). p_s = density of water (1000 kg/m^3).	(7)

Table 3 Sapwood water content and wood density determined by tree species.

Tree species	Water content of sapwood (m_c; g/cm^3)	Wood density (P_b; kg/m^3)	Provider
F. grandifolia	0.505	600	√
A. saccharum	0.346	620	√
B. alleghaniensis	0.458	550	√
A. rubrum	0.445	633	*
B. papyrifera	0.419	505	*

Check mark (√) indicates values provided by Hernandez-Santana and Asbjornsen (in prep.), and an asterisk (*) indicates values determined by the current work.

Table 4 Allometric equation between DBH and A_s.

Forest stand	Tree species	n	a	b	r^2
C8	*A. saccharum*	24	0.7848	1.9697	0.95
C8	*B. alleghaniensis*	21	1.171	1.7936	0.95
C8	*F. grandifolia*	47	0.6727	1.9187	0.98
C2	*B. papyrifera*	20	0.7098	2.0396	0.99
C2	*P. pensylvanica*	20	1.4444	1.3302	0.65
C2	*A. rubrum*	20	0.8279	1.9707	0.99

The allometric equation was fitted in a power function form ($Y=a X^{b}$).
The n is the sample sizes or tree number measured. On the power function form (Y) is A_s, (a) is a normalization constant, (X) is DBH (cm) and (b) is the allometric scaling exponent.

Table 5 Sample trees for V_s calculation in C2 and C8 stands.

	Stand							
	C2				C8			
	Treatment				Treatment			
Tree species	C	N	NP	P	C	N	NP	P
F. grandifolia	3	2	3	3	2	3	3	3
A. rubrum	3	2	3	3	0	0	0	0
A. saccharum	0	0	0	0	3	3	3	3
B. papyrifera	3	3	3	3	0	0	0	0
B. alleghaniensis	0	0	0	0	2	3	3	3

Table 6 Mean DBH of tree sampled for V_s in C8 and C2 stands.

	Treatments							
	C		P		NP		N	
Tree species	DBH (cm)	+1SE	DBH (cm)	+1SE	DBH (cm)	+1SE	DBH (cm)	+1SE
a) *C8 stand*								
F. grandifolia	36.8	7.4	38.1	4.7	52.9	3.4	39.6	2.6
B. alleghaniensis	42.0	13.4	38.7	5.0	33.8	3.2	39.6	6.7
A. saccharum	42.6	5.1	47.6	8.1	35.6	2.0	37.53	2.9
b) *C2 stand*								
F. grandifolia	11.1	0.2	14.4	0.9	10.4	0.5	15.0	1.9
B. papyrifera	13.4	1.6	11.3	1.2	11.1	0.7	10.3	0.4
A. rubrum	12.0	0.08	11.1	0.5	12.0	0.3	13.5	0.1

Table 7 Days having maximum VPD ≥1 kPa for V_s calculation in both stands.

Date	Day-of-year (2013)	Stand	
		C8	C2
July, 5th	186	√	√
July, 12th	193	√	√
July, 15th	196	√	√
July, 17th	198	√	√
July, 18th	199	√	√
July, 19th	200	√	√
August, 3rd	215	√	√
August, 5th	217	√	√
August, 6th	218	√	√
August, 10th	222	√	
August, 11th	223	√	
August, 12th	224	√	

Table 8 Mean V_s among trees species and treatments for both stands.

Tree species	DBH (cm)	A_s (cm^2)	Treatments				
			C	P	NP	N	Prob >F
a) C8 stand							
F. grandifolia	42.4	924.6	23.3	38.1	46.2	37.3	0.87
B. alleghaniensis	38.7	857.4	19.5	33.7	23.6	25.4	0.40
A. saccharum	40.8	1201.9	29.3	15.1	19.2	26.8	0.17
b) C2 stand							
F. grandifolia	12.4	86.9	39.5	50.6	24.7	41.0	0.40
B. papyrifera	11.5	107.6	46.1	49.4	46.6	38.9	0.78
A. rubrum	11.9	110.2	20.2	28.4	23.6	45.3	0.05

Table 9 Means of A, E, iWUE among tree species and among treatments in the C2 stand, obtained from one-way ANOVA.

Tree species	Tree (n)	Treatments				
		C	P	NP	N	Prob > F
a) Photosynthetic rate *(μmol CO_2 m^{-2} s^{-1})*						
F. grandifolia	3	10.22	9.04	11.34	11.43	0.60
B. papyrifera	3	12.40	12.42	8.88	10.23	0.19
A. rubrum	3	7.62	6.48	8.54	5.04	0.33
b) Transpiration rate *(mmol H_2O m^{-2} s^{-1})*						
F. grandifolia	3	2.28	1.99	2.64	2.28	0.42
B. papyrifera	3	3.50	2.63	2.05	2.63	0.15
A. rubrum	3	1.37	1.11	1.80	1.15	0.27
c) Conductance to H2O *(mol H_2O m^{-2} s^{-1})*						
F. grandifolia	3	0.18	0.15	0.18	0.16	0.80
B. papyrifera	3	0.28	0.22	0.13	0.18	0.08
A. rubrum	3	0.10	0.07	0.11	0.07	0.31
d) Instantaneous WUE *(μmol $mmol^{-1}$)*						
F. grandifolia	3	4.63	4.45	4.30	4.65	0.78
B. papyrifera	3	3.56	4.85	4.37	4.09	0.16
A. rubrum	3	5.64	5.88	4.87	4.12	0.0162*

Table 10 Sap velocity means for days having VPD ≥1 kPa between 12:00 to 13:45 hours. Only dates marked with a symbol (√) in C2 from table 7 were included for the calculation.

Tree species	Outer thermocouple (cm h^{-1})	Middle thermocouple (cm h^{-1})	Inner thermocouple (cm h^{-1})	Mean (cm h^{-1})
a) C8 stand				
F. grandifolia	29.8	39.1	26.9	31.9**
A. saccharum	14.7	25.9	26.7	22.4
B. alleghaniensis	15.3	26.2	22.8	21.4
Mean	19.9*	30.4*	25.5*	25.3
b) C2 stand				
F. grandifolia	32.9	38.8	21.5	31.0**
A. rubrum	19.6	28.0	25.9	24.5**
B. papyrifera	24.2	45.2	39.4	36.3**
Mean	25.6*	37.3*	28.9*	30.6***

* used for non-dominant tree species having DBH >10 cm, ** dominant tree species having between 2-10 cm in DBH, and *** used for non-dominant tree species having DBH between 2-10 cm.

