Project Summary

The productivity of temperate forests on glaciated soils has been presumed to be nitrogen (N) limited, although theory suggests that ecosystem productivity should be co-limited by multiple nutrients, especially including phosphorus (P). Experimental tests of N vs. P limitation in temperate forests are needed to better understand the capacity of ecosystems to balance the acquisition of limiting resources. In 2011 we began a full-factorial NxP fertilization experiment in 13 stands of young, mid-aged, and mature northern hardwoods in three contrasting sites that span a range of native soil fertility. Surprisingly, early results showed tree growth responding more to P than to N fertilization. We propose to continue these treatments to test whether P limitation will persist, possibly as a consequence of decades of anthropogenic N enrichment, or whether adjustment of effort to acquire N versus P will result in co-limitation. We will measure aboveground and belowground productivity and evaluate shifts in allocation of effort after a decade of nutrient manipulation. Mechanisms that could enable the ecosystem to acquire and conserve N and P and to maintain stoichiometric balance in the face of changing nutrient availability include foliar N and P resorption, differential rooting depth, soil enzymes, mycorrhizae and changes in tree species composition. The Multiple Element Limitation model will be used to integrate data from the various components of the study, advance understanding of nutrient limitation, and extrapolate the results to improve management of forest ecosystems in the face of increasing CO2, changing climate, and a legacy of atmospheric deposition.

Nutrient Limitation and Co-Limitation

Understanding the limitations of soil nutrient supply to agricultural productivity is critical in forests, which are quintessentially low-input production systems. Northern hardwoods are the most extensive forest type in the northeastern USA (Godman 1992). The Northern States annually produce 2.3 billion cubic feet of roundwood (saw logs, pulpwood, fuelwood, etc.) from hardwoods and 0.7 billion cubic feet from softwoods (Shifley *et al.* 2012). The value of wood products from this region is estimated at $112 billion annually, with an added value of $52 billion from primary wood products manufacturing (Shifley et al 2012). These forests have typically been managed without fertilization (Nyland 2016). However, repeated harvesting depletes the soil stores of available nutrients (Federer *et al.* 1989, Vadeboncoeur *et al.* 2014), and the intensity of removal increases dramatically when biomass production for energy is maximized, compared to harvesting for maximum timber value (Mann *et al.* 1988, Yanai 1998). Thus the sustainability of forest production systems in the absence of fertilization, or an evaluation of the need for future fertilization to maintain ecosystem products and services, depends on understanding the controls on plant demand and soil nutrient supply.

Managing nutrient supply was once understood to depend on identifying the most limiting nutrient, or perhaps the response to the addition of the most limiting nutrient and the nutrients subsequently revealed to be next most limiting (von Leibig, 1840). In forests, this meant that fertilization trials in regions presumed to be nitrogen limited (e.g., hardwoods in the northeastern USA) included N addition and N + P addition (Vadeboncoeur 2010), while in regions of phosphorus limitation (e.g., pines in the southeastern USA) the common treatments were P addition and N + P addition (Fox et al 2007), but there was no reason to test the response to a single nutrient presumed not to be limiting. This approach failed to recognize the possibility that organisms and ecosystems have mechanisms that drive the system toward balanced mineral nutrition (described below). **The purpose of this project is to evaluate the importance of a suite of processes that affect the relative supply of N and P to northern hardwood forests,**

using the first long-term, full-factorial N by P addition experiment in a temperate forest system. Another important benefit of comparing forest response to N vs. P addition is to answer the very basic question of which is more limiting, which, obviously, is difficult to achieve in the absence of trials involving both elements separately.

Ecosystem theory has suggested that productivity should be limited by nitrogen on young soils and phosphorus on older soils (Walker and Syers 1976, Vitousek 2004); thus, northern hardwood forests on glacially derived soils are expected to be N limited. In 2011 we initiated a long-term NxP factorial nutrient manipulation in temperate forests, with 13 stands of three ages distributed across three research sites with contrasting native soil fertility. We were surprised to find that aboveground tree growth increased in response to added P but not N (Goswami *et al.* 2018) and that foliar N:P in the untreated mid-age and mature stands suggests that most of our study sites may be P-limited rather than N-limited (Güsewell 2004, Koerselman and Meuleman). Consistent with P limitation, foliar N:P in plots receiving P has moved into a range that is considered co-limited, and foliar N:P in plots receiving N is even further in the P- limited range after 5 years of treatment (Fig. 1).

