

Investigating species-specific nutrient limitation in northern hardwoods

Shinjini Goswami

Dissertation proposal

April 29th 2013

For consideration by the Graduate Committee:

Dr. Melany Fisk (Advisor)

Dr. Michael Vanni

Dr. Tom Crist

Dr. Dave Gorchov

Dr. Hank Stevens

Overview

Availability and recycling of nutrients constrain primary productivity in many ecosystems. Current ecosystem theories have evolved from an earlier paradigm of limitation of ecosystem productivity by a single nutrient to that of co-limitation by multiple nutrients. Nutrient co-limitation is inferred when ecosystem productivity increases more in response to two nutrients added together than to either nutrient added alone. The increase in productivity can be attributed to responses at the biochemical level (cellular or organ level), at the level of allocation within a plant, or at the community level. For an ecosystem in which productivity is co-limited by N and P, community level co-limitation occurs when some species respond primarily to N and others to P.

Here I propose several related studies to understand potential mechanisms by which nutrient co-limitation of forest productivity could operate at a community level, by studying nutrient responses at three levels of biological organization: organ (roots), whole plant (seedlings) and stand (mature trees). My approach integrates related ecological concepts that include: 1) synergistic response of ecosystem productivity to nitrogen and phosphorus additions (Elser et al., 2007; Harpole et al., 2011), 2) a new paradigm that attributes this synergistic response to biochemical- and community-level responses (Arrigo 2005), and 3) variable allocation mechanisms by plants to minimize limitation by any single resource that can vary at the ecosystem- or at the species-level (Chapin et al., 2002; Naples and Fisk, 2010). Ecosystem research has seldom integrated these concepts effectively to interpret patterns of plant productivity. Research on nutrient-related physiological processes is mostly focused at the organ or cellular level; however nutrient controls on growth simultaneously operate at a hierarchy of biological organization. Therefore, nutrient related physiological responses should also be considered at whole plant and stand levels. I will integrate these concepts to understand nutrient limitation in northern hardwood forest ecosystems, with a focus on three levels of organization at which species differ in their response to resources.

Table Of Contents

Overview.....	2
Nutrient co-limitation.....	4-7
Study system.....	7-10
Study sites and Experimental design of the MELNHE project.....	10-12
Chapter 1: Species-specific fine root responses to nutrient additions.....	13-19
The role of fine roots in understanding nutrient limitation	13-14
Proposed Methodology.....	14-16
Current and predicted results.....	16-19
Timeline.....	19
Chapter 2: Species-specific seedling growth, survivorship and allocation responses to nutrient additions.....	20-26
The role of seedlings in understanding nutrient limitation.....	20-21
Proposed Methodology.....	21-23
Current and predicted results.....	23-26
Timeline.....	26
Chapter 3: Species-specific nutrient limitation of productivity in mature trees.....	27-33
Nutrient co-limitation and community level co-limitation.....	27
Proposed Methodology.....	28-29
Current and predicted results.....	29-32
Timeline.....	33
References.....	34-42

Background and Introduction

Nutrient co-limitation

Ecosystem theory has suggested that productivity is limited by a single nutrient, nitrogen (N) in temperate forests and phosphorus (P) in tropical forests (Vitousek 2004). The underlying concepts of single nutrient limitation were derived from Liebig's Law of the Minimum (Liebig 1842), which states that plant growth is limited by the nutrient in shortest supply relative to plant demand. This idea was originally applied to maximizing agricultural production of individual crop plants; however the concept was extended to complex ecosystems like forests that are comprised of multiple species with a diversity of traits (Harpole et al., 2011). Experimental fertilization studies both in agricultural and natural systems have shown single- nutrient limitation of primary production but the majority of those studies manipulated only a single nutrient at any particular point in time (Menge et al., 2009).

Recent recognition of synergistic interactions between limiting nutrients, in particular N and P, has highlighted the prevalence of nutrient co-limitation across terrestrial and aquatic ecosystems, and has raised many new questions about controls of ecosystem productivity (Elser et al., 2007). The possibility of nutrient co-limitation is consistent with resource optimization theories suggesting that plants can adjust their physiology and allocation patterns to minimize limitation by any single resource (Bloom et al., 1985; Chapin et al., 1987). Plants can allocate their energy and the nutrients that they have in abundance toward acquiring more limiting resources. Over time this could create a condition in which plant growth is equally limited by multiple resources (Chapin et al., 1987, 2002). However, if all plant species could effectively optimize resource acquisition, we would expect the whole ecosystem to be perfectly co-limited. In contrast, if none of the species could optimize resource acquisition, we would expect extreme single-nutrient limitation (consistent with Liebig's Law). And in a situation where some species can optimize while others cannot, aggregate productivity responses are likely to be co-limited.

Temperate forest ecosystems are under-represented in the co-limitation results summarized by Elser et al. (2007). What evidence there is from northern hardwood temperate forests does not clearly support limitation solely by N but instead suggests limitation by P or co-limitation by N and P. Fahey et al. (1998) observed a reduction in foliar N:P ratios in young forest stands after 5 years of nutrient fertilization in multiple stands throughout the White Mountain region of New Hampshire (NH). This experimental result is consistent with the increase in foliar N:P over a period of 34 years at the Hubbard Brook Experimental Forest (HBEF), which suggests an increase in P limitation that could be associated with chronic atmospheric N deposition (Fig. 1). Fine root responses to nutrient additions suggested P limitation in young forests and N limitation in mature forests at the Bartlett Experimental Forest (BEF) in central NH (Naples and Fisk 2010; Fig. 2), and a meta-analysis of fertilization experiments in hardwood forests of northeastern USA and southeastern Canada found evidence of P limitation and NP co-limitation, in addition to N limitation (Vadeboncoeur, 2010).

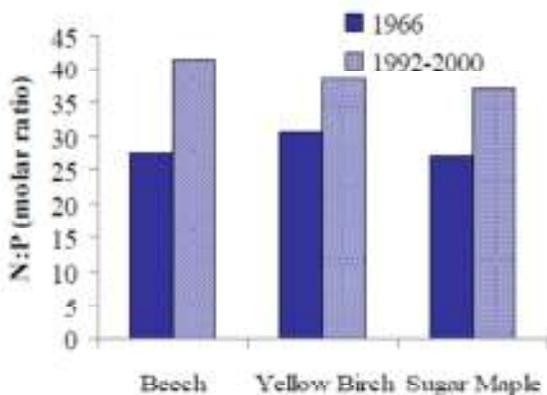


Fig 1. Foliar N:P for dominant tree species at HBEF (Data: Likens and Bormann, 1970; Fahey, unpublished data)

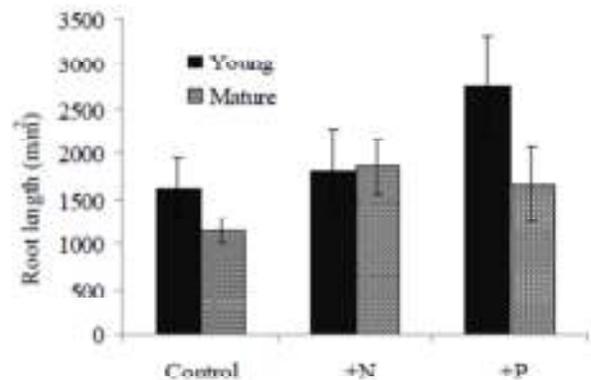


Fig 2. Root proliferation in ingrowth cores (10 cm depth, incubated for one growing season) receiving N and P additions in early successional and mature forest stands at BEF (from Naples and Fisk, 2011)

The concept of “co-limitation” lacks a strict definition (Harpole et al., 2011), but can be defined most simply as the condition in which ecosystem productivity increases more in response to two nutrients added together than to either nutrient added alone. Convincingly testing whether productivity in northern hardwood forests is co-limited by N and P requires a large-scale ecosystem study evaluating forest response to a full factorial NXP treatment. The available evidence suggests that we should find co-limitation (Elser et al., 2007; Vadeboncoeur

2010; Rastetter et al., 2013); if we do, then we can contribute to developing co-limitation theory by elucidating mechanisms that contribute to the additive or synergistic responses to N and P. If we do not find evidence of co-limitation, we can contribute to developing co-limitation theory by learning about the nutrient-use mechanisms that mediate single-nutrient limitation or correspond with a lack of apparent nutrient limitation in the northern hardwood forest ecosystem. Examining the mechanisms underlying co-limitation will also be necessary for predicting the implications for vegetation dynamics during times of anthropogenic change.

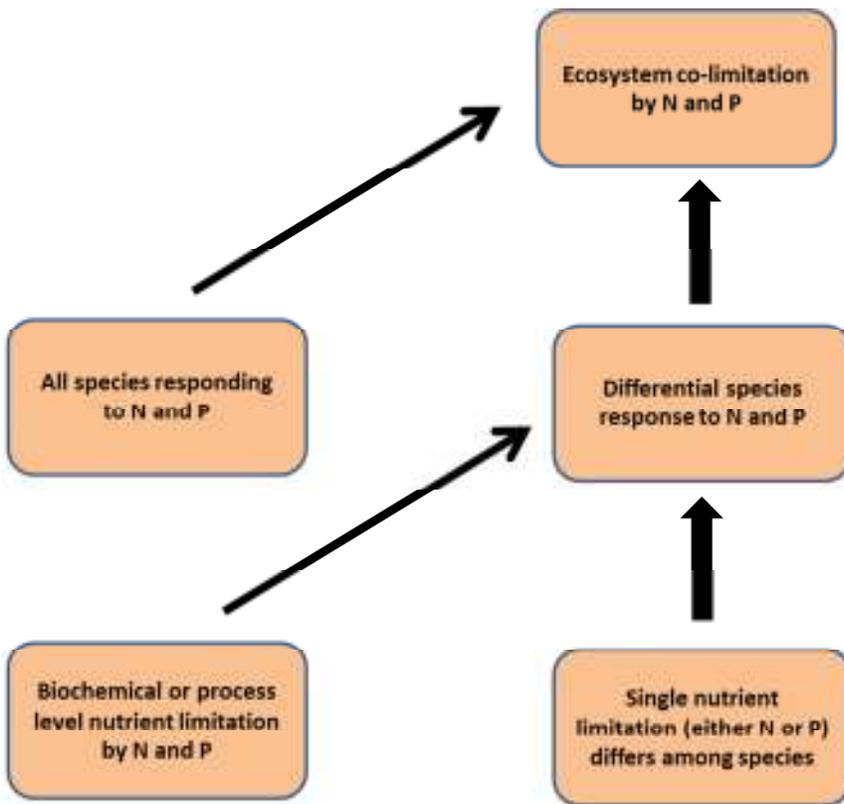


Fig 3. Breakdown of different mechanisms by which ecosystem nutrient co-limitation can arise.

Ecosystem co-limitation by N and P could arise from different underlying mechanisms (Harpole et al., 2011; illustrated in Fig. 3). Biochemical or process level co-limitation occurs when two mutually exclusive elemental nutrients co-limit cellular growth by substituting each other for a certain biochemical function or the same biochemical function is regulated by different enzymes utilizing the two different nutrients (Saito et al., 2008). Co-limitation can also

occur at a level of allocation if there is a tradeoff in allocating resources to acquire N vs. P; for example, limitation by N is likely to constrain the ability of plants to allocate more resources towards belowground structures (like deep roots and mycorrhizae) to acquire P. Furthermore, at the community level, differential species responses to N vs. P could contribute to co-limitation. The species that make up the communities tend to differ in their adaptations to environmental gradients (Chapin and Shaver, 1985), which could potentially include the most limiting nutrients (Harpole et al., 2011). Thus, traditional theories of single nutrient limitations of different species can also translate into co-limitation by differential species response to nutrients. However, nutrient co-limitation could also occur when multiple species in a community are either limited by the same nutrient with identical underlying biochemical co-limitation (Hutchinson 1961; Hubbell 2001; Harpole et al., 2011), or show increased growth responses to both nutrients added together. Moreover, plants can vary their allocation effort for multiple resource acquisition to efficiently balance their needs in species-specific resource limited environments (Chapin et al., 2002). Therefore, community level co-limitation can stem from a set of interactive mechanisms ranging from those that cause individual members to be limited by only a single nutrient, to others that lead to an assemblage of multi-nutrient co-limited species (Arrigo 2005). Distinguishing among these multiple pathways will help us understand community dynamics, nutrient availability and recycling, and subsequent controls on ecosystem productivity.

Study system

Edaphically similar sites in north eastern US, have been chosen by the “Multiple Element Limitation in Northern Hardwood Ecosystems” project (MELNHE), which aims to combine modeling and field studies to explore processes mediating nutrient co-limitation by N and P, in relation to successional change in typically managed hardwood forest systems. MELNHE is a long-term collaborative project with the general goal of examining nutrient co-limitation in a northern hardwood forest ecosystem. It brings together a team of ecosystem scientists studying different aspects including ecosystem modelling, forest structure, forest soils, root and litter dynamics, microbial activity, geochemistry and mineral weathering. Our research team at

Miami University focuses on soil nutrient dynamics, soil microbial work and plant-soil interface (above- and belowground) research.