Table 11 Total sap flow for the C8 calculated upon V_s in days having VPD ≥ 1 kPa between 12:00 to 13:45 hrs.

Tree species	Trees (ha^{-1})	BA (m^2 ha^{-1})	A_s (m^2 ha^{-1})	T (mm h^{-1})
a) *DBH > 10 cm*				
F. grandifolia	306	14.54	9.41	0.2603
B. alleghaniensis	28	2.46	1.71	0.0387
A. saccharum	122	12.89	11.51	0.3017
A. rubrum	6	0.41	0.39	0.0100
T. canadensis	17	0.21	0.19	0.0049
F. americana	14	2.35	2.09	0.0532
T. americana	3	0.56	0.50	0.0128
b) *DBH 2-10 cm*				
F. grandifolia	940	1.86	1.38	0.0443
T. canadensis	20	0.01	0.01	0.0003
Total	1456	35	27	0.7267

Table 12 Total sap flow for the C2 calculated upon V_s in days having VPD ≥ 1 kPa between 12:00 to 13:45 hrs.

Tree species	Trees (ha^{-1})	BA ($m^2\ ha^{-1}$)	A_s ($m^2\ ha^{-1}$)	T ($mm\ h^{-1}$)
a) *DBH > 10 cm*				
F. grandifolia	64	0.75	0.52	0.0130
A. rubrum	144	1.51	1.48	0.0376
B. papyrifera	58	0.60	0.60	0.0229
P. pensylvanica	39	0.38	0.13	0.0042
T. canadensis	8	0.10	0.07	0.0021
B. alleghaniensis	6	0.06	0.05	0.0012
b) *DBH 2-10 cm*				
F. grandifolia	5780	7.01	5.30	0.1649
A. rubrum	1580	3.23	3.23	0.0795
B. papyrifera	1080	2.19	2.13	0.0774
P. pensylvanica	1660	3.95	2.18	0.0670
T. canadensis	140	0.37	0.37	0.0115
B. alleghaniensis	640	1.01	1.08	0.0233
A. pennsylvanicum	900	1.52	1.52	0.0467
F. americana	40	0.07	0.07	0.0022
Total	12139	23	19	0.5542

Table 13 Foliar N(%) concentration in trees growing in C2 and C8 stands.

	Treatments							
	C		P		NP		N	
Tree species	N (%)	+1SE	N (%)	+1SE	N (%)	+1SE	N (%)	+1SE
a) *C8 stand*								
F. grandifolia	2.01	0.05	2.05	0.02	.	.	2.25	0.07
B. alleghaniensis	2.54	0.18	1.9	0.08	2.32	0.15	2.16	0.09
A. saccharum	1.56	0.10	1.47	0.03	1.69	0.10	1.78	0.05
b) *C2 stand*								
F. grandifolia	2.27	0.09	1.77	0.15	2.44	0.05	2.17	0.15
B. papyrifera	2.07	0.11	1.82	0.21	1.87	0.20	2.10	0.15
A. rubrum	1.71	0.04	1.53	0.01	1.68	0.05	1.71	0.01

FIGURES

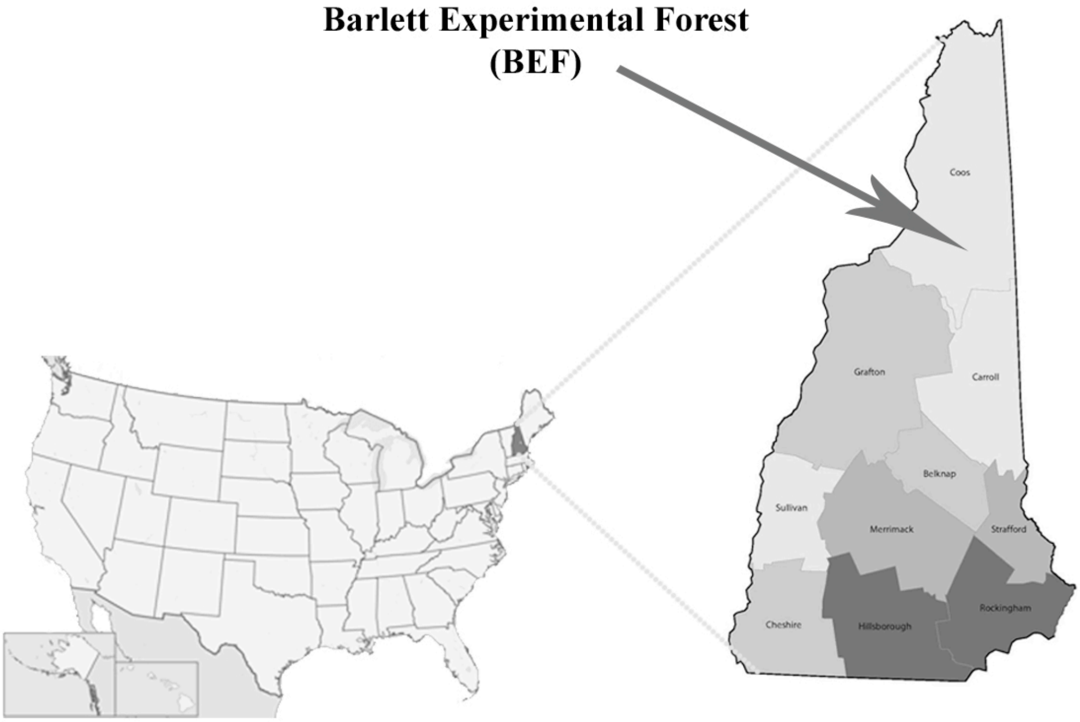

Figure 1 Map of the Bartlett Experimental Forest (BEF), located in NH (USA).