Co-limitation has gained increasing attention in recent years as a concept for characterizing how environmental resources constrain the productivity and dynamics of ecosystems (Harpole 2011). Although defining co-limitation can be challenging, in part because of its dependence on time scales of observation (Davidson and Howarth 2007), a key feature of a system exhibiting co-limitation is a synergistic response to multiple environmental resources. The nature and mechanisms of such synergistic responses, as well as the identity of the limiting resources, differ depending upon the ecosystem or subsystem under consideration, e.g., aquatic vs. terrestrial, primary producer vs. herbivore or consumer,
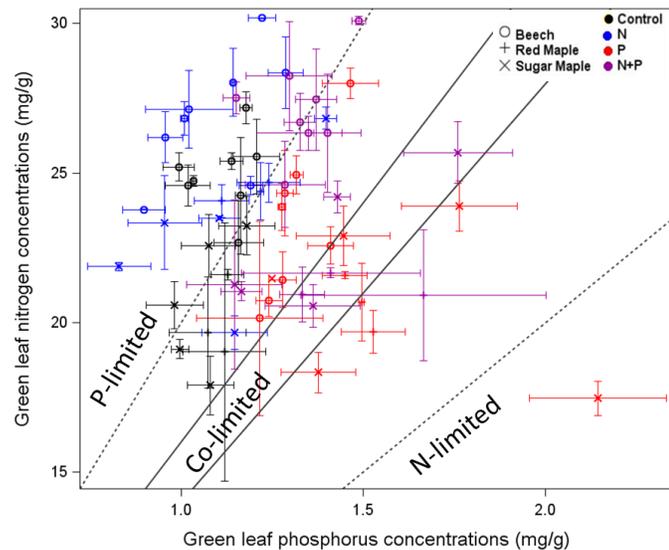

Figure 1. Foliar P and N in 2015 for maple and beech in mid-aged and mature stands. N:P ratios between 14 and 16 are considered to indicate co-limitation (Güsewell 2004). Those below 10 and above 20 indicate N and P co-limitation, respectively (Koerselman and Meuleman 1996).

or forest vs. grassland (Craine *et al.* 2008, Harpole 2011, Sperfeld *et al.* 2012). In all these cases, the line of evidence for co-limitation is that biological activity is stimulated more by the addition of multiple resources than by any single resource alone.

The Multiple Element Limitation (MEL) model has served as the foundation for the development of a theory of nutrient co-limitation, the synchronization of nutrient cycles within ecosystems, and the coupling of these nutrient cycles with energy, carbon, and water fluxes through ecosystems (Rastetter and Shaver 1992, Rastetter *et al.* 1997, 2001, 2005, 2013). The MEL model simulates interactions among C, N, P, light, and water in terrestrial ecosystems based on resource optimization theory (Bloom et al 1985, Chapin *et al.* 1987). In theory, plants

and ecosystems will adjust toward a condition where all resources are equally limiting, if environmental conditions are not changing.

The heart of the MEL model is a dynamic algorithm that drives vegetation toward this optimal acquisition of resources (nutrients, light, CO_2, H_2O) through the reallocation of plant "effort" toward the more limiting resources. "Effort" is an aggregate representation of all allocatable assets such as biomass, enzymes, and carbohydrates. At the optimal allocation of effort, the ratio of acquisition to requirement will be the same for all resources. In this sense, all the resources are equally limiting to growth at steady state.
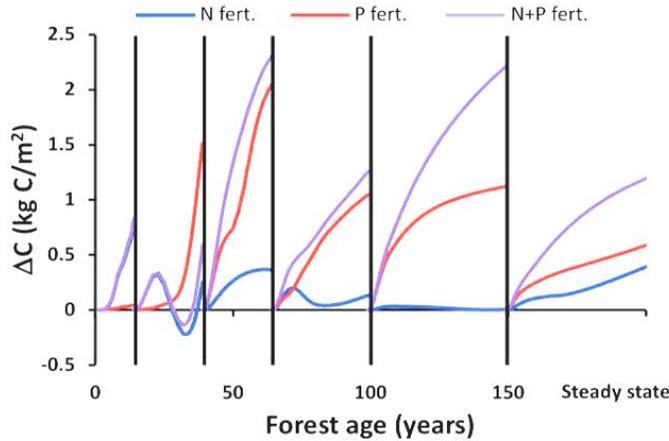

Figure 2. Response of forest biomass relative to the control to simulated additions of N (30 kg/ha/yr), P (10 kg/ha/yr) or N+P beginning at various ages since clearcutting (vertical lines) (Rastetter et al. 2013). The greater response to N in young stands and to P in mid-aged and older stands is consistent with our observations (Fig. 3).

We have adapted and parameterized MEL for northern hardwood forests (Rastetter *et al.* 2013) and are now testing the model predictions of responses to the long-term nutrient addition experiment described in this proposal (Fig 2). We are especially interested in co-limitation of these forests by N and P, the two most commonly limiting nutrients in forest vegetation worldwide.

MEL predicted that following harvest of a northern hardwood forest N and P co-limitation would eventually develop as the cycles of these limiting nutrients were gradually re-synchronized, correcting the disruption of synchrony associated with nutrient losses during and after harvest (Rastetter *et al.* 2013). The model predicted greater N limitation in early stages of recovery (stands 0 or 15 years old grew more with N or NP addition than P addition), followed by P limitation later (stands 40 years old grew more in response to P or NP addition than N addition); the greatest advantage of NP addition was predicted in stands over 100 years old (Fig. 2). Early results from our experimental N and P additions to northern hardwood stands provide some tentative support for the model, with weak evidence for N limitation in early succession, and significant P limitation in mid-aged and mature stands (Goswami *et al.* 2018; Fig. 3).