The MELNHE project will measure ecosystem productivity following N, P, and N+P additions (fertilization initiated in summer 2011). In this project we will also examine variable allocation of ecosystem effort toward acquisition of P vs. N. In this dissertation I argue that it is necessary to judge plant responses to nutrient availability not only at an ecosystem scale, but also at the scale of individual species and my proposed research aims to take advantage of the long term fertilization project (MELNHE) to test community-level nutrient co-limitation by comparing productivity responses by individual tree species.

Understanding the dynamics of nutrient co-limitation is especially important as increasing anthropogenic inputs alter nutrient conditions globally. Forest ecosystems in the north-eastern US have been exposed to long term anthropogenic N deposition, which has altered soil properties and nutrient cycling processes (Fenn et al., 1998; Aber et al., 2003). This could cause N-saturation induced secondary limitation of productivity by P (Mohren et al., 1986; Aber et al., 1989; Stevens et al., 1993). Simultaneously, acid deposition has accelerated leaching loss of base cations (Likens et al., 1996, 1998, Lawrence et al., 1999, Bailey et al., 1996), promoting soil acidification and possibly reducing P availability (Fiorentino et al., 2003). Furthermore, Ca limitation of sugar maple regeneration and mycorrhizal colonization in these forests indicates the direct link between species composition and nutrient status of soil (Driscoll et al., 2001; Juice et al., 2006). Decreased allocation to fine roots and mycorrhizal colonization can further reduce P uptake by forest trees (Naples and Fisk, 2010). All of these processes operating gradually over the course of succession can deplete the nutrient capital of these forest systems and can shift species composition (Vitousek and Reiners, 1975; Bormann and Likens, 1979). Moreover, plant responses to nutrient availability are important in managed forests that experience differential nutrient removal associated with biomass harvest and hydrologic nutrient exports following disturbance and are thus likely to be sensitive to biogeochemical changes (Federer et al., 1989). Hence, it is important to study nutrient dynamics at different stages of succession in a managed forest landscape comprised of multiple

ages. For example, the “Multiple Element Limitation” (MEL) model predicts oscillations between N and P limitation as forests recover from harvest, until recycling of N and P become stoichiometrically balanced and ecosystem productivity becomes co-limited (Rastetter et al., 2013). Model results predict that young forests are limited by P and over time as the ecosystem develop the limitation shifts to N (Rastetter et al., 2013). Therefore, I plan to study mechanisms of community-level nutrient co-limitation over successional time in northern hardwood forests of central NH as part of the broader MELNHE project.

Species traits can influence ecosystem productivity by altering availability or use of limiting nutrients (Chapin and Shaver, 1985; Chapin et al., 1986, 1987), thereby influencing our interpretations of nutrient limitation of productivity. Tree species can modify their local environment because they vary in their life history and physiological traits, such as shade tolerance, nutrient acquisition from soil, nutrient-use and conservation, canopy interception and growth (Augusto et al., 2002; Reich et al., 2005; Lang and Polle, 2011). Northern hardwood forests are a multi-species system (dominated by beech/birch/maple) where species are segregated along axes of soil nutrient availability, sugar maple occurring more on high Ca, high N soils, while American beech occurs more on lower ends of Ca and N gradients (Finzi et al., 1998a, b; Schwarz et al., 2003; Bigelow and Canham, 2007). As the forest develops, different species inhabit specific successional niches (Pacala and Rees, 1998) and there is evidence of trade-offs between traits like species ability to persist in low resource environments (for example, shade tolerance) vs. species ability to exploit temporary pulses of resource rich conditions (for example, disturbance generated high light conditions utilized efficiently by fast growth; Grime 1977, 1979; Muscarella et al., 2013). The three dominant species in the mature forests (beech, yellow birch and sugar maple) differ in life history traits (Table 1). Less shade tolerant species tend to have higher N concentration in their fresh foliage and often have lower resorption and nutrient-use efficiency (Aber and Mellilo, 2001; Bormann and Likens, 1979; Marks 1974; Mellilo et al., 1982; Likens and Bormann, 1970; USDA 1974). Furthermore, experimental observations in Adirondack Park, NY, showed less shade tolerant species like yellow birch having larger increases in foliar N than more shade tolerant species like beech along the N deposition gradient, possibly attributed to a greater photosynthetic gain per unit N

invested by yellow birch (McNeil et al., 2007). In contrast, sugar maple responsiveness to N in northern hardwood forests varies depending on the availability of other nutrients like Ca, on light availability, and also on the stage of tree development (Duchesne et al., 2002; Finzi and Canham, 2000; St.Clair et al., 2008). Therefore, we can ask whether species that differ in their life history traits and nutrient use respond differentially to the addition of N, P and N+P influencing ecosystem-level productivity responses to nutrient additions.

Table 1. Comparing life history traits for the 3 dominant species in northern hardwoods.

Life history traits	Beech	Sugar Maple	Yellow Birch
Shade tolerance	Highest	Intermediate	Least
Seed size	Bigger	Smaller	Smallest
Vegetative reproduction	Very active	No	No

Study sites and experimental design of MELNHE

MELNHE has established 13 experimental northern hardwood forest stands (replicated stands of three ages) in central NH, in the Bartlett Experimental Forest (BEF; stands C1 through C9), the Hubbard Brook Experimental Forest (HBEF; stands HBM and HBO) and in the White Mountain National Forest near Jeffers Brook (JB; stands JBM and JBO). Forest composition is typical of northern hardwoods (Fig. 4), mature forests having an overstory dominated by sugar maple (*Acer Saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula Alleghniensis* Britton), with occasional presence of white ash (*Fraxinus Americana* L.), white birch (*Betula papyrifera* Marsh.) and red maple (*Acer rubrum* L.). Mid-age forests are generally dominated by white birch, yellow birch, American beech, followed by pin cherry (*Prunus pensylvanica* L.f.), red maple and aspen (*Populus grandidentata* Michx.). Pin cherry, white birch and red maple dominate the young forests. Soils are spodosols (typic and aquic Haplorthods) that developed on granite and gneiss derived glacial tills.

At BEF, we are studying 9 forest stands, three each of mature (>100 years; stands C7 through C9), mid-age (30-35 years; stands C4 through C6) and young (15-20 years; stands C1 through C3). At HBEF and JB, we are studying two mature (stands HBO and JBO) and two mid-age stands (HBM and JBM). Forest age is determined as time since clear cut. Stand elevations

range from 250-400 m. All stands have four 50X50 m plots, where control, N, P and N+P fertilizer treatments have been randomly assigned. Fertilizer is applied to the entire 50 X 50 m plot; measurements are made in the inner 30 X 30 m area (Fig. 5 shows an example forest stand). Six out of 13 stands have an additional Ca-treated plot (C1, C6, C8, HBCa, JBM and JBO).

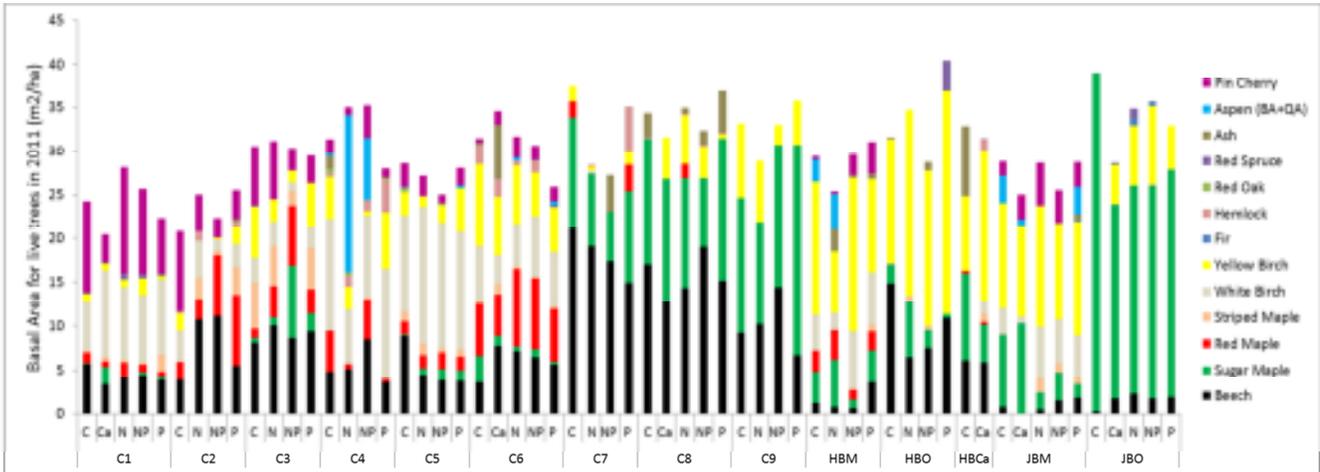


Fig 4. Basal area of live trees by species in 2011 for each plot (m²/ha) in replicated young, mid-age and mature stands.

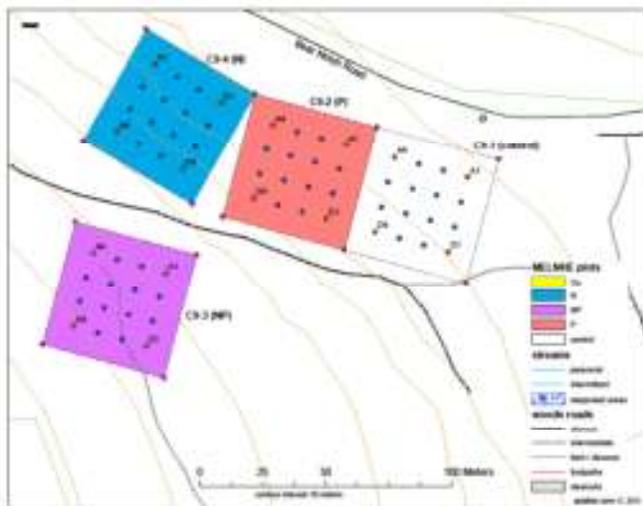


Fig 5. Stand C9 at BEF; picture courtesy: Matt Vadeboncoeur.

Nutrient additions began in May 2011. N is added at a rate of 30 kg/ha/year as NH₄NO₃, P is added at a rate of 10 kg/ha/year as NaH₂PO₄. Nitrogen and P amendments are applied twice during the growing season (late May and early July) and fertilizer is spread by hand evenly over 2.5X10 m sub-plots. Ca was added one time in the form of wollastonite mineral (CaSiO₃; 1150 kg/ha).

I propose to take advantage of the MELNHE study sites and nutrient manipulations to address my general questions of mechanisms of community-level nutrient co-limitation of northern hardwoods by N and P. I will examine the possibility of community-level co-limitation by linking research at three levels of biological organization: organ (roots), whole plant (seedlings) and stand (mature trees) (Fig. 6; individually discussed in the following three chapters).

Chapter 1: Species-specific fine root responses to nutrient additions (organ level)

Chapter 2: Species-specific seedling growth, survivorship and allocation responses to nutrient additions (whole plant level)

Chapter 3: Species-specific nutrient limitation of productivity in mature trees (stand level)

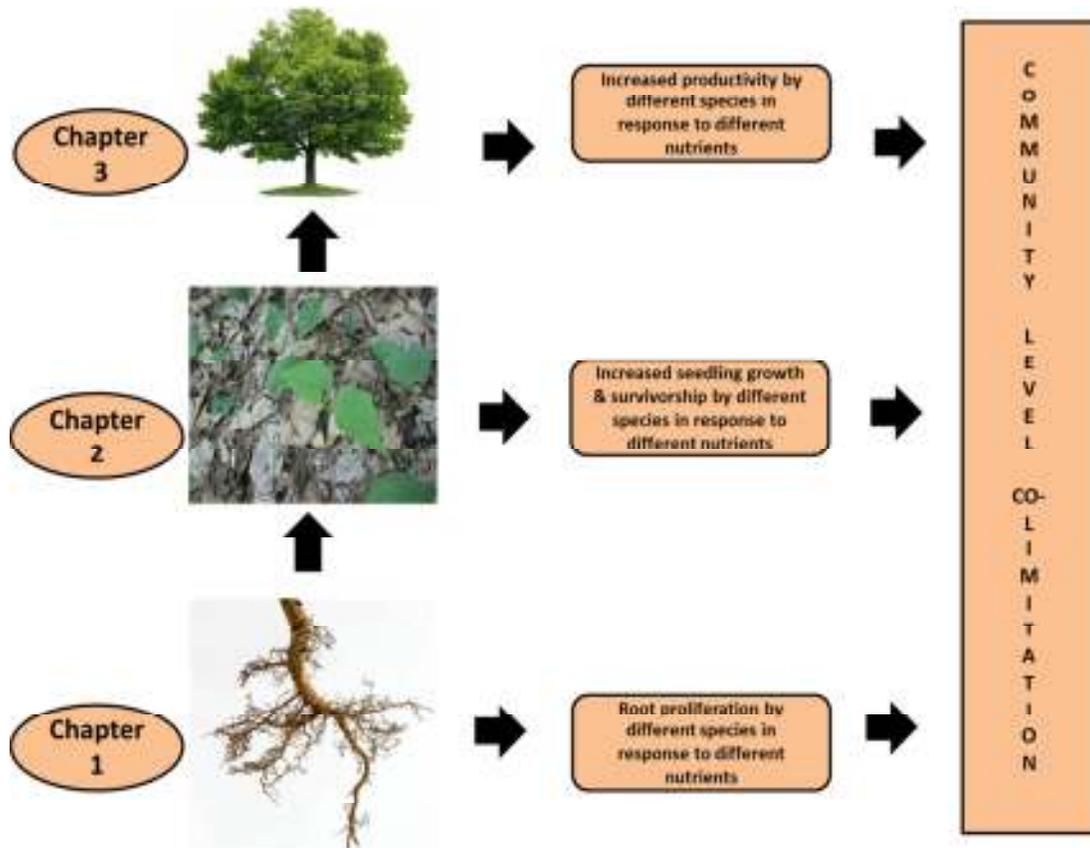


Fig 6. Simplified conceptual model followed in this research, demonstrating links between different species-specific nutrient responses at a hierarchy of biological organization, proposed for understanding mechanisms of community-level co-limitation of productivity by N and P.