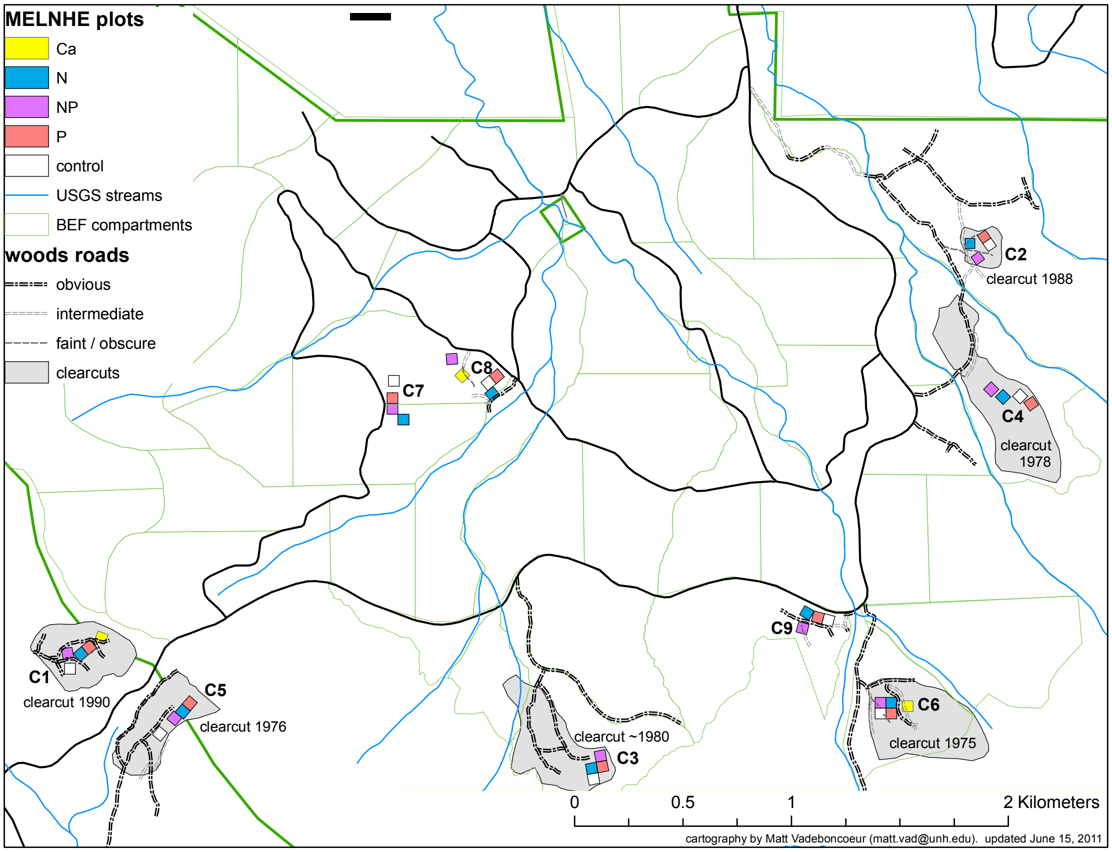

Figure 2 Map of the study site locations and treatment plot layouts.
The C2 is the young stand (25-year old after a cut occurred), and the C8 is the old stand (>100-year-old) by Matthew Vadeboncoeur.

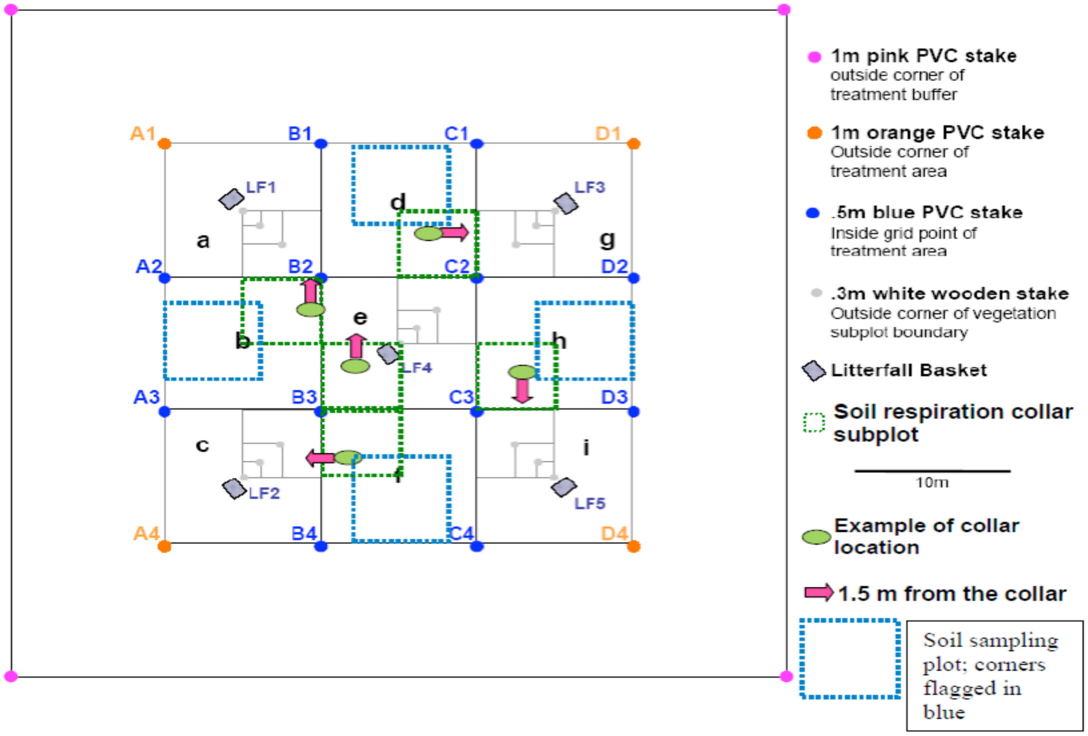

Figure 3 MELNHE General Plot Layout.
Sample trees for this study were located in the outside corner of the treatment buffer (1m pink PVC stake), which it is the full 50 x 50 m^2 fertilized area, and the inventory trees were done in the 30 x 30 m2 in the outside corner of the treatment area gridded area (1m orange PVC stake), figure produced by Matthew Vadeboncoeur.