Although non-significant, the trend was towards the greatest response to NP, and we suggested that spatial heterogeneity in growth responses to N or P among our thirteen forest stands might indicate that forests in the region are close to a transition between P and N limitation (Goswami *et al.* 2018). Consistent with co-limitation, the model predicts that the response to N and P together should be greater than the response to the more limiting nutrient (Fig. 2), but it is not surprising that these responses could not be distinguished before about a decade of treatment (the time frame of our proposed remeasurement).
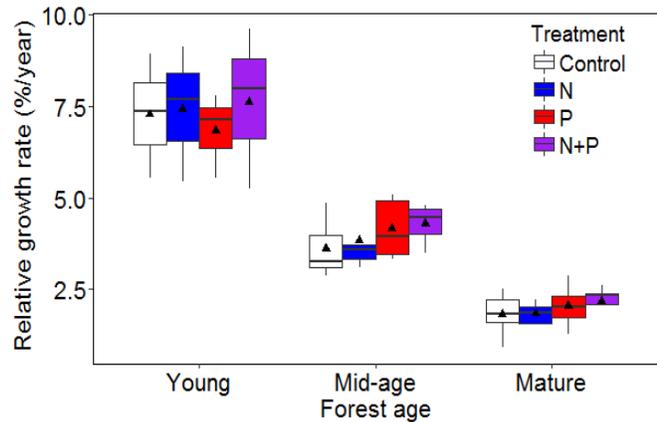

Figure 3. Growth rate (relative basal area increment) of individual trees > 10 cm dbh over 4 growing seasons post-treatment. We detected a significant response to P (p=0.02) but not to N (p=0.73), and did not detect an NxP interaction (p=0.68).

A variety of mechanisms could contribute to achieving N and P co-limitation in forests. Our conceptual model illustrates various natural and anthropogenic factors that challenge the maintenance of co-limitation by N and P (Fig. 4), forcing greater limitation by one or the other element. In response, several mechanisms counteract these challenges to favor balanced nutrition that tends towards N and P co-limitation. Because of the differing time scales of the mechanisms contributing to co-limitation, observing the responses of forest ecosystems to changing nutrient availability requires long-term study. **By adding relatively low levels of N and P over a long (decadal) time period, we will test for nutrient co-limitation of temperate forest productivity and evaluate the importance of various mechanisms contributing to co-limitation.** We recognize that not all of the possible responses to nutrient additions will lead towards co-limitation by N and P, and it is entirely possible that co-limitation will not be attained in spite of the many possible mechanisms favoring balance. As environmental resources and conditions continue to change as a result of natural and anthropogenic forcing, a better understanding of forest responses is needed to inform forest management decisions and predict long-term changes in forest productivity, C sequestration, timber value, and other ecosystem services. The proposed research is designed to contribute to such a goal.

Ongoing and Completed Activities

The proposed research builds upon previously funded projects that established the plots, described pre-treatment conditions, and began the nutrient treatments to address N and P co-limitation in young and mature northern hardwood forests. A summary of the findings from this work demonstrates the productivity of our research team and the value of continuing to build upon this knowledge base (Table 1). In addition to publications and presentations, products of this work include: data sets, provided on our project web site and that of the Hubbard Brook LTER; archived samples, located in the Long-Term Storage system at SUNY-ESF and in the physical archive building at Hubbard Brook; and code and compiled software for the MEL model, hosted by the Ecosystems Center at MBL. This previous work includes pre-treatment studies characterizing spatial heterogeneity and coupling of N and P recycling across 13 forest stands in central NH. We have documented ecosystem responses to up to 5 years of fertilizing,

including P limitation of tree growth and soil microbial activity. The next several years of this experiment are a crucial time to intensify our measurements of response mechanisms to test questions about the development of a co-limitation response and to elucidate the plant and microbial mechanisms that could help to drive the ecosystem toward a condition of co-limitation.

Rationale and Significance

It is generally understood that productivity of terrestrial vegetation is limited by P on old soils and by N on young soils, based on the depletion of primary mineral P and accumulation of N as soils develop (Walker and Syers 1976). Thus, N should be the primary limiting nutrient in ecosystems with relatively young soils, such as recently glaciated temperate hardwood forests. However, this is not always found to be the case (Vadeboncoeur 2010), and recent demonstration of the prevalence of N and P co-limitation in aquatic and terrestrial ecosystems (Elser *et al.* 2007, Harpole *et al.* 2011) has stimulated interest in the processes regulating nutrient limitation of productivity.

Optimization of resource acquisition, the accumulation of nutrients within ecosystem cycles, and the consequent synchronization of those cycles should operate to minimize limitation by any single nutrient (Bloom *et al.* 1985, Chapin *et al.* 1987, Rastetter *et al.* 2013). We therefore expect the availability and acquisition of multiple nutrients to maintain a condition near nutrient co-limitation (Elser *et al.* 2007). In our conceptual model of forest nutrient limitation, both natural and anthropogenic factors challenge the maintenance of balanced nutrition in forested ecosystems (Fig. 4). The history of high atmospheric deposition of N should force greater P limitation as it relieves N limitation (Vitousek *et al.* 2010). Also forcing systems towards P limitation is the changing allometry of trees, with a greater demand for P relative to N as forests grow older, due to the proportion of tissues with different N:P ratios (Vitousek *et al.* 1988). Later in forest development, delayed recycling of detrital P may also exacerbate P limitation (Wardle *et al.* 2004). Forcing systems in the other direction, towards N limitation, are the high N losses associated with disturbances (Vitousek *et al.* 1979), which are particularly relevant for young forests developing following clearcutting. Also, increasing precipitation associated with climate change could drive higher losses by leaching and denitrification (Groffman *et al.* 2012). In our experiment, we use N and P fertilization to challenge balanced nutrition and provide an opportunity to study systems in a state of greater P limitation or N limitation.