Chapter 1

Species-specific fine root responses to nutrient additions

Introduction

The role of fine roots in understanding nutrient limitation

Fine root proliferation in response to nutrients can indicate nutrient limitation in forest ecosystems (Gleeson and Good, 2003). Fine roots are extremely important in nutrient acquisition for trees and their growth and physiology can be highly plastic (Pregitzer et al., 2002; Meinen et al., 2009). This plasticity can cause proliferation in response to patches of limiting nutrients, which we call “foraging” (Fitter, 1994). Foraging by roots has a significant C cost, but can be of benefit if it improves acquisition of the nutrient that most limits growth (Chapin et al., 1986; Naples and Fisk, 2010). Experimental evidence has directly linked such foraging responses to the nutrient that limits aboveground net primary productivity in forest systems (Raich et al., 1994; Gleeson and Good, 2003).

Available evidence suggests that fine root foraging is a species-specific trait. Fine roots proliferate in the presence of nutrients, and the extent of foraging can differ among species (Caldwell 1994; Robinson 1994). Furthermore, species can differ in their strategies of fine root foraging (Rajaniemi and Reynolds, 2004). In some instances, fine roots of different species have been found to detect the presence of neighboring roots in addition to sensing small-scale variation in resource availability (Mahall et al., 1991; Gersani et al., 2001; Falik et al., 2003; Hodge 2009; Chen et al., 2012). The difference in fine root foraging by species in response to nutrients, and the ability of fine roots to variably allocate resources, further justifies the need to study fine root responses at a species scale, which would contribute significantly in understanding ecosystem-level nutrient responses in a mixed species ecosystem.

However, it is not clear whether foraging is genetically constrained or it is a plastic trait in different species (Naples and Fisk, 2010). Root proliferation in response to a nutrient can indicate that productivity is limited by that nutrient (Gleeson and Good, 2003; Fig. 7). Nevertheless, preferential root foraging could also provide a possible mechanism to improve

acquisition of a particular nutrient that is in high demand, but not necessarily limiting to productivity, if the effort to acquire that certain nutrient provides it in sufficient supply. To understand this difference requires measuring productivity in response to that particular nutrient, which I will do in Chapter 3. Learning whether species-specific nutrient foraging patterns exist will improve our understanding of competitive interactions among northern hardwood species regardless of whether they correspond to nutrient limitation to productivity.

Whereas, if fine roots respond to the same nutrients that are found to limit productivity, then this will contribute to interpreting whether species-specific fine root foraging is genetically constrained or is a plastic trait.

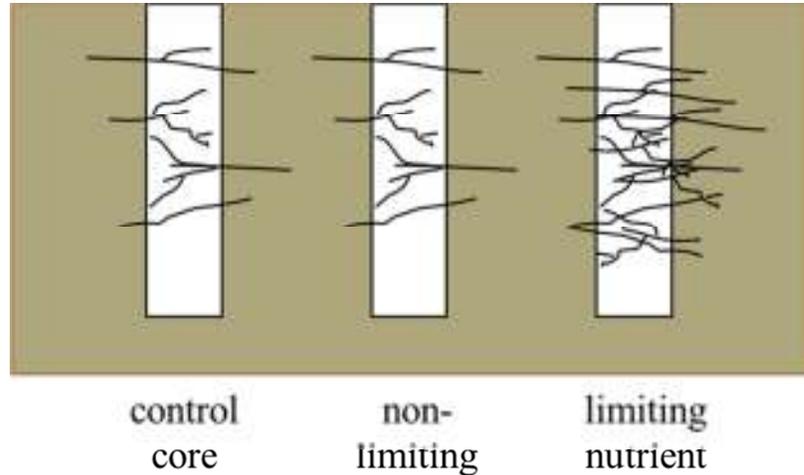


Fig 7. Illustration of more extensive root proliferation (referred to as foraging) in root ingrowth cores that received treatment with a limiting nutrient, compared to that in controls and cores with non-limiting nutrients.

Studies that examined the physiological response of sugar maple to Ca (Juice et al., 2006) and recent observations on foliar chemistry of beech and birch

(Goswami, unpublished), suggest species-specific nutrient requirements in northern hardwood ecosystems. **I will address the general question of whether fine root foraging indicates demand for different nutrients among species, potentially contributing to community-level co-limitation in northern hardwood forests by testing the hypothesis that roots of beech, birch and sugar maple preferentially forage for P, N and Ca respectively.**

Proposed methodology

Root foraging responses will be tested by comparing root proliferation in fertilized and unfertilized cores of root-free soil (ingrowth cores). I established 60 ingrowth cores (5 cm diameter, 10 cm depth) in each of three mid-age forest stands. I chose to work in mid-age (30-35 years) rather than mature or young stands because mid-age stands have higher diversity and

tend to have the most even representation by common species, dominated by yellow birch, white birch and beech, followed by pin cherry, red maple and aspen (indicated below in the “current and predicted results”; Fig. 9). To minimize bias in the ingrowth core assay associated with uneven relative abundance, I used inventory data to select plots with the most evenness in composition; these were in stands C5, C6 and HBM. Cores were arranged in groups of 5 each and placed where tree species composition was evenly mixed. One core of each group received one of the treatments (+N, +P, +water, +Ca and Control). Nutrients were added as solutions four times a year. +N cores received 40 g/m²/year of N as NH₄NO₃, +P cores received 20 g/m²/year of P as NaH₂PO₄ and +Ca cores received 10 g/m²/year of Ca as CaCl₂·2H₂O; these quantities were intended to increase the annual availability of N by approximately three times, of P by approximately greater than three times (to account for weathering of mineral P, and

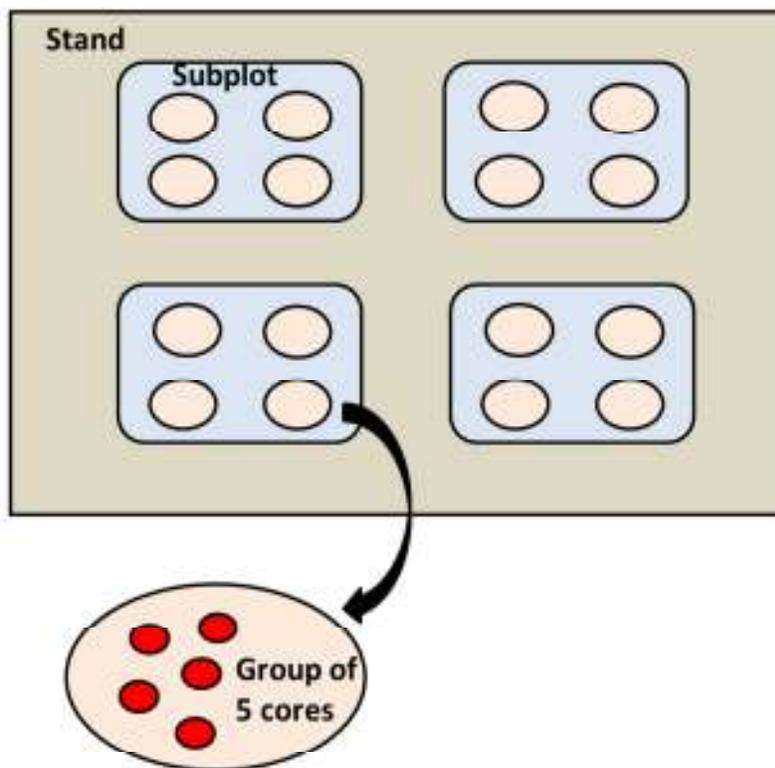


Fig 8. Experimental design to test species-specific response to nutrient additions: four 4 X 4 m subplots of cores within each forest stand (C5, C6 and HB) to account for within-stand variation, four groups of cores within each subplot to maximize exposure of each nutrient treatment to all species present in a mixed-species stand; and five cores (one of each treatment) per group.

fixation of available P) and Ca by approximately three times. I distributed cores in a nested design of groups within subplots in order to minimize effects of heterogeneity of root distributions (Fig. 8). Cores were incubated for two growing seasons to allow maximum root colonization with minimal turnover. C5 cores were deployed in May 2011 and were harvested in October 2012; C6 and HB cores were deployed in April 2012 and will be harvested in October 2013.

Cores were harvested by

re-coring (4.5 cm diameter, 7 cm depth; following Fahey and Hughes, 1994). Fine roots were

hand sorted, cleaned, and visually identified into distinct morphological categories depending on differences in root architecture and branching and presence of different root mycorrhizal tips (based on existing literature on root morphologies of northern hardwood forest tree species and Yanai et al., 2008). Fine root length was quantified using a line-intersect method (Tennet 1975) and roots were frozen for later molecular identification of fine roots. DNA will be extracted from representative root fragments of each morphological category using the MoBio Powerplant DNA isolation kit. The plastid *trnL* intron will be amplified by PCR using primers C and D (Taberlet et al., 1991), a primer set that has successfully distinguished a wide variety of tree species (Brunner et al., 2001). The amplification reaction will be performed following Brunner et al., (2001), and the species identity of individual root fragments will be confirmed by sequencing. Sequences will be identified to species by BLAST match (Altschul et al., 1997) to the GenBank nonredundant database. The results will be reported as the proportion of total sequences in samples contributed by each species. Ectomycorrhizal (EM) fungi associated with the different morphological categories of fine roots will be identified by extracting DNA from representative root tips using the MoBio technique as described earlier, using fungal rRNA gene primers ITS1F and ITS4 (Gardes and Bruns, 1993). Metrics like fine root length, biomass and mycorrhizal colonization will be measured as indicators of root foraging by species. Increased root proliferation by different species in response to different nutrients would support my hypothesis, suggesting species-specific nutrient limitation of productivity.

Current and predicted results

Tree species composition in mid-age forest stands C5, C6 and HBM, was uniformly dominated by white birch, yellow birch and beech, followed by pin cherry, red maple and aspen (Fig. 9).

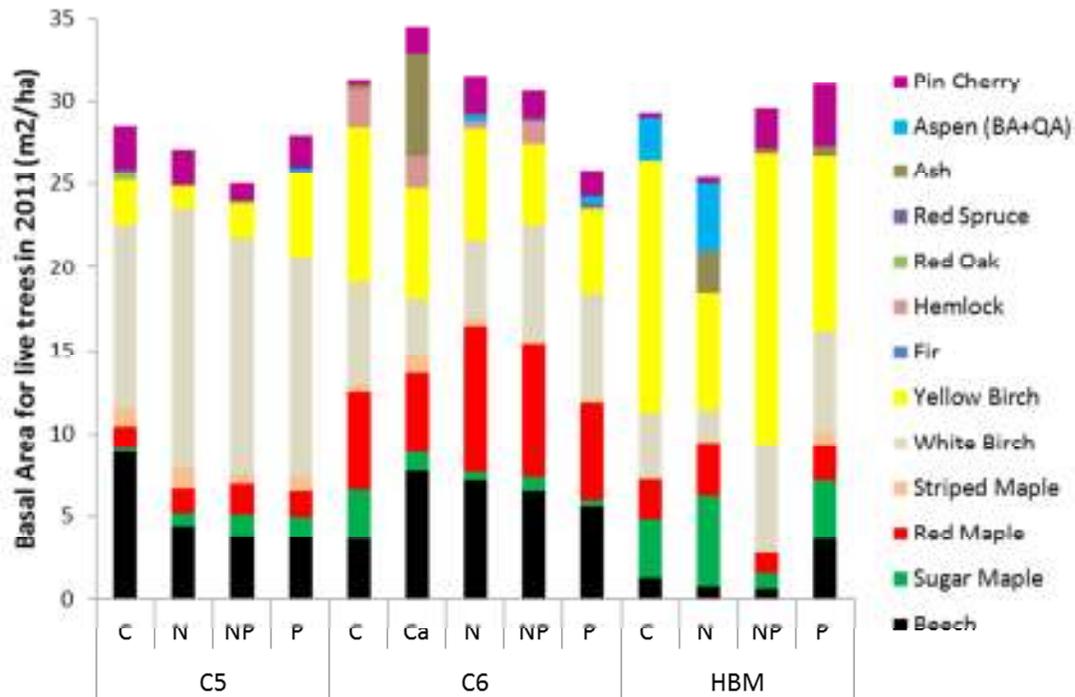


Fig 9. Basal area of live trees by species in 2011 for each plot (m²/ha) in replicated mid-age stands.