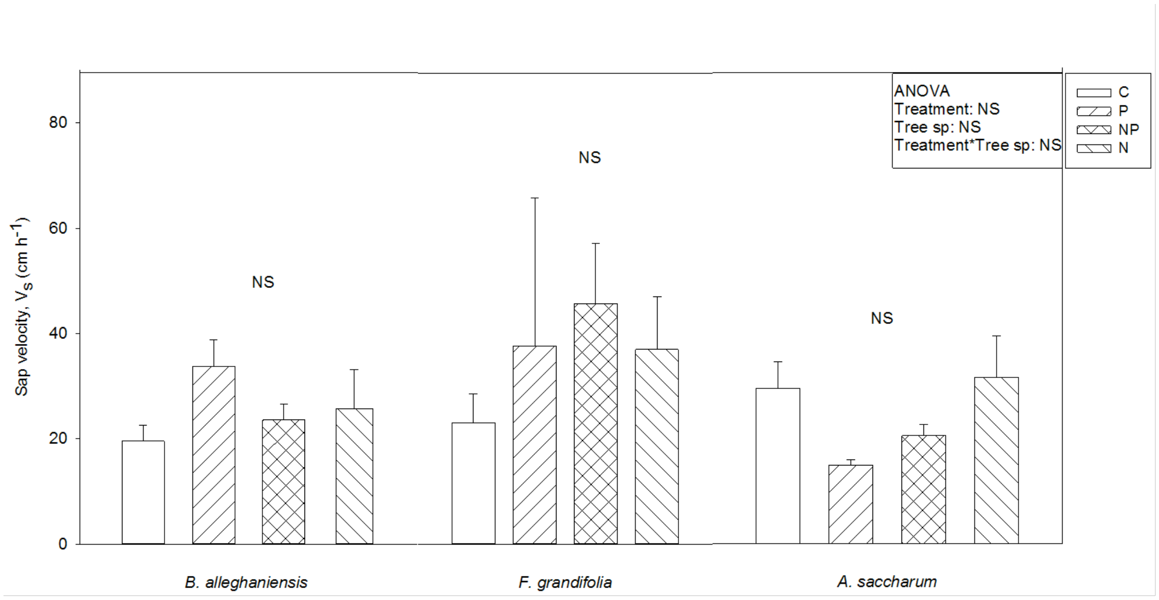

Figure 4 Mean sap velocity compared among species and treatments in the old stand (C8). Data show days having maximum VPD ≥1 kPa, between 12:00 to 13:45 hours during daylights saving time. Error bars denote a standard error of the mean.

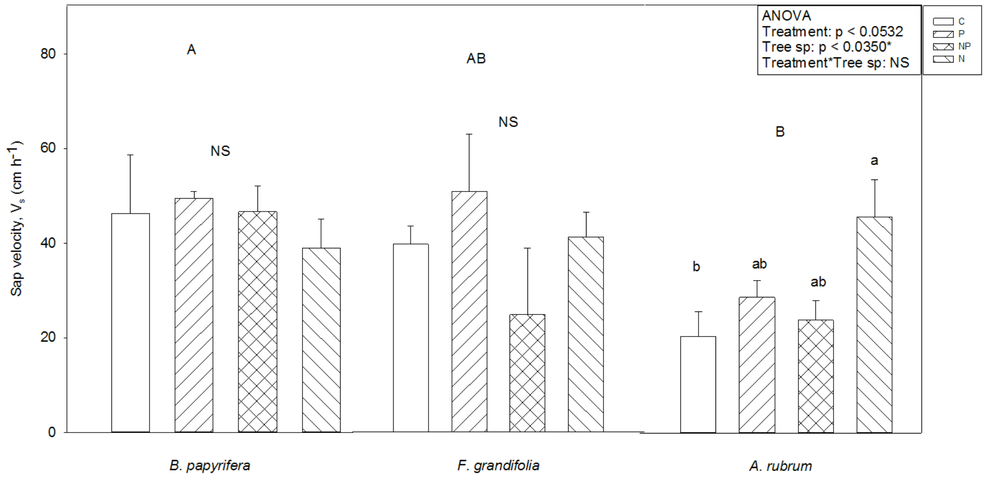

Figure 5 Mean sap velocity compared among species and treatments in the young stand (C2). Data show days having maximum VPD ≥1 kPa, between12:00 to 13:45 hours during daylights saving time. *Capital letters* denote differences among tree species. L*ower case letters* indicate the differences among treatments. Error bars denote standard error of the mean.

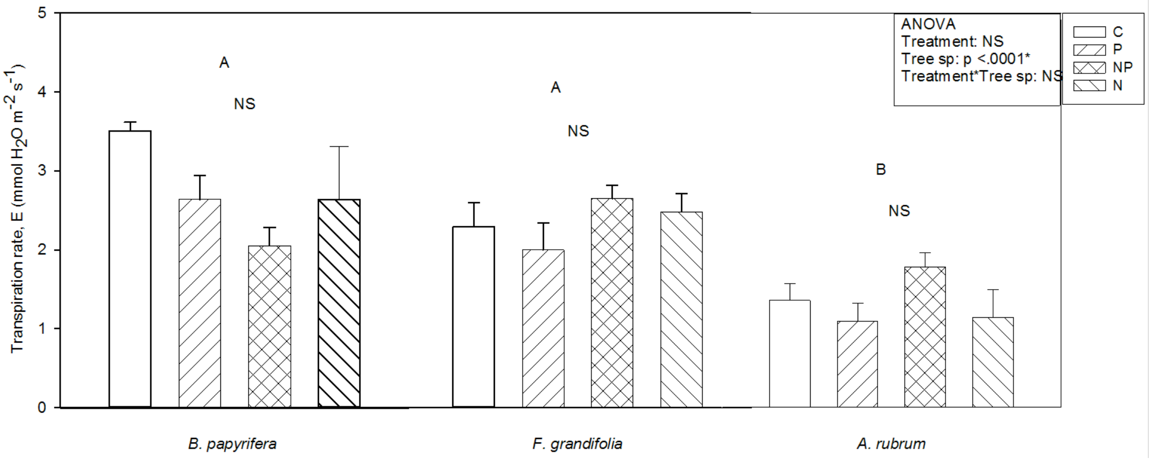

Figure 6 Mean leaf transpiration rates compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean.

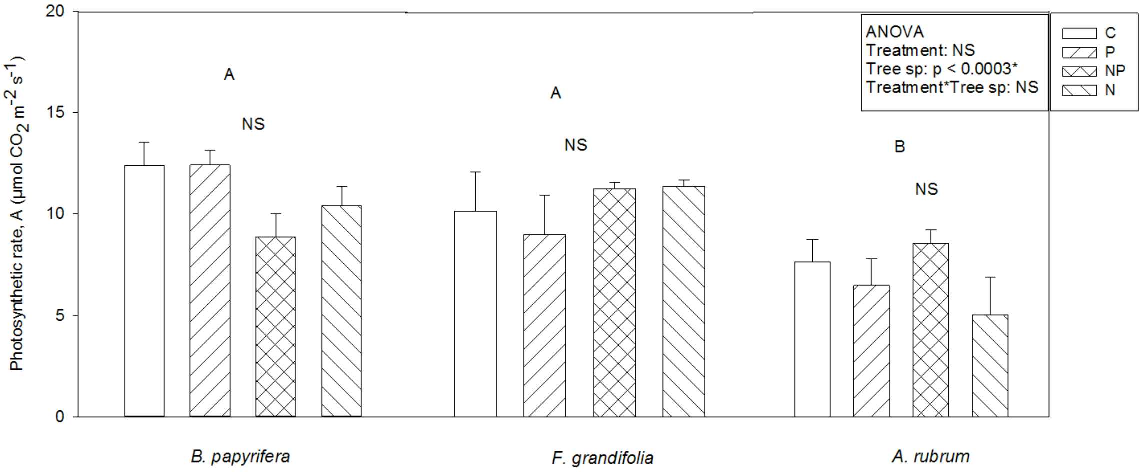

Figure 7 Mean leaf photosynthetic rates compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean.