Table 1. Previous work in our study sites includes pre-treatment characterization of soil and vegetation, preliminary results describing responses to nutrient additions, and ongoing work by our graduate students.

Pre-treatment

Root ingrowth cores showing foraging for P in mid-age stands and suggesting P limitation (Naples and Fisk 2010)

Characterization of among-stand variation in soil nutrients (Vadeboncoeur *et al.* 2012, 2014), weathering rates (Schaller *et al.* 2010), root biomass (Yanai *et al.* 2006, Park *et al.* 2007), and aboveground biomass and nutrient stocks (Fatemi *et al.* 2011)

An improved version of the Multiple Element Limitation (MEL) model based in part on data from these stands, showing changes in limiting nutrients and shifts in optimal allocation with stand age and management (Fig. 1, Rastetter *et al.* 2013)

A stand-scale nutrient depletion budget with implications for sustainable forest management (Vadeboncoeur *et al.* 2014)

Variation in foliar nutrient resorption (See 2013, See *et al.* 2015) and soil respiration (Bae 2013, Bae *et al.* 2015) showing significant effects related to site, stand age, and nutrient availability

Coupling of soil N and P availability across all study sites (Ratliff and Fisk 2016)

AM and EM colonization rates, which declined equally with soil depth (Diggs 2014)

Treatment effects

Soil microbial responses indicating limitation by P and suppression by N of microbial respiration (Fisk *et al.* 2015)

Responses of foliar chemistry and sugar content of maple sap, showing greater sap sweetness in response to N addition (Wild 2014, Wild and Yanai 2015)

Sap flow responses in multiple species in a subset of stands and treatments (Hernandez-Hernandez 2015, Zahor 2014, A. Rice and M. Johnston *et al.,* unpublished data)

Negative effects of N and P treatments on survivorship of maple and beech germinants, corresponding with improved N and P nutrition and greater leaf damage (Goswami 2017)

Initial post-treatment soil respiration, showing a greater response to N addition where soil N availability was low (Kang *et al.* 2016)

Responses by resin-available soil nutrients, showing that treatments elevate availability of the added nutrient and that, over time, P addition suppresses N availability (Fisk *et al.* 2014; Goswami 2017)

Growth of trees post treatment, indicating P limitation in mid-age and mature forests (Fig. 2, Goswami *et al.* 2018)

Foliar nutrient concentrations and resorption post-treatment that support ambient P limitation and suggest the development of N limitation in response to P addition (Fig. 1, Gonzales 2017)

Mycorrhizal fungal communities on beech seedlings post-treatment (Barner 2016) and in soil ingrowth bags (Horton *et al.* 2018)

Counteracting these challenges to balanced nutrition are the factors favoring co-limitation in forests, such as processes regulating the stoichiometry of nutrient supply, the relative uptake or conservation of nutrients (e.g., foliar resorption), and shifts in dominance of tree species in the canopy, rooting depth in soil, or fungal partners in the rhizosphere (Fig. 4).

Processes restoring nutrient co-limitation operate over a wide range of time scales. Changes in resource allocation by soil microbes can influence stoichiometry of nutrient availability fairly rapidly, for example via production of different enzymes (Allison *et al.* 2011) and regulation of microbial growth and metabolic activity (Hartman and Richardson 2013) which influence nutrient uptake and turnover into organic matter. Changes in foliar nutrition influencing photosynthetic performance and changes in resorption and nutrient use efficiency should also be relatively rapid. Shifts in plant allocation belowground for nutrient acquisition may be more gradual. Eventually changes in the relative abundance of plant species with differing traits (Fahey *et al.* 1998) could contribute to maintaining long-term co-limitation at the ecosystem level. We will not observe the longest of these response times only 10 years into our study (fertilizer additions commenced in 2011); however, we can test for early signs of change in canopy dominance and use models to project the effects of those changes in the long term.
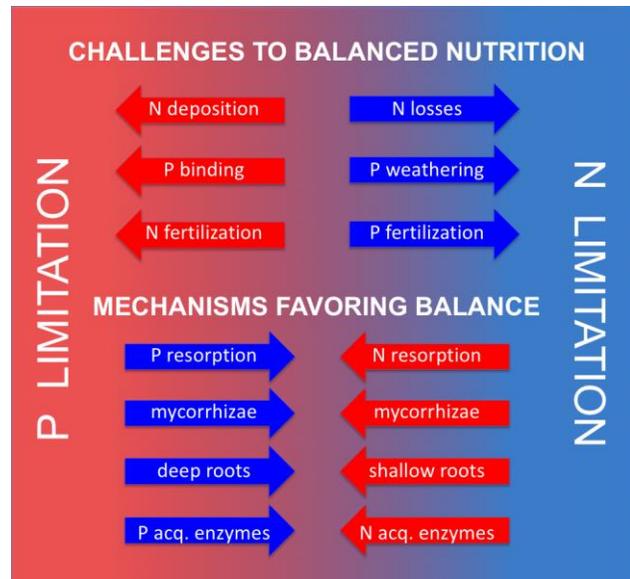

Figure 4. Conceptual model of N vs P limitation in forests, showing factors that cause systems to deviate from co-limitation by N and P and the mechanisms that favor co-limitation by conserving or acquiring the more limiting nutrient.