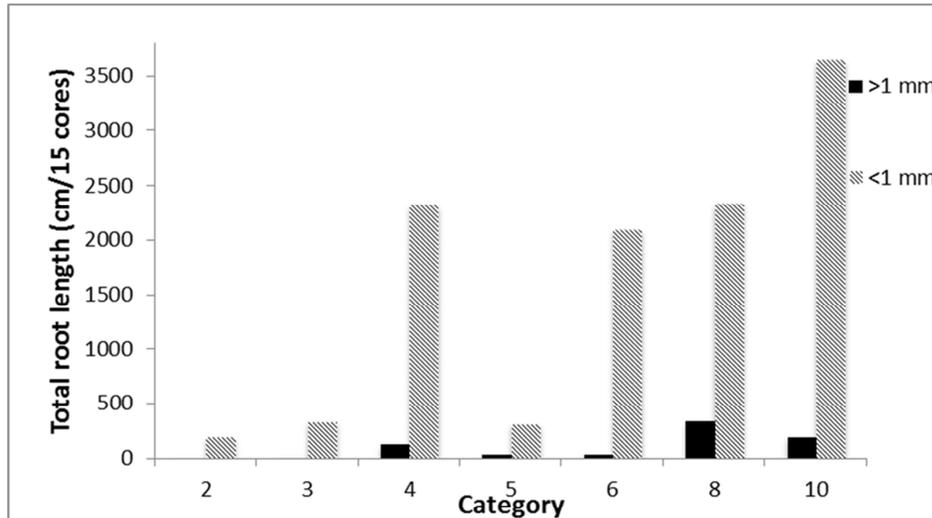


Fig 10. Fine root length by morphological category in ingrowth cores incubated in stand C5, May 2011-October 2012

Fine root length data have been analyzed for 15 of a total of 60 cores in stand C5. This subset of the data shows that fine root length colonizing ingrowth cores differed among morphological categories (Fig. 10). Furthermore, root foraging response to nutrients differed among

morphological categories, with category 4 responding more to Ca and P, and category 10 responding more to N (data from all cores are not processed yet; Fig. 11), indicating a possible difference between species in nutrient responses.

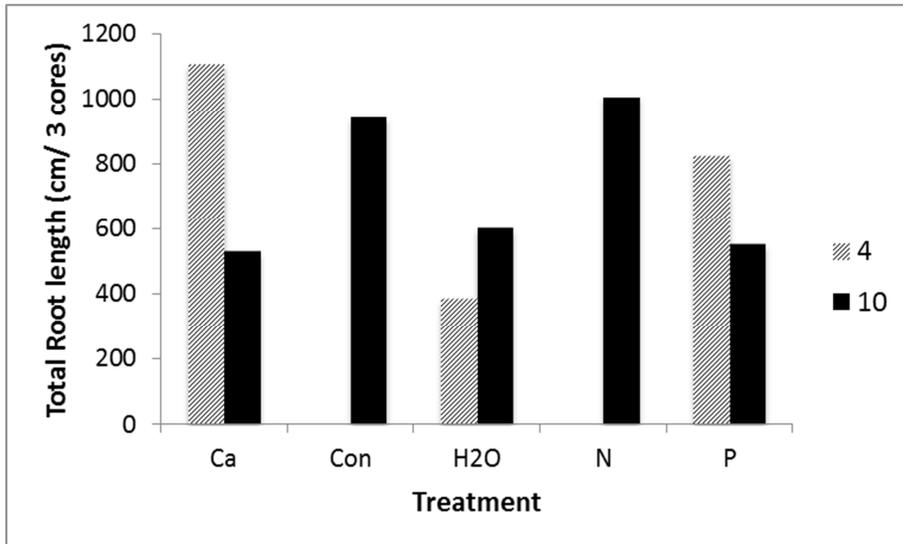


Fig 11. Total fine root length of morphological categories 4 and 10 from ingrowth cores incubated in stand C5, May 2011-October 2012

Root tips differed among root morphological categories (Fig. 12), suggesting that morphological categories are colonized by different types of EM fungi.

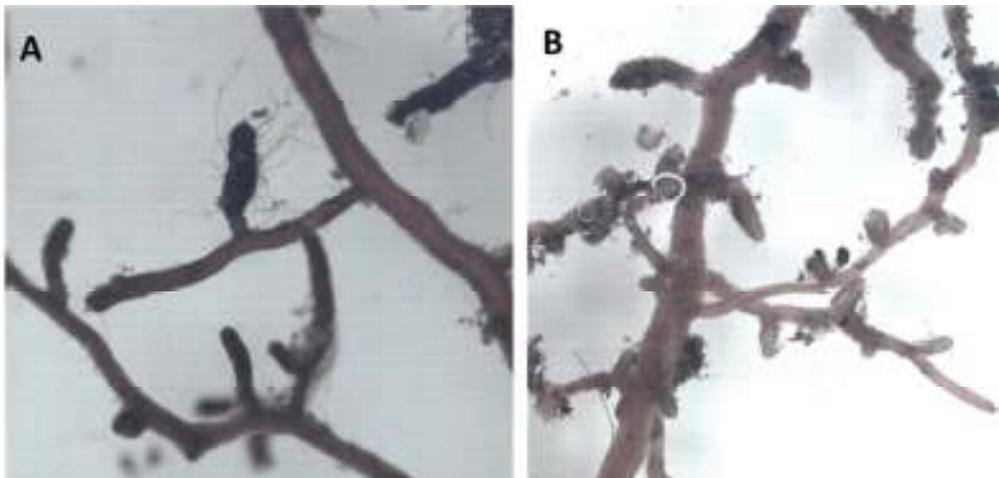
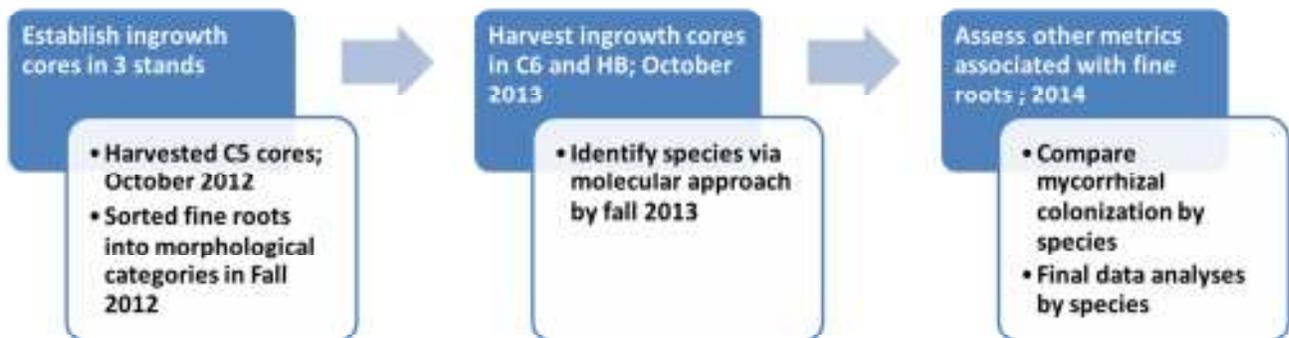


Fig 12. Images showing difference in mycorrhizal root tips between morphological categories 4 (A) and 10 (B).

These patterns indicate differential nutrient responses and need further confirmation by completing root length analyses and by identifying tree roots to species using molecular methods. Differentiating roots based on EM and arbuscular mycorrhizal (AM) colonization can further assist in estimating differences in species nutrient responses, since beech and yellow birch predominantly favors EM colonization, while sugar maple and pin cherry are likely to favor AM colonization.

TIMELINE



Chapter 2

Species-specific seedling growth, survivorship and allocation responses to nutrient additions

Introduction

The role of seedlings in understanding nutrient limitation

Understanding nutrient controls on seedling growth and survivorship is essential to interpret species-specific nutrient limitation. Although not necessarily representative of mature trees, seedlings are useful from several perspectives. First, they are likely to respond to nutrient fertilization more rapidly than mature trees. Second, it is possible to study whole-plant allocation patterns in seedlings. Finally, survivorship of seedlings in the forest understory can indicate future regeneration potential, especially for those shade-tolerant species that require advance regeneration to utilize gaps that arise over time (Aber and Mellilo, 2001; Marks 1974; Mellilo et al., 1982). Allocation to roots vs. shoots is a key means by which plants respond to the relative demand for above- vs belowground resources, in order to maximize acquisition of the most limiting resource (Chapin et al., 1986, 1987; Grime, 1994); and allocation patterns can vary by species (Ehrenfeld 2001). Ecophysiological studies have suggested that variable allocation can contribute to interpreting nutrient limitation in northern hardwood forest ecosystems (Fahey et al., 1998). Less shade tolerant species like pin cherry can only persist if its leaves are in the top of the canopy; high nutrient conditions are required for this, presumably because allocation can be shifted aboveground. In contrast, shade tolerant species like beech and sugar maple are flexible in preferentially allocating to above- and belowground growth. Alleviating belowground resource limitations tend to reduce plant root: shoot ratio (Harris, 1992). Therefore, we can expect root: shoot ratio to respond to limiting nutrients and also to water.

Fertilization in the MELNHE stands began in summer 2011, which coincided with the masting of beech, birch and sugar maple in some of the mid age and mature stands; this provided an opportunity to study species-specific traits by which seedlings of different species utilize nutrients and to test effects on growth and survivorship. **Therefore, I propose to**

examine species-specific nutrient requirements by testing the following hypotheses, a) A species will reduce its root: shoot ratio in response to its limiting nutrient, b) Lower root: shoot ratios correspond to higher aboveground growth, and c) survivorship of a species increases in response to its limiting nutrient.

Proposed methodology

Studying survivorship in response to nutrients

I will study nutrient effects on seedling survivorship in 10 MELNHE forest stands (it was impractical to conduct seedling inventory in all the stands, so we omitted stands C3, C5 and C7): C1 and C2 (young); C4, C6, HBM and JBM (mid aged); C8, C9, HBO, and JBO (mature). Each stand has four 50 X 50 m plots that have received fertilizer treatments beginning summer 2011. In each plot, 10 1 X 1 m seedling subplots were established in summer 2012 (Fig. 13). All seedlings (<50 cm in height) in these subplots were identified to species and surveyed in May and August 2012. Seedlings will be re-surveyed in May 2013 and again in August 2013 to test effects of nutrients on survivorship. I plan to mark surviving seedlings in May 2013 to follow up on the survivorship patterns over successive years. Additionally, I quantified soil moisture in

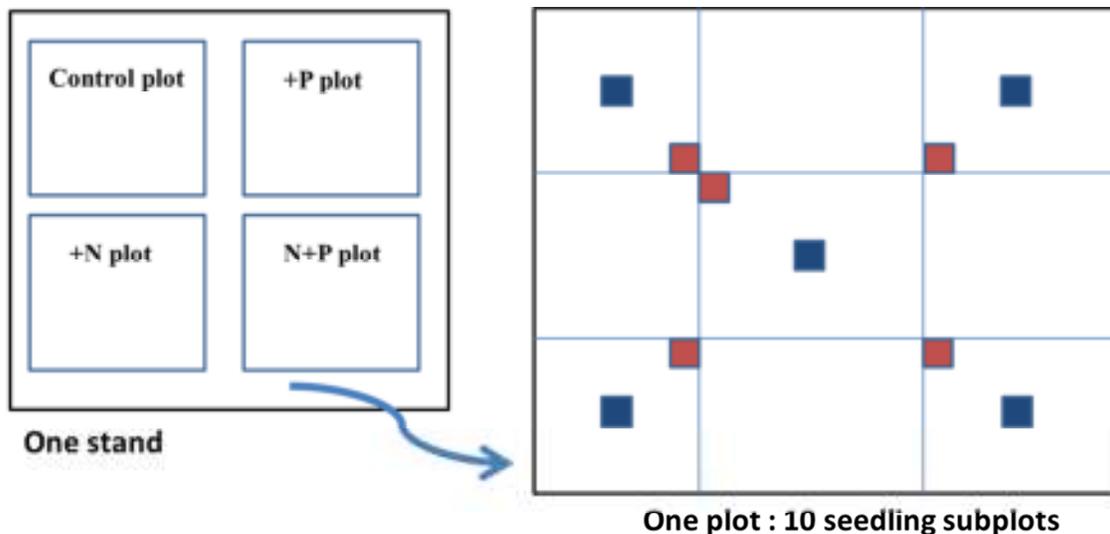


Fig 13. Experimental design for studying effects of nutrient treatments on seedling growth and survivorship.

each of the seedling subplots to understand whether small scale soil moisture variability could affect survivorship of different species.

Studying variable allocation patterns in response to nutrients

In July 2012 I collected seedlings of two dominant northern hardwood species, American beech and sugar maple, from the vicinity of each seedling subplot of three mature MELNHE forest stands (C8, C9 and HBO), to study mechanisms of variable allocation effort in different species in response to nutrient availability. Two seedlings of each species were randomly selected and destructively harvested from the nearby 50 cm (at all directions) of each seedling subplot. Pairing the harvested seedlings with the seedling subplots allows me to relate seedling survivorship of beech and sugar maple (quantified in the subplot) with the allocation efforts measured, and also to investigate whether soil moisture affects seedling allocation patterns. Seedlings were carefully extracted with root systems intact, following sampling methods presented in Juice et al., (2006). Seedlings were transported to the laboratory in plastic bags and processed the same day as collected. Approximately 20-24 seedlings per species were collected from each of the nutrient treated plots. Seedlings will be harvested again to test nutrient limitation of seedling growth.