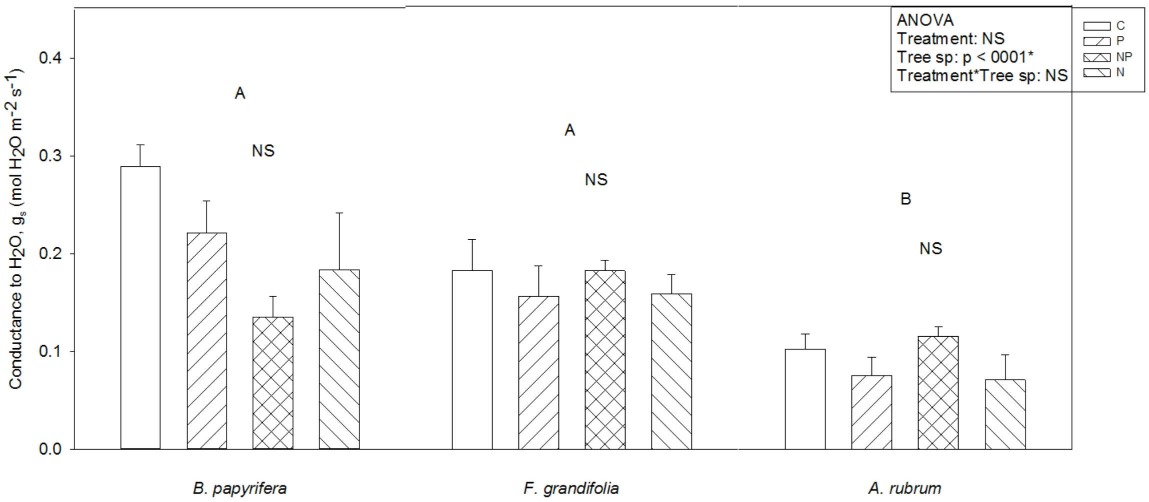

Figure 8 Mean leaf conductance compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. Error bars denote standard error of the mean.

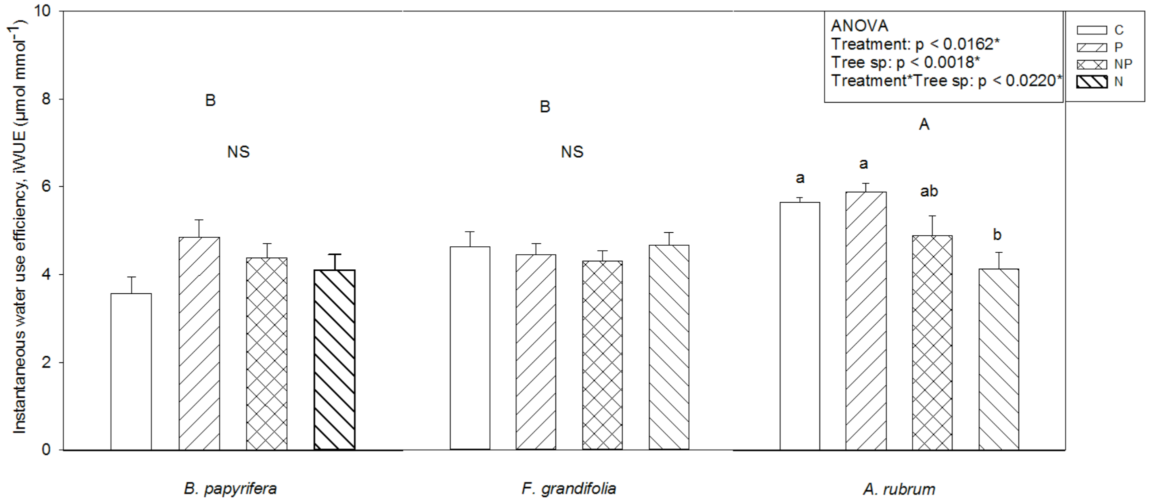

Figure 9 Mean instantaneous water use efficiency compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. L*ower case letters* indicate differences among treatments. Error bars denote standard error of the mean.

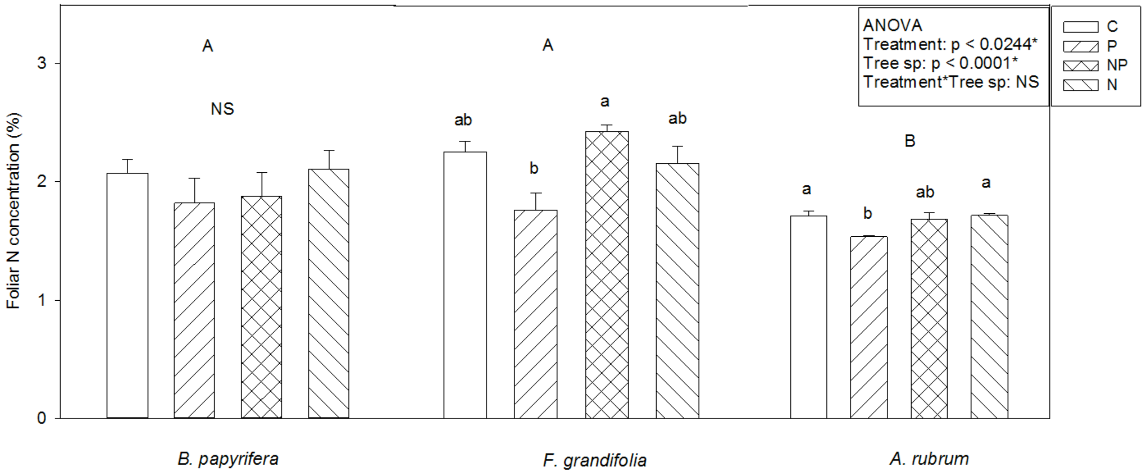

Figure 10 Mean foliar nitrogen concentration compared among species and treatments in the young stand (C2). *Capital letters* denote differences among tree species. L*ower case letters* indicate differences among treatments. Error bars denote standard error of the mean.