There have been few attempts to test for nutrient co-limitation in temperate forests. Of the 346 terrestrial studies summarized by Elser *et al.* (2007), only 4 were conducted in temperate forests, and of these, none was a full factorial manipulation of N and P. We are aware of only two full factorial studies of response to N and P in temperate forests, and these were both short term with very high rates of nutrient additions (young Douglas-fir in the Pacific Northwest by Mainwaring *et al.* (2014) and mature hardwoods in southern New England by Finzi (2009)). Adding nutrients to test for limitation disrupts the balance among processes regulating the supply and acquisition of different nutrients. Responses are likely to change over time, depending on how rapidly various process can re-equilibrate. Therefore, a full understanding of the limitations to forest productivity will require factorial experiments that are sufficiently long-term to accommodate adjustments by biota that occur over time scales ranging from soil enzyme production to tree species replacement.

We have undertaken the first long-term NxP factorial nutrient manipulation in temperate forests, in 13 stands of three ages distributed across three research sites. Foliar N:P in the controls of the mid-age and mature stands suggest that most of our study sites are P-limited rather than N-limited (Fig. 1). After 5 years of treatment, plots receiving P have moved into the co-limited range, and those receiving N are clearly P limited (Fig. 1). Similarly, aboveground

tree growth by 2015 had increased in response to P but not N additions in mid-age and mature stands, and the growth response to N+P was not significantly greater than the response to P alone (Fig. 3). In contrast, aboveground growth did not respond to P addition in the youngest stands but showed a trend toward greater growth with N addition (Fig. 3). These observations are consistent with MEL model predictions of greater N limitation early in forest succession and P limitation in mid-age forests (Fig. 2). Our failure to demonstrate co-limitation in mature forest is not surprising only 4 years into the nutrient manipulation especially at our low addition levels, because of the gradual effects of some of the important mechanisms of response that mediate nutrient limitation in forest ecosystems. It is also possible that the legacy of anthropogenic N deposition has shifted these ecosystems toward P limitation, and that the restoration of a co-limited state may be slow or even impossible for these ecosystems to achieve.

Mechanisms Favoring Balance

Pre-treatment measurements across our 13 stands, which vary in native soil fertility, illustrate some of the possible mechanisms for the development of N and P co-limitation. For example, soil phosphatase activity was higher in stands where pretreatment soil N availability was high (Ratliff and Fisk 2016), suggesting greater effort towards P acquisition. Leaf P resorption was also greater where soil N availability was high (See *et al.* 2015), which contributes to P conservation where P is most limiting. Belowground C allocation was greatest where N availability was low (Bae *et al.* 2015), which illustrates effort to obtain N where N is most limiting. Consistent with this pre-treatment observation, the initial response of soil respiration to N addition was greatest where soil N availability was low (Kang *et al.* 2016).

Figure 5. Adding P for 4 years reduced resin-available N, especially where resin-available P was high.

Soil and foliar responses to nutrient addition in early years of treatment illustrate additional mechanisms of N and P interaction. For example, the activity of soil phosphatase declined in response to added P (p <0.004 in Oe and Oa, p= 0.06 in mineral soil). Adding P also reduced the resin-available N in soil, most notably where resin-available P was high (Fig. 5), suggesting that relieving P limitation induces greater uptake of N by plants or soil microorganisms to maintain favorable N:P ratios. Hence, suppression of plant-available N through sequestration in plant biomass and in microbially derived organic pools could cause N to become limiting where P limitation is alleviated, and contribute to maintaining these forests near a condition of N and P co-limitation.

Mycorrhizal associations could play an important role in the balance of N and P limitation in these forests. All 13 of our stands are composed of roughly equal mixtures of ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) trees (Table 2). These different mycorrhizal types differ in their carbon-nutrient tradeoffs, but the situation is complex as C, N and P limitation of both plant and fungus can be involved (Hoeksema *et al.* 2010). We state hypotheses (below) that are based on the simplistic generalization that AM are more important in P acquisition (George *et al.* 1995) while EM associations facilitate efficient N acquisition from
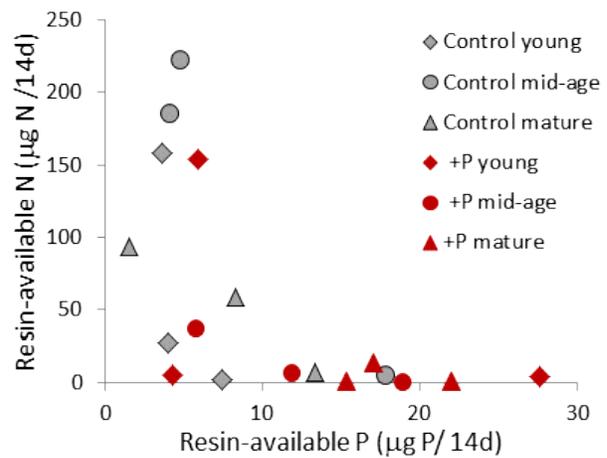
organic N sources (Lilleskov *et al.* 2002). However, it is well established that addition of N or P can reduce mycorrhizal colonization of both types, with more consistent declines in response to elevated P than to elevated N (Treseder 2004). Although AM exert a greater influence on P than N nutrition, their response to N can depend on P status (Johnson *et al.* 2003, Blanke *et al.* 2005). As for EM affecting only N acquisition, some studies find that EM may provide greater cost-effectiveness of P acquisition than AM (Jones *et al.* 1998) or enhance weathering of primary mineral P (Blum *et al.* 2002). Recent evidence suggests that in temperate deciduous forest supporting a mixture of AM and EM trees, foliar N resorption is higher in the EM species (Zhang *et al.* 2018), suggesting greater N limitation. We will examine N by P interactions in our factorial addition experiment; no studies have yet evaluated the effects of changing N and P availability on the outcome of competition among AM and EM trees in temperate forests. In our early results, white birch responded significantly to P (Goswami *et al.* 2018), consistent with EM species being more competitive where N is low. The particular fungal taxa involved in the AM and EM symbioses play a key role in mycorrhizal function, and this topic is the subject of a separate proposal for work in our study system (in preparation by T. Horton, SUNY-ESF). Other collaborators are evaluating interactions with nutrient additions and whether mineral weathering has been enhanced (J. Blum, University of Michigan).