Images of all seedlings of both species were taken using a flatbed scanner in the laboratory. These images will be used for quantifying root and shoot lengths and leaf area of each seedling. All seedlings were divided in to root and shoot tissues. Root and shoot biomass were quantified for half of the seedlings per species, by drying to constant mass at 60° C and weighing. The remaining root tissues were gently washed with tap water and preserved in 60% ethanol for mycorrhizal analysis. The remaining shoot tissues were weighed fresh, oven dried to constant mass at 60° C, and reweighed. Root: shoot ratios were calculated on a mass basis. Seedling leaf area will be quantified from scanned images using ImageJ software. Seedling root length will be estimated from scanned images using a line intercept method (Tennet 1975). Specific Root Length (SRL) and Specific Leaf Area (SLA) will be calculated by dividing these parameters by mass. Dried and preserved tissues will be analyzed for foliar and root chemistry to calculate root: shoot and whole plant N, P and Ca. I used pre-treatment soil N and P

availability to explore nutrient effects on seedling growth. Pre-treatment soil samples separated by horizons (Oe, Oa; organic horizons and B; mineral horizon) were collected in June 2009 and net N mineralization was measured by 21 day lab incubations and resin available P was measured by incubating resin bags in the Oa horizon for one growing season. I also used pre-treatment soil moisture data from 2009 (quantified from the soil samples described above) and soil moisture quantified in seedling subplots in summer 2012 to examine potential effects of soil moisture on seedling growth.

Current and predicted results

Seedling survivorship, growth and nutrient limitation

A nutrient will be identified as limiting if it promotes seedling growth (mass) relative to controls. Survivorship, the next relevant index, is of significant importance for conservative species like beech and sugar maple that need to survive and regenerate, filling in the gaps over the course of forest development. Growth and survivorship of seedlings in our experimental plots will address the question of whether species-specific nutrient requirements are a potential mechanism for community level nutrient co-limitation.

Addition of N and N+P decreased survivorship of all three species, whereas P alone had no effect (Fig. 14). I did not find any evidence of nutrient effects on seedling survivorship. Survivorship will be quantified again after two growing seasons (in August 2013) to test nutrient effects.

There was no evidence of nutrient limitation of seedling growth. However, whole seedling biomass of both species (beech and sugar maple) after one growing season showed a negative trend with pre-treatment P availability consistently across Oe, Oa and B soil horizons and sugar maple seedling biomass was significantly related with Oa horizon pre-treatment soil P availability ($R^2= 0.47$, $p= 0.01$; Fig. 15). I will test limitation again by measuring seedling biomass in 2013.

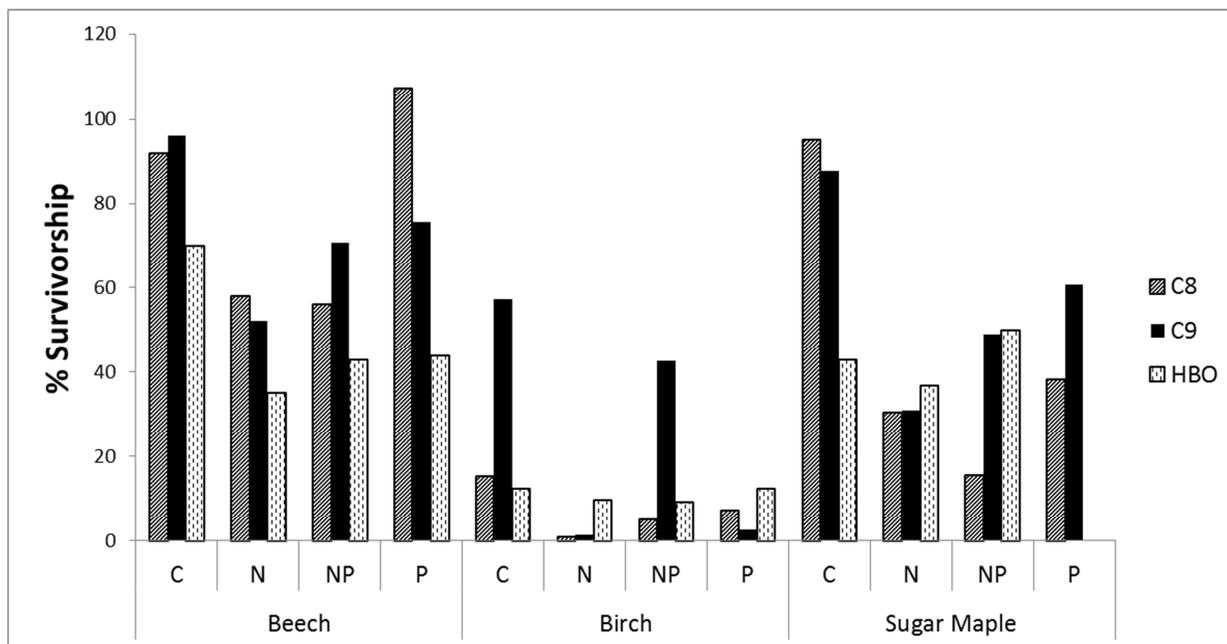


Fig 14. Survivorship of beech, yellow birch and sugar maple seedlings in three mature stands C8, C9 and HBO (P = P treated plot, N = N treated plot, NP = N+P treated plot, Ca = Ca treated plot and C = Control plot), between June and August 2012.

Exploring allocation patterns in response to different nutrients

Root: shoot ratios of both beech and sugar maple were not affected by fertilizer treatments. I will assess treatment effects again in 2013.

Soil moisture appeared to have a positive influence on seedling root: shoot ratios. Average root: shoot ratios for beech and sugar maple showed an increasing trend with pre-treatment soil moisture consistently across Oe, Oa and B soil horizons (Fig. 16; Sugar maple root: shoot significantly related to Oa and B horizons, $R^2 = 0.42$ and 0.41 , $p = 0.01$ and 0.02 , respectively;). This pattern was also found for post-treatment soil moisture measured in the top 10 cm by a LICOR in August, 2012. The relation between sugar maple root: shoot and soil moisture was stronger ($R^2 = 0.44$, $p = 0.01$) than that of beech and soil moisture (Fig. 17). These results indicate an influence of soil moisture on allocation patterns in beech and sugar maple.

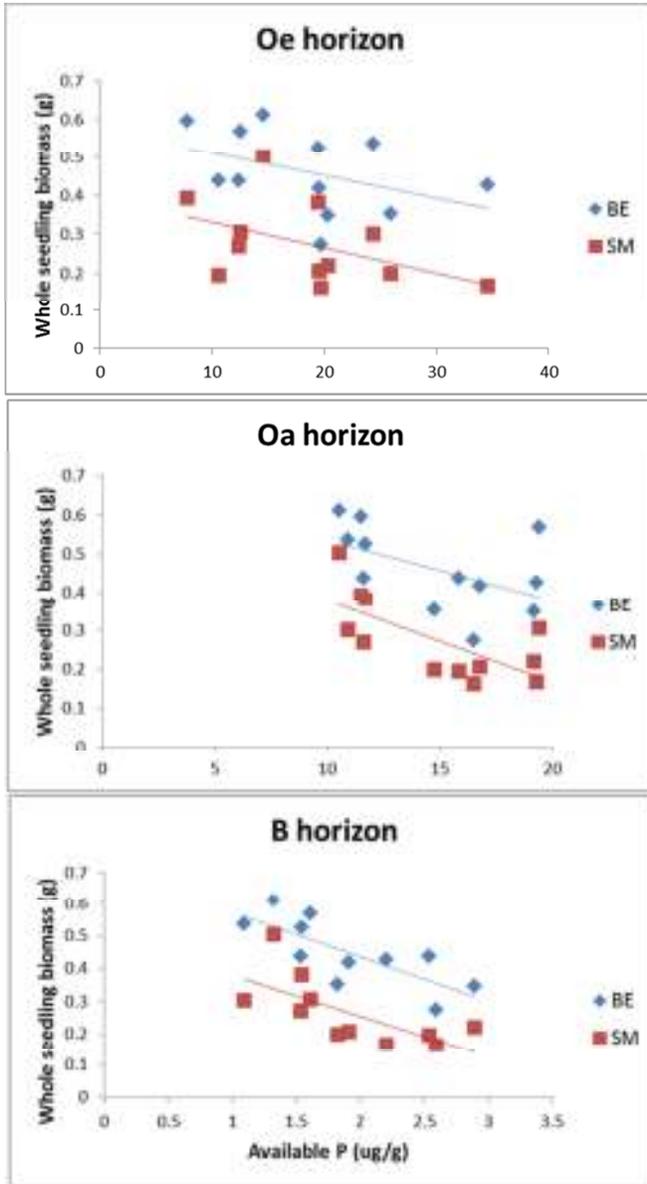


Fig 15. Relationship between whole seedling biomass (plot means) for both species in August 2012 and pre-treatment soil P availability in the Oe, Oa and B horizons in three mature stands C8, C9 and HBO.

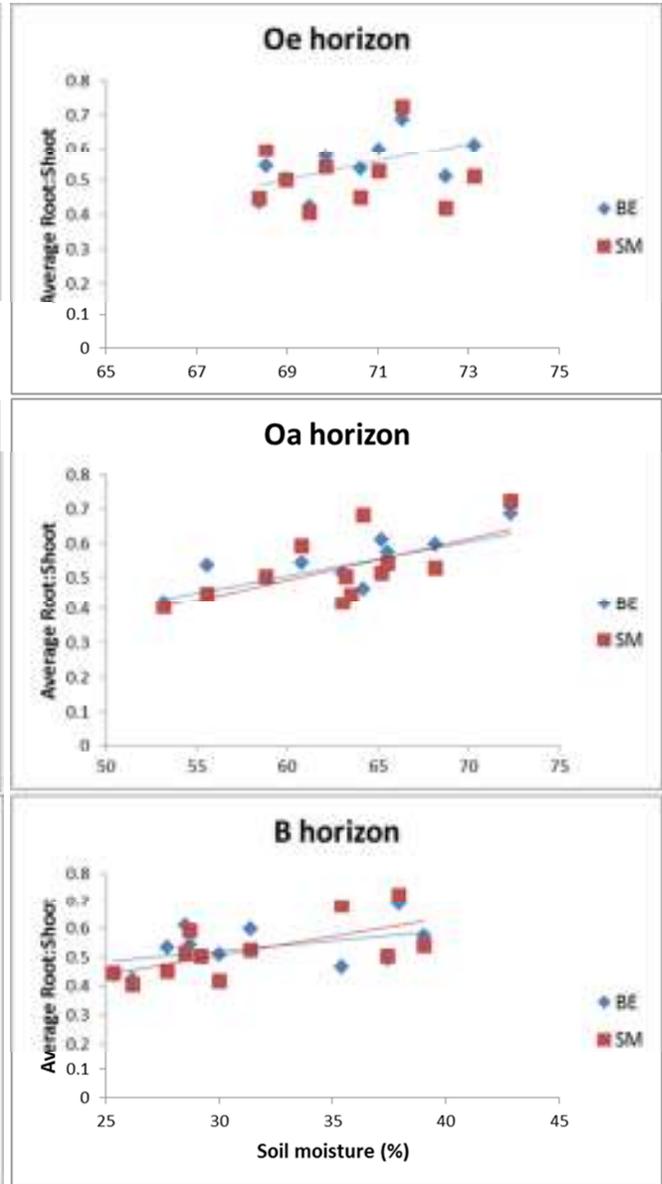


Fig 16. Relationship between root:shoot ratios (plot means) for beech and sugar maple seedlings and pre-treatment soil moisture (%) in the Oe, Oa and B horizons in three mature stands C8, C9 and HBO.

There was no evidence of nutrient limitation on seedling growth and survivorship. I will measure growth and survivorship of seedlings again in 2013 to test nutrient limitation. I will explore different patterns of variable allocation efforts by species (metrics like root and shoot allocation, SRL, percent mycorrhizal colonization, allocation to photosynthetic tissue in relation

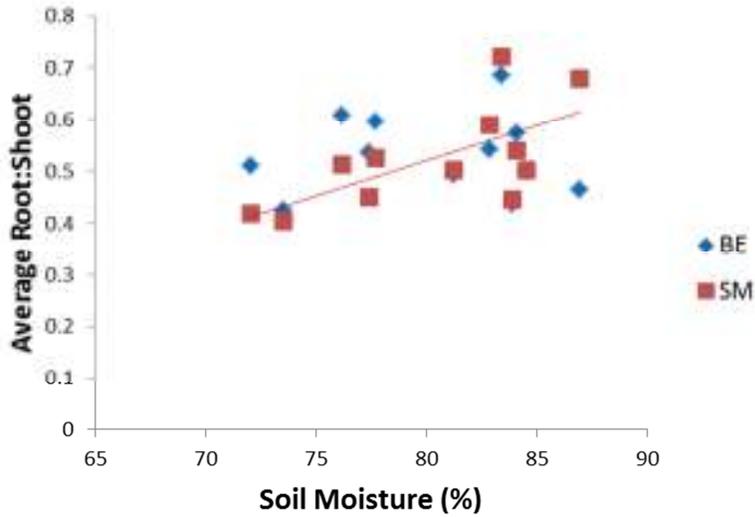
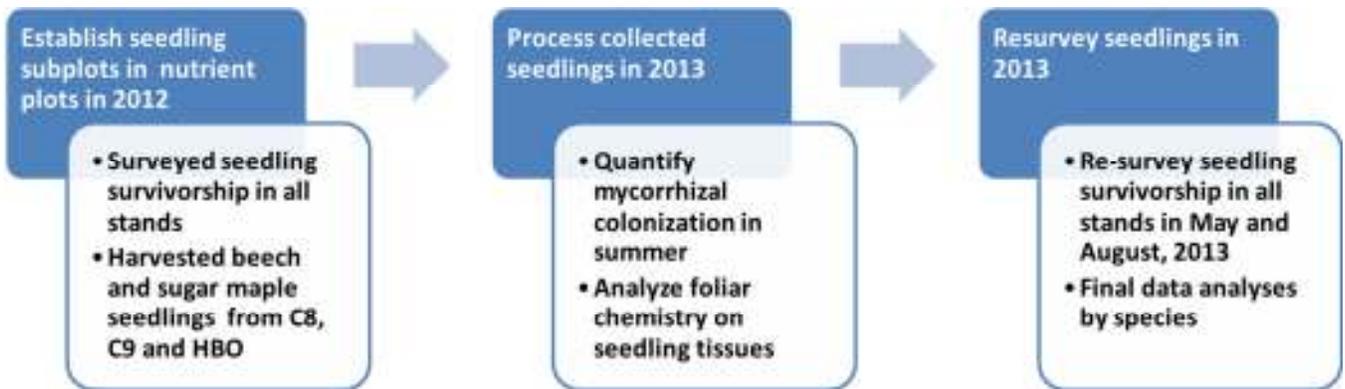


Fig 17. Relationship between root:shoot ratios (plot means) for beech and sugar maple seedlings and post-treatment soil moisture in three mature stands C8, C9 and HBO.

to mycorrhizal colonization etc.) to understand whether allocation is related to growth and survivorship.