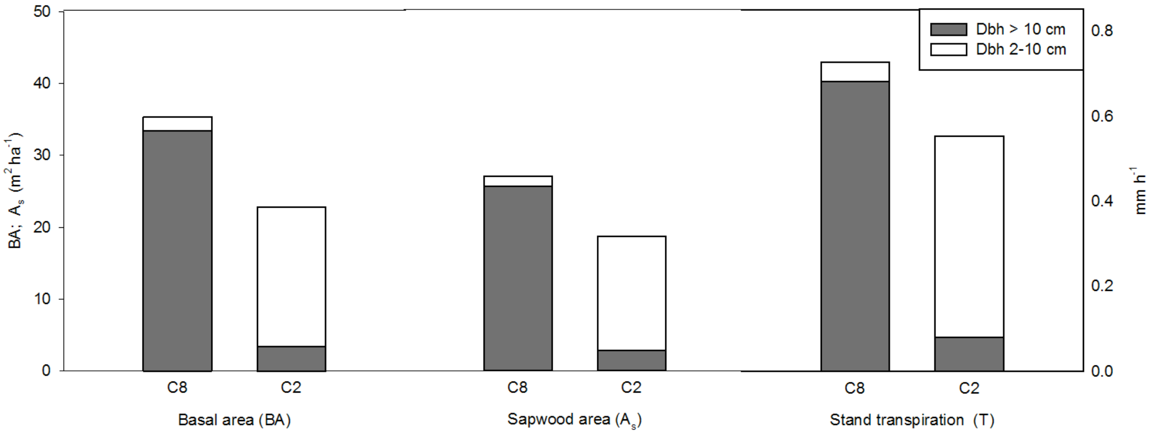

Figure 11 Basal area (BA) and sapwood area (A_s) are to the left axis, and stand transpiration (T) is to the right axis. Grey bar represents trees having DBH > 10 cm, while empty bar represent trees having DBH between 2-10 cm.

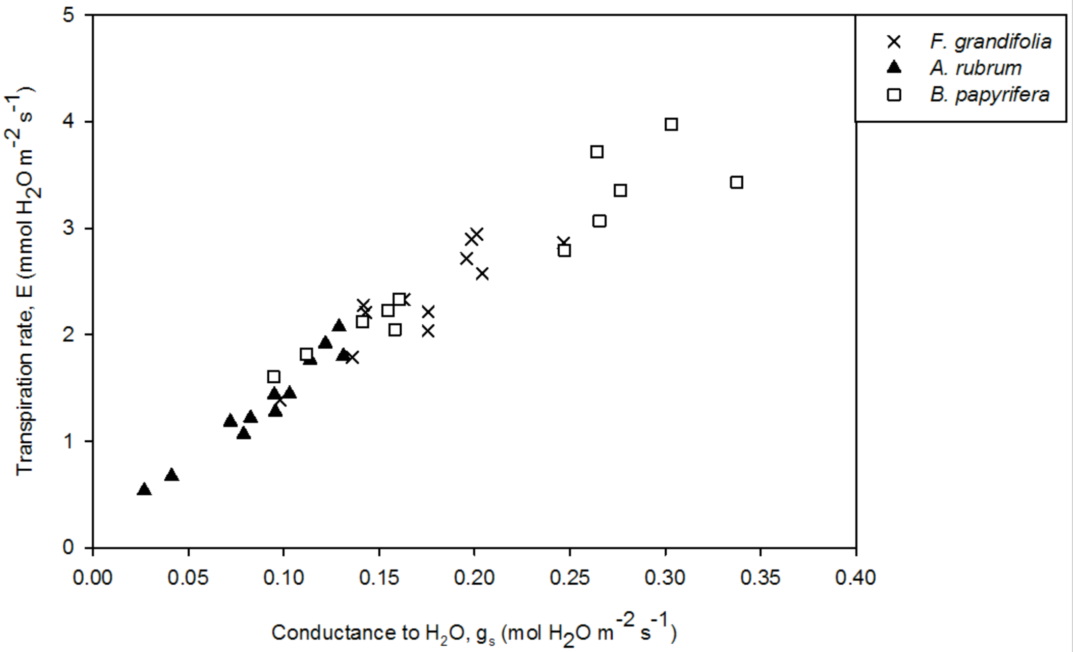

Figure 12 Transpiration rate as a function of conductance of the three dominant tree species in the C2 in the BEF, Bartlett, NH.

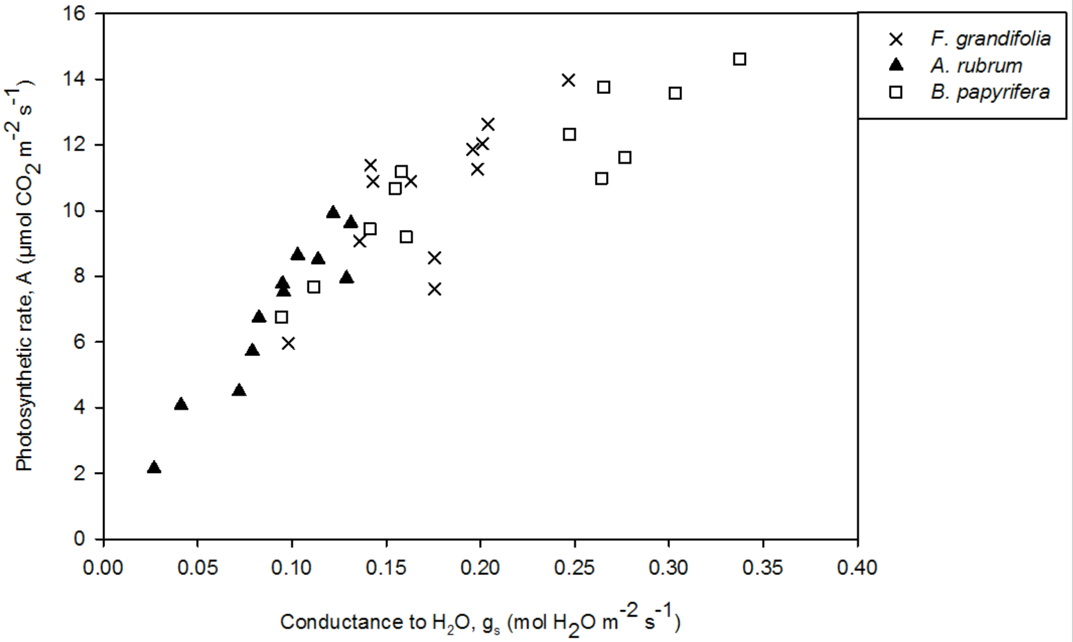

Figure 13 Photosynthesis rate as a function of conductance of the three dominant tree species in the C2 in the BEF, Bartlett, NH.

LITERATURE CITED

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39, 378-386.

Abrams, M.D., 1998. The red maple paradox. Bioscience 48, 355-364.