The overall objective of this proposed research is to test and elaborate the theory of temperate forest co-limitation and to evaluate the importance of various mechanisms that may contribute to maintaining co-limitation. We propose to continue the factorial NxP manipulation experiment in 13 northern hardwood forest stands distributed across three sites differing in native fertility. We will test the hypothesis that co-limitation of aboveground production will become apparent after 10 years of the treatments, as predicted by the MEL model for some stand ages (Fig. 2). An alternative hypothesis is that the P limitation we have observed in older stands will persist, as might be expected as a consequence of decades of anthropogenic N enrichment and the limits to P weathering rates, a condition termed "transactional P limitation" by Vitousek *et al.* (2010). In addition to determining whether these temperate forest ecosystems are limited by N, P, or N+P, we propose to measure the importance of a suite of mechanisms that could contribute to the maintenance of balanced N and P nutrition (Fig. 3), including foliar N and P resorption, differential rooting depth, soil enzyme activities, changes in tree species dominance, and mycorrhizal abundance and colonization. This work takes on particular importance in the rapidly changing northern forest environment; decreased loading of atmospheric pollutants, especially acid deposition and N (Bernal *et al.* 2012), are interacting with changing climate to alter soil nutrient availability with highly uncertain consequences for forest production and health.

Approach

We propose to continue plot-scale fertilization in 13 forest stands distributed across three sites in the White Mountain National Forest of New Hampshire. At the Bartlett Experimental Forest (BEF), which is underlain by granite, we have three young, three mid-aged, and three mature stands. At both Hubbard Brook Experimental Forest (HB), on granodiorite, and Jeffers Brook (JB) on amphibolite (metamorphosed basalt), we have one mature and one mid-aged forest stand. Differing mineralogy of the glacial till at the three sites leads to a range in soil nutrient availability (Vadeboncoeur *et al.* 2014). These sites were chosen to represent a wide range of environmental conditions representative of the diversity of northern hardwood ecosystems across northern New England. Thus the responses to treatment could vary

significantly across our sites (Kang *et al.* 2016), and our detailed characterization of soil fertility and tree nutrition prior to treatment will allow us to evaluate relationships between site quality and treatment responses. This is a strength of our study, as the generality of results from this experiment will be greater than from a typical single-site nutrient addition experiment.

Each stand has four plots treated with N (30 kg N/ha/yr as NH_4NO_3), P (10 kg P/ha/yr as NaH_2PO_4), both N and P, and control, added annually since spring 2011. These relatively modest rates are designed to alter site fertility while minimizing artifacts associated with high doses of fertilizer. These treatments allow us to test for NP co-limitation and to differentially challenge balanced forest nutrition and thereby induce mechanisms that maintain co-limitation (Fig. 4).

Objective I. Test for limitation or co-limitation of forest productivity

Hypothesis 1: **Aboveground productivity is co-limited by N and P.**

Prediction: Tree diameter growth and aboveground productivity will be greatest in response to N+P, consistent with theories of co-limitation.
Prediction: Growth responses to either N alone or P alone will exceed those in controls because of the tradeoffs addressed under Objective II.
Alternative Prediction: If only one element were limiting, this would be exhibited by the response to addition of the other element being no different from the control.

Hypothesis 2: **Belowground productivity is least under N+P addition.**

We will measure three indicators of belowground productivity, because it cannot be measured directly: belowground carbon allocation, fine root biomass, and fine root growth.

Prediction: Belowground productivity will be least in response to N+P, consistent with co-limitation to aboveground growth. Alleviating nutrient limitation should reduce allocation of effort belowground.
Prediction: Belowground responses to additions of either N or P alone will be similar, if N and P are co-limiting, and less than in the controls.
Alternative Prediction: If only one element is limiting, then addition of that element will reduce belowground productivity, while addition of the other element will have no effect.