TIMELINE



Chapter 3

Species-specific nutrient limitation of productivity in mature trees

Introduction

Nutrient co-limitation and community level co-limitation

It is required to test whole-ecosystem level co-limitation before community level co-limitation can be interpreted. I will test ecosystem level co-limitation by measuring whole plot productivity response (of all tree species) to the nutrient treatment. Referring back to the conceptual model (Fig. 3), nutrient co-limitation occurs when whole-plot productivity response to N+P addition exceeds the response to N and to P added alone. If co-limitation is supported at the ecosystem level, then I can infer a community level co-limitation if productivity by individual species is limited by different nutrients. Alternatively, individual species might show N and P co-limitation. Nevertheless, even in absence of a support for ecosystem level co-limitation, studying species-specific nutrient requirements is important for understanding community dynamics across environmental gradients. Moreover, species-specific traits could aid interpretation of successional changes in nutrient limitation or nutrient cycling. Conversely, a lack of species differentiation in nutrient use traits would suggest that the northern hardwood community should be fairly stable across fertility gradients or in response to nutrient enrichment.

The objective of this proposed investigation is to test nutrient co-limitation at whole-ecosystem and at community levels. I hypothesize that northern hardwood forest productivity is co-limited by the availability of N and P, and that productivity of beech will respond to P, yellow birch to N and sugar maple to Ca. I will also test productivity responses by other early successional tree species to different nutrients.

Proposed methodology

I will study all the 13 MELNHE forest stands in NH for this investigation; all stands having four 50 X 50 m plots are receiving fertilizer treatments (N, P, N+P and control) beginning in May 2011.

Pre-treatment data

Prior to treatment, forest composition was inventoried in all stands in 2004 and 2011. These inventories provide baseline values for future calculations of aboveground productivity. DBH and species were recorded for all overstory trees ≥ 10.0 cm DBH and DBH and species were recorded for trees 2.0 to 9.9 cm DBH in five 5 X 5 m subplots in each plot.

Growth efficiency is measured as productivity per unit leaf mass. I used the change in basal area (BA) per year per plot (as an index of productivity) and litterfall mass (or foliar mass) to estimate growth efficiency for each of the three dominant tree species: American beech, sugar maple and yellow birch. Litterfall was collected four times a year in five litter baskets per plot. Leaf litter was sorted by species, dried and weighed. To interpret trends in productivity, I compared growth efficiency to foliar chemistry. Litter for foliar chemistry was collected using a shotgun or pole pruners. Leaf tissue samples were analyzed for P using ICP-OES (Perkin-Elmer Optima 5000) at SUNY ESF. N was analyzed by dry combustion (modified Dumas Combustion analyzer) at Cornell University. To further explore productivity trends, I compared pre-treatment soil N and P availability (described under Chapter 2 methods) with growth efficiency of tree species. I explored the pre-treatment patterns by comparing productivity, foliar and soil N and P data available from three forest stands (stand choice was restricted to three because of unavailability of foliar data elsewhere): one mid aged (C6; ~35 years old), and two mature (C8 and C9; >100 years old). Species compositions of these stands are indicated below in “current and predicted results” (Fig. 18).

Post-treatment data

To test productivity response to nutrient treatments, all stands will be re-inventoried in 2014. The change in BA per year per plot will be used as one index to estimate productivity and

species-specific allometric equations developed and validated in our forest stands (Whittaker et al., 1974; Siccama et al., 1994; Hamburg et al., 1997; Fahey et al., 1998; Arthur et al., 2001; Fatemi 2007) will be used to estimate change in total biomass in all plots. Litterfall collection and foliar chemistry will be continued in these stands in a similar manner as described earlier and growth efficiency calculations will be repeated.

In 2012, post-treatment soil N and P availability was detected by using anion exchange resin strips (Ionics AR-204-SZRA; Maltz Sales) to quantify available PO_4^- and NO_3^- , and cation exchange resin strips (Ionics CR67-HMR; Maltz Sales) to quantify available NH_4^+ in soils. Resin strips (2 x 6 cm) were prepared by rinsing in weak HCl and DI H_2O and then soaking in 1M NaCl (cation strips and anion strips for NO_3^-) or alternating rinses in DI H_2O and 0.5M NaHCO_3 (anion strips for PO_4^-). All strips were rinsed with DI H_2O immediately prior to placement in the field. We deployed resin strips in the Oa horizon by inserting them under the blade of a knife used to cut the surface organic horizon at a 30 - 45° angle. Eight strips per plot were deployed for each nutrient. Strips were retrieved after 14 days and rinsed in deionized H_2O prior to extraction for nutrient analyses. Anion exchange resins were extracted by shaking rinsed strips in 30mL of 1M KCl for NO_3^- and 30mL of 0.5M HCl for PO_4^- for 1 hour each. Cation strips were extracted by shaking rinsed strips in 30 mL of 1M KCl for 1 hour. We used a phenolate-hypochlorite method to quantify NH_4^+ (method 351.2, US EPA 1983) and a cadmium reduction method to quantify NO_3^- (method 353.2, US EPA 1983) in extracts. Extract P concentration was analyzed by the ammonium-molybdate-ascorbic acid method (Murphy and Riley, 1962). In addition to resin strips, we collected soil samples (separated by horizons) in July 2012, and measured net N mineralization by 21 day lab incubations. I will repeat these measurements in 2014 to document changes in soil nutrient availability with treatments.

Current and predicted results

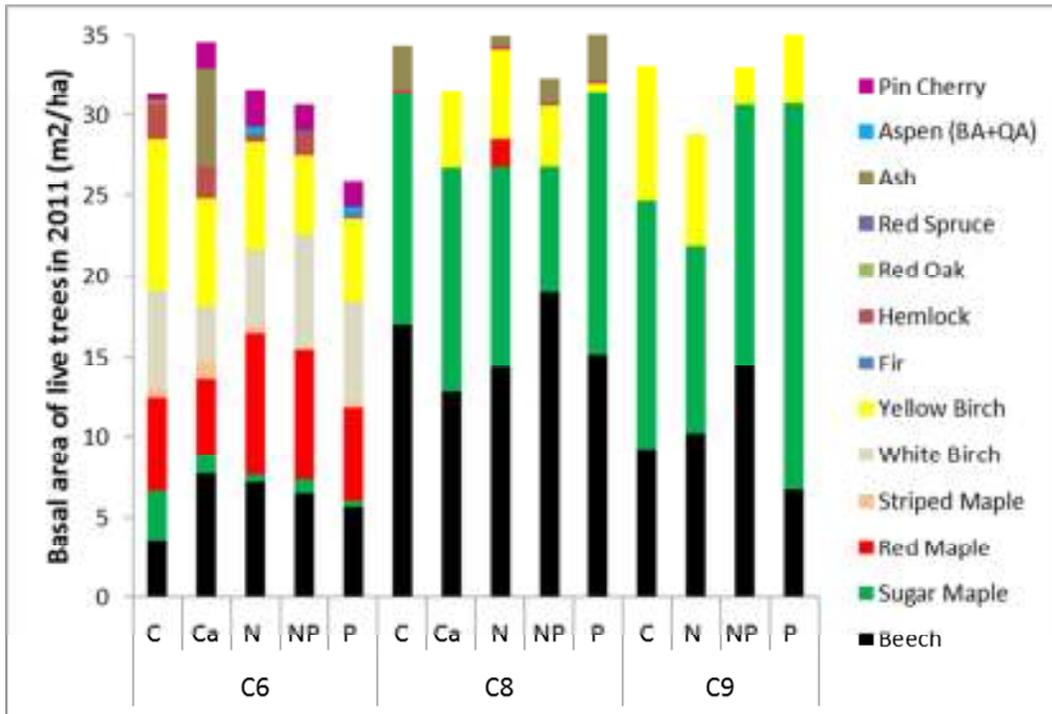


Fig 18. Basal area of live trees by species in 2011 for each plot (m²/ha) in mid-age (C6) and mature (C8 and C9) stands; used for growth efficiency estimates.

I will test nutrient limitation by comparing productivity response of our treatment plots relative to the controls. The productivity data will be summed up at the ecosystem level (by adding productivity response of all tree species to the added nutrient), and then successively broken down into constituent species and analyzed at the species level.

Pre-treatment productivity trends by species

I explored pre-treatment productivity patterns in relation to nutrient availability by using growth efficiency as a measure of productivity. The growth efficiency of beech appears to be limited by foliar P and that of yellow birch appears to be limited by foliar N. Beech growth efficiency was negatively related ($p < 0.05$) to foliar N:P and positively related to foliar P concentration within the mature stands (C8 and C9; Fig. 19). A similar pattern was also observed when the mid-age stand was included (Fig. 20).

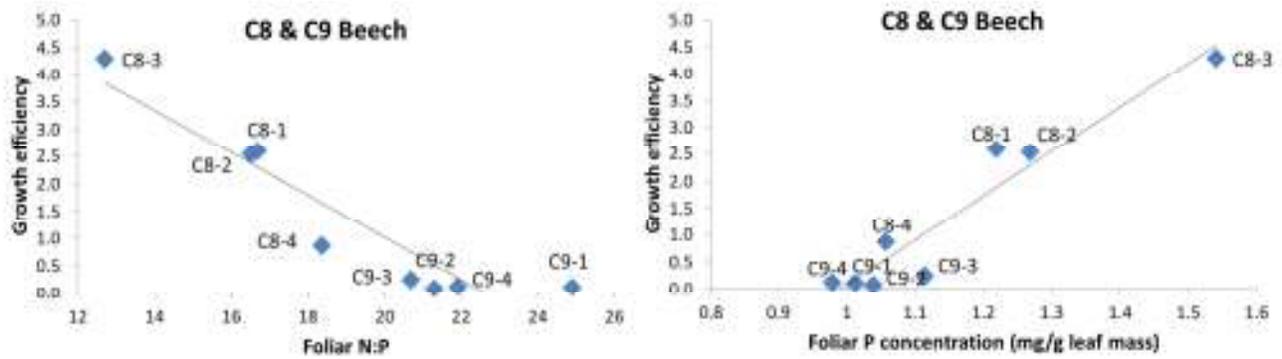


Fig 19. Growth efficiency (cm² basal area increment/kg leaf litter) of beech in mature stands with foliar N:P ($R^2= 0.86$, $p= 0.00$) and foliar P concentration ($R^2= 0.92$, $p= 0.00$).

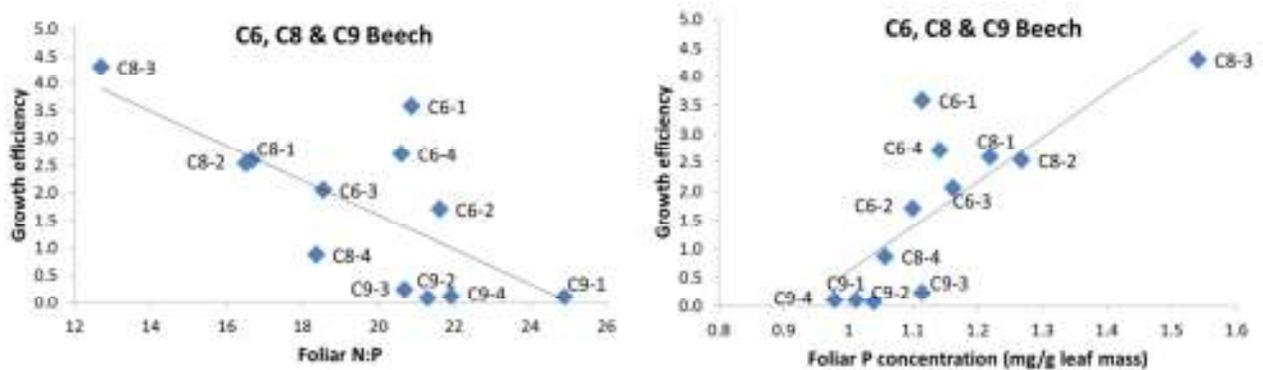


Fig 20. Growth efficiency (cm² basal area increment/kg leaf litter) of beech in mid and mature stands with foliar N:P ($R^2= 0.48$, $p= 0.01$) and foliar P concentration ($R^2= 0.63$, $p= 0.00$).