Abrams, M.D., Kubiske, M.E., 1990. Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin-influence of light regime and shade-tolerance rank. Forest Ecology and Management 31, 245-253.

Allison, T.D., Art, H.W., Cunningham, F.E., Teed, R., 2003. Forty-two years of succession following strip clearcutting in a northern hardwoods forest in northwestern Massachusetts. Forest Ecology and Management 182, 285-301.

Ambrose, A.R., Sillett, S.C., Dawson, T.E., 2009. Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. Plant Cell and Environment 32, 743-757.

Bleby, T.M., Burgess, S.S.O., Adams, M.A., 2004. A validation, comparison and error analysis of two heat-pulse methods for measuring sap flow in Eucalyptus marginata saplings. Funct. Plant Biol. 31, 645-658.

Bobbink, R., Bik, L., Willems, J.H., 1988. Effects of nitrogen-fertilization on vegetation structure and dominance of brachypodium-pinnatum (l) beauv in chalk grassland. Acta Bot. Neerl. 37, 231-242.

Bormann, F.H., Siccama, T.G., Likens, G.E., Whittake.Rh, 1970. Hubbard Brook Ecosystem Study - composition and dynamics of tree stratum. Ecol. Monogr. 40, 373-&.

Boxman, A.W., Vandam, D., Vandijk, H.F.G., Hogervorst, R.F., Koopmans, C.J., 1995. Ecosystem responses to reduced nitrogen and sulfur inputs into 2 coniferous forest stands in the Netherlands. Forest Ecology and Management 71, 7-29.

Brown, S., 1997. Estimating Biomass and Biomass Change of Tropical Forests: A Primer. FAO Forestry Paper 134, Food and Agriculture Organization, Rome, Italy.

Bucci, S.J., Scholz, F.G., Goldstein, G., Meinzer, F.C., Franco, A.C., Campanello, P.I., Villalobos-Vega, R., Bustamante, M., Miralles-Wilhelm, F., 2006. Nutrient availability constrains the hydraulic architecture and water relations of savannah trees. Plant Cell and Environment 29, 2153-2167.

Buckley, T.N., Turnbull, T.L., Pfautsch, S., Gharun, M., Adams, M.A., 2012. Differences in water use between mature and post-fire regrowth stands of subalpine Eucalyptus delegatensis R. Baker. Forest Ecology and Management 270, 1-10.

Burgess, S.S.O., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A.H., Bleby, T.M., 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiol. 21, 589-598.

Colin, W., Mark, F., 1996. Stomata. Chapman & Hall, 2-6 Boundary Row, London SE1 *HN, UK.

Cramer, J., Fahey, T., Battles, J., 2000. Patterns of leaf mass, area and nitrogen in young northern hardwood forests. American Midland Naturalist 144, 253-264.

Cramer, M.D., Hoffmann, V., Verboom, G.A., 2008. Nutrient availability moderates transpiration in Ehrharta calycina. New Phytologist 179, 1048-1057.

de Groot, R.S., Wilson, M.A., Boumans, R.M.J., 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecol. Econ. 41, 393-408.

de Vries, W., Solberg, S., Dobbertin, M., Sterba, H., Laubhann, D., van Oijen, M., Evans, C., Gundersen, P., Kros, J., Wamelink, G.W.W., Reinds, G.J., Sutton, M.A., 2009. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management 258, 1814-1823.

Dietze, M.C., Moorcroft, P.R., 2011. Tree mortality in the eastern and central United States: patterns and drivers. Glob. Change Biol. 17, 3312-3326.

Elvir, J.A., Wiersma, G.B., Day, M.E., Greenwood, M.S., Fernandez, I.J., 2006. Effects of enhanced nitrogen deposition on foliar chemistry and physiological processes of forest trees at the Bear Brook Watershed in Maine. Forest Ecology and Management 221, 207-214.

Fahey, T.J., Battles, J.J., Wilson, G.F., 1998. Responses of early successional northern hardwood forests to changes in nutrient availability. Ecol. Monogr. 68, 183-212.

Fahey, T.J., Siccama, T.G., Driscoll, C.T., Likens, G.E., Campbell, J., Johnson, C.E., Battles, J.J., Aber, J.D., Cole, J.J., Fisk, M.C., Groffman, P.M., Hamburg, S.P., Holmes, R.T., Schwarz, P.A., Yanai, R.D., 2005. The biogeochemistry of carbon at Hubbard Brook. Biogeochemistry 75, 109-176.

Finzi, A.C., 2009. Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. Biogeochemistry 92, 217-229.

Fisk, M.C., Ratliff, T.J., Goswami, S., Yanai, R.D., 2013. Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. Biogeochemistry.

Forrester, D.I., Collopy, J.J., Beadle, C.L., Warren, C.R., Baker, T.G., 2012. Effect of thinning, pruning and nitrogen fertilizer application on transpiration, photosynthesis and water-use efficiency in a young Eucalyptus nitens plantation. Forest Ecology and Management 266, 286-300.

Gebauer, T., Horna, V., Leuschner, C., 2008. Variability in radial sap flux density patterns and sapwood area among seven co-occurring temperate broad-leaved tree species. Tree Physiol. 28, 1821-1830.

Giday, H., Kjaer, K.H., Fanourakis, D., Ottosen, C.O., 2013. Smaller stomata require less severe leaf drying to close: A case study in Rosa hydrida. Journal of Plant Physiology 170, 1309-1316.

Gusewell, S., 2004. N : P ratios in terrestrial plants: variation and functional significance. New Phytologist 164, 243-266.

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. Ecology Letters 14, 852-862.

Hatton, T.J., Catchpole, E.A., Vertessy, R.A., 1990. Integration of sap flow velocity to estimate plant water-use. Tree Physiol 6, 201-209.

Hernandez-Santana, V., Asbjornsen, H., in prep. Water use in a northern hardwood forest is mainly controlled by solar radiation and stand structure rather than by species or short-term fertilization.

Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901-908.

Hoover, C.M., Leak, W.B., Keel, B.G., 2012. Benchmark carbon stocks from old-growth forests in northern New England, USA. Forest Ecology and Management 266, 108-114.

Hubbard, R.M., Ryan, M.G., Giardina, C.P., Barnard, H., 2004. The effect of fertilization on sap flux and canopy conductance in a Eucalyptus saligna experimental forest. Glob. Change Biol. 10, 427-436.

Huntington, T.G., Richardson, A.D., McGuire, K.J., Hayhoe, K., 2009. Climate and hydrological changes in the northeastern United States: recent trends and implications for forested and aquatic ecosystems. Can. J. For. Res.-Rev. Can. Rech. For. 39, 199-212.