Objective II. Explore mechanisms that restore balance between N and P limitation

Hypothesis 3: **Plant and microbial responses to nutrient limitation act to restore co-limitation.**

Prediction: Foliar N resorption will be greatest under P addition, and foliar P resorption will be greatest under N addition.
Prediction: Where N is limiting (P fertilized plots), root exploration and mycorrhizal colonization will be greater in the surface horizons to access N from organic matter. Where P is limiting, root exploration and mycorrhizal colonization will be greater at depth to access slowly-available P and P weathered from soil minerals.
Prediction: Phosphatase enzyme activity will decline in response to P addition and increase in response to N addition, and the activity of enzymes involved in N acquisition (leucine amino peptidase and N-acetyl glucosaminidase) will decline in response to N addition and increase in response to P addition.

Hypothesis 4: **Changing species dominance will result in species with nutrient requirements and acquisition mechanisms that are more closely matched to N and P supply rates, thus driving the ecosystem toward co-limitation.**

Prediction: Belowground, AM species will gain dominance when N is added and EM will gain when P is added. Belowground dominance will be indicated by the species composition of fine roots and by mycorrhizal colonization rates.

Prediction: Aboveground, EM species will gain canopy dominance when P is added, whereas AM will gain dominance when N is added. This change will be most pronounced in young stands where tree mortality is high during the self-thinning phase of stand development.

Prediction: In mature stands where tree replacement is very slow, differences in performance by EM vs AM tree species will be reflected in greater stem growth and reproductive output, as opposed to canopy and belowground dominance.

Alternative prediction: Other tree traits, such as maximum shoot growth rate or plasticity in fine root:shoot ratio, play a more important role than mycorrhizal status in determining the outcome of competition among species.

Objective III. Evaluate improved understanding of co-limitation via the MEL model and predict long-term forest response to anticipated future conditions

The MEL model will serve as a means to synthesize results of the project and link those results to the large volume of data already available from northern hardwood forest ecosystems. The underlying question to be addressed in this synthesis is: Do all the data currently available fit together in a self-consistent representation of the linked C, N, P, and water dynamics of northern hardwood forests? If not, why not, and how does the model have to be changed to accommodate the discrepancies?

The model will also serve as a means to examine the whole-ecosystem consequences of our experimental results. For example, we expect the results to show differences among our sites and in response to the factorial N and P additions in aboveground and belowground tree biomass and productivity, degree and type of mycorrhizal colonization, and exo-enzyme activity. These changes can be studied in the model by changing the parameterization of the affected processes. In the model, these parameter changes will result in changes in process rates, which will then have effects that propagate all through the system. Do such changes result in ecosystem-level feedbacks that dampen or amplify the direct effects? If the parameter changes can be associated with other dynamics in the model (e.g., increased light use efficiency as the canopy closes), then the parameters can be replaced by equations describing the association, thereby allowing a more dynamic examination of the long-term consequences of our findings. An improved MEL model will allow us to explore the importance of resource limitation to future forest responses to continued anthropogenic N deposition, increasing CO_2, and changing precipitation and temperature scenarios.

Methods

Study Sites

We will test our hypotheses by making measurements in a previously established plot-scale fertilization experiment in the White Mountains of New Hampshire. The experiment involves three sites separated by ~50 km (Fig. 6). The sites are underlain by Spodosols with a C-horizon of glacial origin; the complex geology of the White Mountains results in varied soil parent materials. Bartlett Experimental Forest is on granitic bedrock (Mt. Osceola and Conway Granites), Hubbard Brook is on schist (Rangeley Formation), and Jeffers Brook is on amphibolite, metamorphosed from basalt (Ammonoosuc Volcanics). Soil fertility is thus generally lowest at Bartlett and highest at Jeffers Brook.
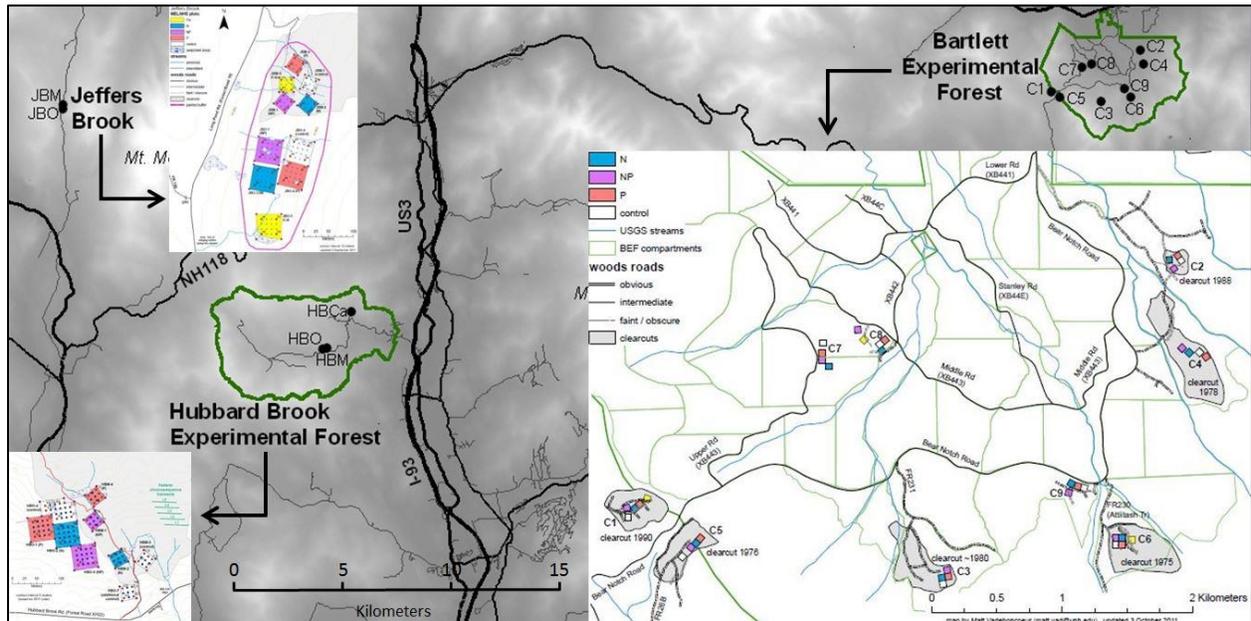