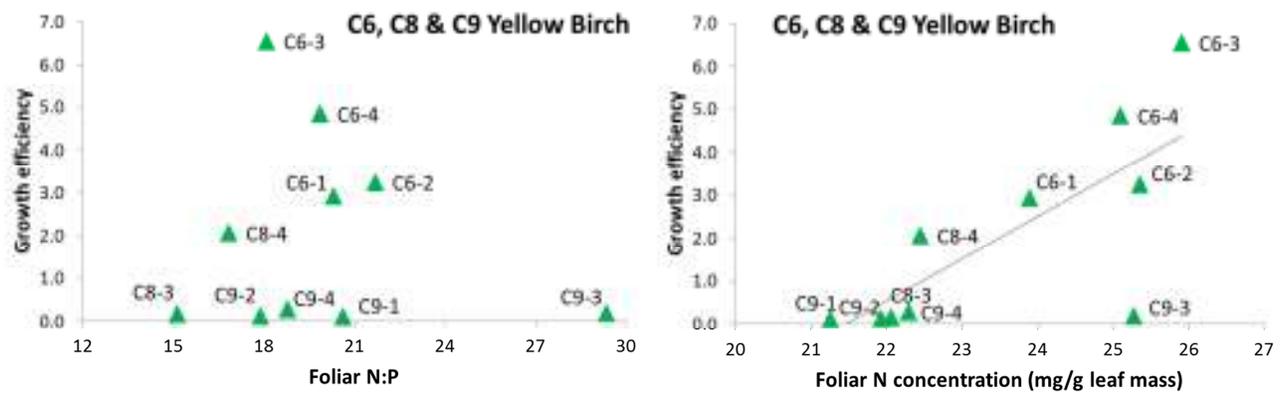


Fig 21. Growth efficiency (cm² basal area increment/kg leaf litter) of yellow birch in mid and mature stands with foliar N:P ($p= 0.69$) and foliar N concentration ($R^2= 0.55$, $p= 0.00$).

Yellow birch growth efficiency showed little pattern in relation to foliar N:P (Fig. 21), but increased in relation to foliar N concentration in mid and mature stands.

Beech growth efficiency was not related to soil P availability. Sugar maple growth efficiency was not related to either soil N or P. Yellow birch showed marginally significant relationship with soil N availability (Fig. 22).

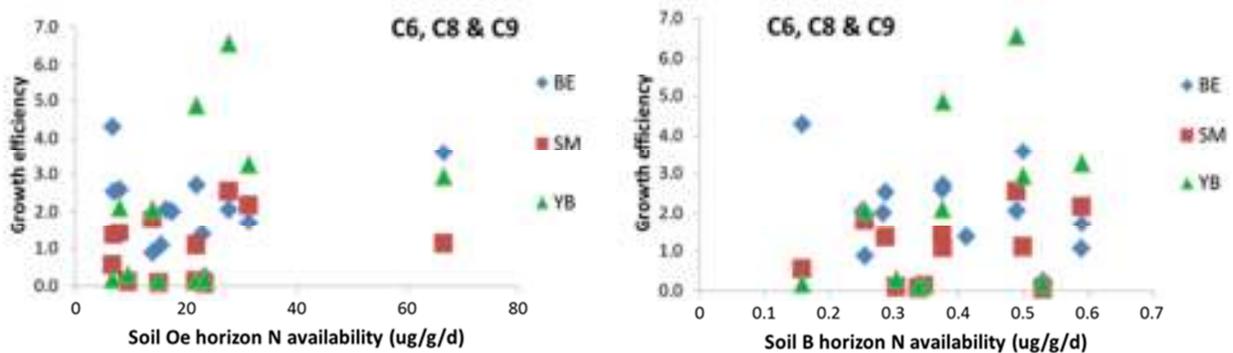
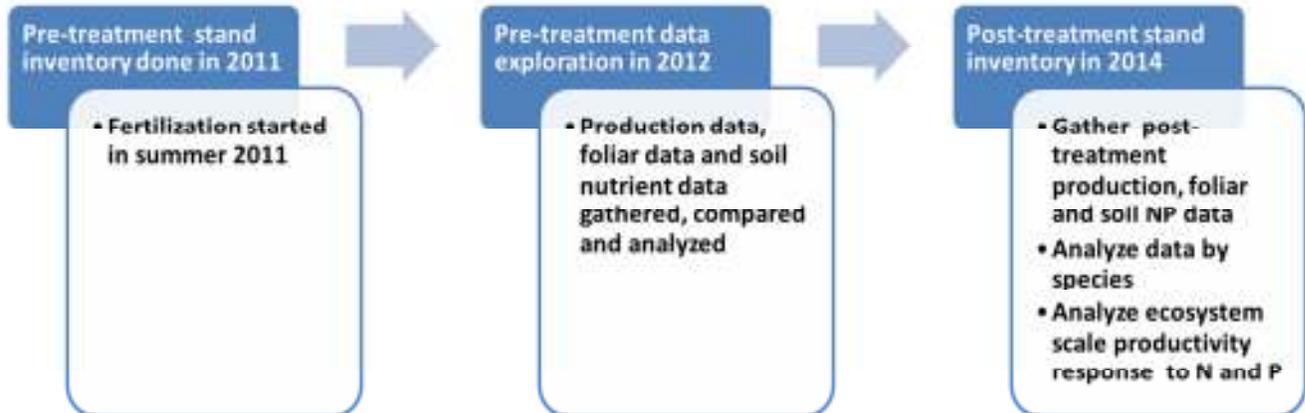


Fig 22. Growth efficiency (cm^2 basal area increment/kg leaf litter) of yellow birch in mid and mature stands with soil N availability ($R^2 = 0.19$; $p = 0.14$).

The above pre-treatment trends of growth efficiency will be repeated for exploring post-treatment productivity responses. I will use the change in BA per plot and species-specific allometric equations to estimate productivity responses to the nutrient treatments at species-specific and ecosystem scales.

TIMELINE



References:

- Aber, J. D., Goodale, C. L., Ollinger, S. V., Smith, M., Magill, A. H., Martin, M. E., Hallett, R. A., Stoddard, J. L. (2003). Is nitrogen deposition altering the nitrogen status of Northeastern forests? *Bioscience* 53:375 -389.
- Aber, J. D. and Melillo, J. M. (2001). *Terrestrial Ecosystems*. Academic Press, Orlando, FL.
- Aber, J. D., Nadelhoffer, K. J., Steudler, P., Melillo, J. M. (1989). Nitrogen saturation in northern forest ecosystems. *Bioscience* 39:378–386.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSL-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402.
- Arrigo, K. R. (2005). Marine microorganisms and global nutrient cycles. *Nature* 437: 349-355.
- Arthur, M. A., Hamburg, S. P., Siccama, T. G. (2001). The accuracy of allometric estimates of aboveground living biomass and nutrient contents of a northern hardwood forest. *Canadian Journal of Forest Research* 31: 11-17.
- Augusto, L., Ranger, J., Binkley, D., Rothe, A. (2002). Impact of several common tree species of European temperate forests on soil fertility. *Annals of Forest Science* 59:233–253.
- Bailey, S. W., Hornbeck, J. M., Driscoll, C. T., Gaudette, H. E. (1996). Calcium inputs and transport in a base poor forest ecosystem as interpreted by Sr isotopes. *Water Resources Research* 32: 707–719.
- Bedison, J. E. and McNeil, B. E. (2009). Is the growth of temperate forest trees enhanced along an ambient nitrogen deposition gradient? *Ecology* 90: 1736-1742.
- Bigelow, S. W. and Canham, C. D. (2007). Nutrient limitation of juvenile trees in a northern hardwood forest: calcium and nitrate are preeminent. *Forest Ecology and Management* 243: 310-319.
- Bingham, M. A. and Simard, S. W. (2013). Seedling genetics and life history outweigh mycorrhizal network potential to improve conifer regeneration under drought. *Forest Ecology and Management* 287: 132 -139.
- Bingham, M. A. and Simard, S. W. (2012). Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings. *Mycorrhiza* 22: 317-326.

- Blair, B. C. and Perfecto, I. (2004). Successional status and root foraging for phosphorus in seven tropical tree species. *Canadian Journal of Forest Research* 34: 1128-1135.
- Bloom, A. J., Chapin, F. S. III, Mooney, H. A. (1985). Resource limitation in plants- an economic analogy. *Annual Review of Ecology and Systematics* 16: 363-392.
- Blum, J. D., Klaue, A., Nezat, C. A., Driscoll, C. T., Johnson, C. E., Siccama, T. G., Eagar, C., Fahey, T. J., Likens, G. E. (2002). Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* 417: 729-731.
- Bormann, F. H. and Likens, G. E. (1979). *Pattern and process in a forested ecosystem*. Springer, New York.
- Brassard, B. W., Chen, H. Y. H., Cavard, X., Laganier, J., Reich, P. B., Bergeron, Y., Pare, D., Yuan, Z. (2013). Tree species diversity increases fine root productivity through increased soil volume filling. *Journal of Ecology* 101: 210-219.
- Brunner, I., Brodbeck, S., B  chler, U., Sperisen, C. (2001). Molecular identification of fine roots of trees from the Alps: reliable and fast DNA extraction and PCR-RFLP analyses of plastid DNA. *Molecular Ecology* 10: 2079-2087.
- Caldwell, M. M. (1994). Exploiting nutrients in fertile soil microsites. In: Caldwell, M. M., Pearcy, R. W. (eds) *Exploitation of environmental heterogeneity by plants: ecophysiological processes above- and belowground*. Academic, San Diego, pp 325-347.
- Canham, C. D., Berkowitz, A. R., Kelly, V. R., Lovett, G. M., Ollinger, S. V., Schnurr, J. (1996). Biomass allocation and multiple resource limitation in tree seedlings. *Canadian Journal of Forest Research* 26: 1521-1530.
- Chapin, F.S. III, Matson, P. A., Mooney, H. A. (2002). *Principles of Terrestrial Ecosystem Ecology*. Springer Verlag, New York.
- Chapin, F. S. III, Bloom, A. J., Field, C. B., Waring, R. H. (1987). Plant responses to multiple environmental factors. *BioScience* 37: 49-57.
- Chapin, F. S. III, Vitousek, P. M., Van Cleve, K. (1986). The nature of nutrient limitation in plant communities. *American Naturalist* 127: 48-58.
- Chapin, F. S. III and Shaver, G. R. (1985). Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* 66: 564-576.

- Chen, B. J. W., During, H. J., Anten, N. P. R. (2012). Detect thy neighbor: identity recognition at the root level in plants. *Plant Science* 195: 157-167.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31: 343-366.
- Cleavitt, N. L., Fahey, T. J., Battles, J. J. (2011). Regeneration ecology of sugar maple (*Acer saccharum*): seedling survival in relation to nutrition, site factors, and damage by insects and pathogens. *Canadian Journal of Forest Research* 41: 235-244.
- Cleavitt, N. L., Fairbairn, M., Fahey, T. J. (2008). Growth and survivorship of American Beech (*Fagus grandifolia* Ehrh.) seedlings in a northern hardwood forest following a mast event. *Journal of the Torrey Botanical Society* 135: 328-345.
- Danger, M., Daufresne, T., Lucas, F., Pissard, S., Lacroix, G. (2008). Does Liebig's law of the minimum scale up from species to communities? *Oikos* 117: 1741-1751.
- Driscoll, C. T., Lawrence, G. B., Bulger, A. J., Butler, T. J., Cronan, C. S., Eagar, C., Lambert, K. F., Likens, G. E., Stoddard, J. L., Weathers, K. C. (2001). Acid Rain Revisited: advances in scientific understanding since the passage of the 1970 and 1990 Clean Air Act Amendments. Hubbard Brook Research Foundation. Science Links™ Publication. Vol. 1, no.1.
- Drobyshev, I., Gewehr, S., Berninger, F., Bergeron, Y., McGlone, M. (2013). Species specific growth responses of black spruce and trembling aspen may enhance resilience of boreal forest to climate change. *Journal of Ecology* 101 (1): 231-242.
- Duchesne, L. and Oulmet, R. (2009). Present-day expansion of American beech in northeastern hardwood forests: does soil base status matter? *Canadian Journal of Forest Research* 39: 2273-2282.
- Duchesne, L., Ouimet, R., Houle, D. (2002). Basal area growth of sugar maple in relation to acid deposition, stand health, and soil nutrients. *Journal of Environmental Quality* 31: 1676-1683.
- Ehrenfeld, J. G. (2001). Plant-soil interactions. In S. Levin, ed-in-chief, *Encyclopedia of Biodiversity*, Academic Press, San Diego, CA. pp. 689-709.
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., Smith, J. E. (2007). Global Analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10: 1135-1142.