Ilstedt, U., Malmer, A., Elke, V., Murdiyarso, D., 2007. The effect of afforestation on water infiltration in the tropics: A systematic review and meta-analysis. Forest Ecology and Management 251, 45-51.

Kubiske, M.E., Abrams, M.D., 1994. Ecophysiological analysis of woody species in contrasting temperate communities during wet and dry years. Oecologia 98, 303-312.

Leak, W.B., 1987. Characteristics of five climax stands in New Hampshire.

Leak, W.B., 1991. Secondary forest succession in New-Hampshire, USA. Forest Ecology and Management 43, 69-86.

Likens, G.E., Driscoll, C.T., Buso, D.C., 1996. Long-term effects of acid rain: Response and recovery of a forest ecosystem. Science 272, 244-246.

Likens, G.E., Driscoll, C.T., Buso, D.C., Siccama, T.G., Johnson, C.E., Lovett, G.M., Ryan, D.F., Fahey, T., Reiners, W.A., 1994. The biogeochemistry of potassium at Hubbard Brook. Biogeochemistry 25, 61-125.

Liptzin, D., Ashton, P.M.S., 1999. Early-successional dynamics of single-aged mixed hardwood stands in a southern New England forest, USA. Forest Ecology and Management 116, 141-150.

Liu, X.P., Fan, Y.Y., Long, J.X., Wei, R.F., Kjelgren, R., Gong, C.M., Zhao, J., 2013. Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of Robinia pseudoacacia seedlings. Journal of Environmental Sciences-China 25, 585-595.

Lovett, G.M., Arthur, M.A., Weathers, K.C., Fitzhugh, R.D., Templer, P.H., 2013. Nitrogen Addition Increases Carbon Storage in Soils, But Not in Trees, in an Eastern US Deciduous Forest. Ecosystems 16, 980-1001.

Marcus, A.I., Moore, R.C., Cyr, R.J., 2001. The role of microtubules in guard cell function. Plant Physiology 125, 387-395.

Martin, C.W., Bailey, A.S., 1999. Twenty years of change in a northern hardwood forest. Forest Ecology and Management 123, 253-260.

Matimati, I., Verboom, G.A., Cramer, M.D., 2013. Nitrogen regulation of transpiration controls mass-flow acquisition of nutrients. Experimental Botany McDowell, N.

Barnard, H., Bond, B., Hinckley, T., Hubbard, R., Ishii, H., Köstner, B., Magnani, F., Marshall, J., Meinzer, F., Phillips, N., Ryan, M., Whitehead, D., 2002. The relationship between tree height and leaf area: sapwood area ratio. Oecologia 132, 12-20.

Meinzer, F.C., Bond, B.J., Warren, J.M., Woodruff, D.R., 2005. Does water transport scale universally with tree size? Functional Ecology 19, 558-565.

Mohan, J.E., Cox, R.M., Iverson, L.R., 2009. Composition and carbon dynamics of forests in northeastern North America in a future, warmer world. Can. J. For. Res.-Rev. Can. Rech. For. 39, 213-230.

Moore, G.W., Bond, B.J., Jones, J.A., Phillips, N., Meinzer, F.C., 2004. Structural and compositional controls on transpiration in 40-and 450-year-old riparian forests in western Oregon, USA. Tree Physiol 24, 481-491.

Moreno-Sotomayor, A., Weiss, A., Paparozzi, E.T., Arkebauer, T.J., 2002. Stability of leaf anatomy and light response curves of field grown maize as a function of age and nitrogen status. Journal of Plant Physiology 159, 819-826.

Nadelhoffer, K.J., Emmett, B.A., Gundersen, P., Kjonaas, O.J., Koopmans, C.J., Schleppi, P., Tietema, A., Wright, R.F., 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398, 145-148.

Ollinger, S.V., Aber, J.D., Federer, C.A., 1998. Estimating regional forest productivity and water yield using an ecosystem model linked to a GIS. Landscape Ecology 13, 323-334.

Perlin, J., 1991. A forest journey: The role of wood in the development of civilization. Harvard University Press, Cambridge, MA, 445.

Reich, P.B., Abrams, M.D., Ellsworth, D.S., Kruger, E.L., Tabone, T.J., 1990. Fire effects ecophysiology and community dynamics of central Wisconsin oak forest regeneration. Ecology 71, 2179-2190.

Reiners, W.A., 1992. Twenty years of ecosystem reorganization following experimental deforestation and regrowth suppression. Ecol. Monogr. 62, 503-523.

Ripullone, F., Lauteri, M., Grassi, G., Amato, M., Borghetti, M., 2004. Variation in nitrogen supply changes water-use efficiency of Pseudotsuga menziesii and Populus x euroamericana; a comparison of three approaches to determine water-use efficiency. Tree Physiol 24, 671-679.

Roem, W.J., Berendse, F., 2000. Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. Biological Conservation 92, 151-161.

Rossatto, D.R., Hoffmann, W.A., Franco, A.C., 2009. Stomatal traits of cerrado and gallery forest congeneric pairs growing in a transitional region in Central Brazil. Acta Botanica Brasilica 23, 499-508.

Ryan, M.G., Binkley, D., Fownes, J.H., Giardina, C.P., Senock, R.S., 2004. An experimental test of the causes of forest growth decline with stand age. Ecol. Monogr. 74, 393-414.

Schafer, K.V.R., Oren, R., Tenhunen, J.D., 2000. The effect of tree height on crown level stomatal conductance. Plant Cell and Environment 23, 365-375.

Schulze, E.D., 1989. Air-pollution and forest decline in a spruce (Picea-abies) forest. Science 244, 776-783.

Vadeboncoeur, M.A., 2010. Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. Can. J. For. Res.-Rev. Can. Rech. For. 40, 1766-1780.

Vadeboncoeur, M.A., Hamburg, S.P., Blum, J.D., Pennino, M.J., Yanai, R.D., Johnson, C.E., 2012. The Quantitative Soil Pit Method for Measuring Belowground Carbon and Nitrogen Stocks. Soil Science Society of America Journal 76, 2241-2255.

Vertessy, R.A., Watson, F.G.R., O'Sullivan, S.K., 2001. Factors determining relations between stand age and catchment water balance in mountain ash forests. Forest Ecology and Management 143, 13-26.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D., 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications 7, 737-750.

Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea - how can it occur? Biogeochemistry 13, 87-115.

Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20, 5-15.

Wine, M.L., Zou, C.B., 2012. Long-term stream flow relations with riparian gallery forest expansion into tall grass prairie in the Southern Great Plains, USA. Forest Ecology and Management 266, 170-179.