- Fahey, T. J., Battles, J. J., Wilson, G. F. (1998). Responses of early successional northern hardwood forests to changes in nutrient availability. *Ecological Monographs* 68: 183-212.
- Fahey, T. J. and Hughes, J. W. (1994). Fine root dynamics in a northern hardwood forest ecosystem, Hubbard brook experimental forest, NH. *Journal of Ecology* 82:533–548.
- Falik, O., Reides, P., Gersani, M., Novoplansky, A. (2003). Self/non-self discrimination in roots. *Journal of Ecology* 91: 525–531.
- Fatemi, F. R. (2007). Aboveground biomass and nutrients in developing northern hardwood stands in New Hampshire, USA. State University of New York, College of Environmental Science and Forestry, Syracuse, NY. Master thesis.
- Fédérer, C. A., Hombeck, J. W., Tritton, L. M., Martin, C. W., Pierce, R. S., Smith, C. T. (1989). Long-term depletion of calcium and other nutrients in eastern US forests. *Environmental Management* 13: 593 -601.
- Fenn, M. E., Poth, M. A., Aber, J. D., Baron, J. S., Bormann, B. T., Johnson, D. W., Lemly, A. D., McNulty, S. G., Ryan, D. F., Stottlemeyer, R. (1998). Nitrogen excess in North American ecosystems: a review of predisposing factors, geographic extent, ecosystem responses and management strategies. *Ecological Application* 8:706–733.
- Finzi, A. C. and Canham, C. D. (2000). Sapling growth in response to light and nitrogen availability in a southern New England forest. *Forest Ecology and Management* 131: 153-165.
- Finzi, A.C., van Breemen, N., Canham, C.D., (1998). Canopy tree–soil interactions within temperate forests: species effects on soil carbon and nitrogen. *Ecological Application* 8: 440–446.
- Fiorentino, I., Fahey, T. J., Groffman, P. M., Driscoll, C. T., Eagar, C., Siccama, T. G. (2003). Initial responses of phosphorus biogeochemistry to calcium addition in a northern hardwood forest ecosystem. *Canadian Journal of Forest Research* 33:1864–1873.
- Fisk, M. C., Yanai, R. D., Fierer, N. (2010). A molecular approach to quantify root community composition in a northern hardwood forest- testing effects of root species, relative abundance, and diameter. *Canadian Journal of Forest Research* 40: 836-841.
- Gardes, M. and Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes— application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Gersani, M., Brown, J. S., O’Brien, E. E., Maina, G. M., and Abramsky, Z. (2001). Tragedy of the commons as a result of root competition. *Journal of Ecology* 89: 660–669.

- Gleeson, S. K. and Good, R. E. (2003). Root allocation and multiple nutrient limitation in the New Jersey Pinelands. *Ecology Letters* 6:220–227.
- Grime, J. P. (1994). The role of plasticity in exploiting environmental heterogeneity. In: Caldwell, M. M., Pearcy, R. W. (eds) *Exploitation of environmental heterogeneity by plants: ecophysiological processes above- and belowground*. Academic, San Diego, pp 1-19.
- Grime, J. P. (1979). *Plant strategies and vegetation processes*. Wiley, Chichester.
- Hamburg, S. P., Zamolodchikov, D. G., Korovin, G. N., Nefedjev, V. V., Utkin, A. I., Gulbe, J. I., Gulbe, T. A. (1997). Estimating the carbon content of Russian forests; a comparison of phytomass/volume and allometric projections. *Mitigation and Adaptation Strategies for Global Change* 2: 247-265.
- Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Bracken, 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.
- Harris, R. W. (1992). Root-shoot ratios. *Journal of Arboriculture* 18: 39-42.
- Helmisaari, H., Ostonen, I., Lohmus, K., Derome, J., Lindroos, A., Merila, P., Nojd, P. (2009). Ectomycorrhizal root tips in relation to site and stand characteristics in Norway spruce and Scots pine stands in boreal forests. *Tree Physiology* 29: 445-456.
- Hinckley, T. M., Friend, A. L., Mitchell, A. K. (1992). Foliage, tree, and stand level responses to fertilization: a physiological perspective. In *Forest Fertilization: Sustaining and Improving Nutrition and Growth of Western Forests*. Eds. N.J. Chappell, G. F. Weetman and R. E. Miller. University of Washington, Seattle, Inst. Forest Resources Contrib. No. 73, pp 82-89.
- Hodge, A. (2009). Root decisions. *Plant, Cell and Environment* 32 628–640.
- Hubbell, S. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ, USA.
- Hutchinson, G.E. (1961). The paradox of the plankton. *American Naturalist* 95: 137–147.
- Juice, S. M., Fahey, T. J., Siccama, T. G., Driscoll, C. T., Denny, E. G., Eagar, C., Cleavitt, N. L., Minocha, R., Richardson, A. D. (2006). Response of sugar maple to calcium addition to northern hardwood forest. *Ecology* 87: 1267-1280.
- Lang, C. and Polle, A. (2011). Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed central European forest. *Tree Physiology* 31: 531-538.

- Lawrence, G.B., David, M. B., Bailey, S. W., Shortle, W. C. (1997). Assessment of soil calcium status in red spruce forests in the north-eastern United States. *Biogeochemistry* 38:19–39.
- Lea, R., Tierson, W. C., Leaf, A. L. (1979). Growth responses of northern hardwoods to fertilization. *Forest Science* 25: 597–604.
- Liebig, J. (1842). *Animal Chemistry, or Organic Chemistry in its Application to Physiology and Pathology*. Johnson Reprint Corporation, New York, USA.
- 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., Fahey, T. J., Reiners, W. A., Ryan, D. F., Martin, C. W., Bailey, S. W. (1998). The biogeochemistry of calcium and Hubbard Brook. *Biogeochemistry* 41:89–173.
- Mahall, B.E. and Callaway, R.M. (1991). Root communication among desert shrubs. *PNAS* 88: 874–876.
- Marks, P. L. (1974). The role of pin cherry (*Prunus pennsylvanica* L.) in the maintenance of stability in northern hardwood ecosystems. *Ecological Monographs* 44: 73-88.
- McNeil, B. E., Read, J. M., Driscoll, C. T. (2007). Foliar nitrogen responses to elevated atmospheric nitrogen deposition in nine temperate forest canopy species. *Environmental Science and Technology* 41:5159 -5197.
- Meinen, C., Hertel, D., Leuschner, C. (2009). Root growth and recovery in temperate broad-leaved forest stands differing in tree species diversity. *Ecosystems* 12: 1103-1116.
- Mellilo, J. M., Aber, J. D., Muratore, J. F. (1982). Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. *Ecology* 63: 621-626.
- MELNHE project website <http://www.esf.edu/melnhe/>
- Menge, D. N. L., Ballantyne, F. IV, Weitz, J. S. (2011). Dynamics of nutrient uptake strategies: lessons from the tortoise and the hare. *Theoretical Ecology* 4: 163-177.
- Menge, D. N. L., Pacala, S. W., Hedin, L. O. (2009). Emergence and maintenance of nutrient limitation over multiple timescales in terrestrial ecosystems. *American Naturalist* 173: 164-175.
- Mohren, G. M. J., van den Burg, J., Burger, F. W. (1986). Phosphorus deficiency induced by nitrogen input in Douglas-fir in The Netherlands. *Plant Soil* 95:191–200.

- Moore, J. D. and Ouimet, R., (2006). Ten-year effect of dolomitic lime on the nutrition, crown vigor, and growth of sugar maple. *Canadian Journal of Forest Research* 36: 1834–1841.
- Moore, J., Ouimet, R., Duchesne, L. (2012). Soil and sugar maple response 15 years after dolomitic lime application. *Forest Ecology and Management* 281: 130-139.
- Murphy, J. and Riley, J. P. (1962). A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31-36.
- Muscarella, R., Uriate, M., Forero-Montana, J., Comita, L. S., Swenson, N. G., Thompson, J., Nytch, C. J., Jonckheere, I., Zimmerman, J. K. (2013). Life-history trade-offs during the seed-to-seedling transition in a subtropical wet forest community. *Journal of Ecology* 101: 171-182.
- Nadelhoffer, K. J., Aber, J. D., Melilo, J. M. (1985). Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. *Ecology* 66: 1377-1390.
- Naples, B. K. and Fisk, M. C. (2010). Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry* 97: 109-121.
- North, R. L., Guildford, S. J., Smith, R. E. H. Havens, S. M., Twiss, M. R. (2007). Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnology and Oceanography* 52: 315-328.
- Park, B. B., Yanai, R. D., Fahey, T. J., Bailey, S. W., Siccama, T. G., Shanley, J. B., Cleavitt, N. L. (2008). Fine root dynamics and forest production across a calcium gradient in northern hardwood and conifer ecosystems. *Ecosystems* 11: 325-341.
- Pregitzer, K. S., DeForest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., Hendrick, R. L. (2002). Fine root architecture of nine north American trees. *Ecological Monographs* 72: 293-309.
- Pregitzer, K. S., Laskowski, M. J., Burton, A. J., Lessard, V. C., Zak, D. R. (1998). Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* 18: 665-670.
- Raich, J. W., Riley, R. H., Vitousek, P. M. (1994). Use of root-growth cores to assess nutrient limitations in forest ecosystems. *Canadian Journal of Forest Research* 24: 2135-2138.
- Rajaniemi, T. K. and Reynolds, H. L. (2004). Root foraging for patchy resources in eight herbaceous species. *Oecologia* 141:519-525.
- Reich, P. B., Grigal, D. F., Aber, J. D., Gower, S. T. (1997). Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* 78: 335-347.

- Reich, P., Hobbie, S., Lee, T., Ellsworth, D., West, J., Tilman, D., Knops, J. M. H., Naeem, S., Trost, J. (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* 440: 922-925.
- Reich, P. B., Oleksyn, J., Modrzyński, J., Mrozinski, P., Hobbie, S. E., Eissenstat, D. M., Chorover, J., Chadwick, O. A., Hale, C. M., Tjoelker, M. G. (2005). Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. *Ecology Letters* 8:811–818.
- Rewald, B. and Leuschner, C. (2009). Belowground competition in a broad-leaved temperate mixed forest: pattern analysis and experiments in a four-species stand. *European Journal of Forest Research* 128: 387-398.
- Robinson, D. (1994). The response of plants to non-uniform sources of nutrients. *New Phytologist* 127:635 -674.
- Safford, L. O. (1973). Fertilization increases diameter growth of birch-beech-maple trees in New Hampshire. USDA Forest Service Research Note NE-182. Northeastern Forest Experiment Station.
- Saito, M. A., Goepfert, T. J., Ritt, J. T. (2008). Some thoughts on the concept of colimitation: three definitions and the importance of bioavailability. *Limnology and Oceanography* 53: 276-290.
- Sattelmacher, B., Gerendas, J., Thoms, K., Bruck, H., Bagday, N. H. (1993). Interaction between root growth and mineral nutrition. *Environmental and Experimental Botany* 33: 63-73.
- Schwarz, P.A., Fahey, T.J., McCulloch, C.E. (2003). Factors controlling spatial variation of tree species abundance in a forested landscape. *Ecology* 84: 1862–1878.
- Siccama, T. G., Hamburg, S. P., Arthur, M. A., Yanai, R. D., Bormann, F. H., Likens, G. E. (1994). Corrections to allometric equations and plant tissue chemistry for Hubbard Brook Experimental Forest. *Ecology* 75: 246-248.
- St.Clair, S. B., Sharpe, W. E., Lynch, J. P. (2008). Key interactions between nutrient limitation and climatic factors in temperate forests: a synthesis of the sugar maple literature. *Canadian Journal of Forest Research* 38: 401-414.
- Taberlet, P., Gielly, L., Pautou, G., Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Tennet, D. (1975). A test of modified line intersect method of estimating root length. *Journal of Ecology* 63:995–1001.

- Tilman, D. (2004). Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *PNAS* 101: 10854-10861.
- Tilman, D. (1999). The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* 80: 1455-1474.
- Tilman, D. (1982). *Resource Competition and Community Structure*, Mono-graphs in Population Biology (Princeton Univ. Press, Princeton).
- Vadeboncoeur, M. A. (2010). Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. *Canadian Journal of Forest Research* 40: 1766-1780.
- Vitousek, P. M. (2004). *Nutrient cycling and limitation: Hawai'i as a model System*. Princeton University Press.
- 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.
- Vitousek, P. M. and Reiners, W. A. (1975). Ecosystem succession and nutrient retention: a hypothesis. *Bioscience* 25:376–381.
- Whittaker, R. H., Bormann, F. H., Likens, G. E., Siccama, T. G. (1974). The Hubbard Brook Ecosystem study: forest biomass and production. *Ecological Monographs* 44:233-252.
- Woodwell, G. M. and Whittaker, R. H. (1968). Primary production in terrestrial ecosystems. *American Zoologist* 8:19–30.
- Wright, S. J., Yavitt, J. B., Wurzburger, N., Turner, B. L., Tanner, E. V. J., Sayer, E. J., Santiago, L. S., Kaspari, M., Hedin, L. O., Harms, K. E., Garcia, M. N., Corre, M. D. (2011). Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* 92: 1616-1625.
- Yanai, R. D., Fisk, M. C., Fahey, T. J., Cleavitt, N. L., Park, B. B. (2008). Identifying roots of northern hardwood species: patterns with diameter and depth. *Canadian Journal of Forest Research* 38: 2862-2869.
- Yavitt, J. B., Harms, K. E., Garcia, M. N., Mirabello, M. J., Wright, S. J. (2011). Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology* 36: 433-445.