Figure 6. We have N, P, N+P, and control treatment plots at each of 13 stands in the White Mountain National Forest in New Hampshire: 2 at Jeffers Brook, 2 at Hubbard Brook Experimental Forest, and 9 at Bartlett Experimental Forest. Pay no attention to the Ca treatment plots at 7 of the stands, which do not figure in this proposal.

The climate is humid continental with an average precipitation of 1400 mm, normally with continuous snow cover from December to April. Average monthly temperature ranges from -9 to 18 C. Soil moisture differs across the three sites, as reflected in the gravimetric soil moisture content of samples collected for soil incubations and our seasonal measurements of soil water potential associated with soil respiration. There is also significant interannual variation, but no significant treatment effect (Fig. 7). We will continue to monitor soil moisture for use as a covariate in generalized linear mixed-effects models of our treatment responses.
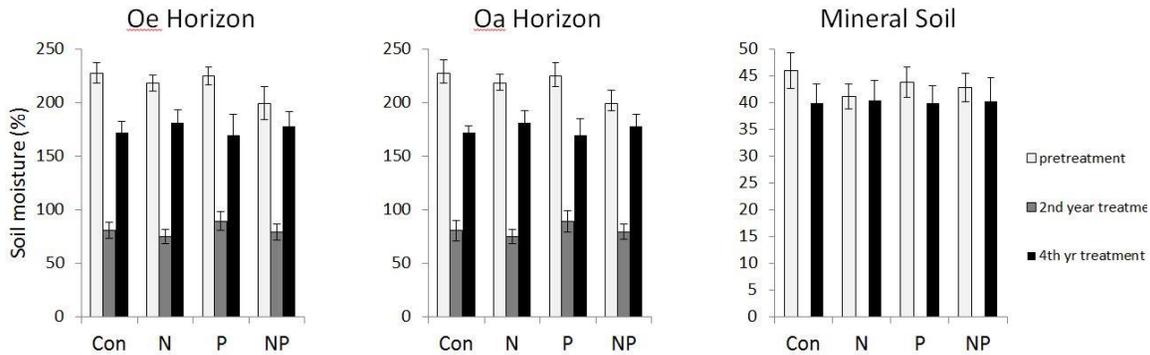
Figure 7. Gravimetric soil moisture content in 2010, 2012, and 2014. Years differ significantly in soil moisture but treatments do not.

The forests at all three sites were naturally regenerated following logging for even-aged management, which is the dominant silvicultural system in this forest type. Stands range in age from 28 to >130 years (Table 2). The forest composition is typical of northern hardwoods, with an overstory dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), and white ash (*Fraxinus americana*) in mature forest stands. Pin cherry (*Prunus pensylvanica*), white birch (*Betula papyrifera*), and red maple (*Acer rubrum*) dominate our young stands (Table 2).

Table 2. Selected characteristics of the 13 stands. Those designated with C are at Bartlett, JB are at Jeffers Brook, and HB are at Hubbard Brook. BE – beech; YB – yellow birch; WB – white birch; SM – sugar maple; RM – red maple; PC – pin cherry.

Stand	Year Cut	Elevation (m)	Aspect	Slope (%)	EM Tree Species			AM Tree Species		
					BE	YB	WB	SM	RM	PC
C1	1990	570	SE	5-20	x	x	x			x
C2	1988	340	NE	15-30	x	x	x		x	x
C3	1980	590	NNE	8-20	x	x	x	x	x	x
C4	1978	410	NE	20-25	x	x	x		x	x
C5	1976	550	NW	20-30	x	x	x		x	x
C6	1975	460	NNW	13-20	x	x	x	x	x	x
C7	1890	440	ENE	5-10	x		x	x	x	
C8	1883	330	NE	5-35	x	x		x		
C9	1890	440	NE	10-35	x	x		x		
JBM	1985	730	WNW	25-35	x	x	x	x		x
JBO	1900	730	WNW	30-40	x	x		x		
HBM	1971	500	S	10-25	x	x	x	x	x	x
HBO	1910	500	S	25-35	x	x		x		

Nutrient Manipulations

Each stand has four plots treated with N (30 kg N/ha/yr as NH_4NO_3), P (10 kg P/ha/yr as NaH_2PO_4), or both, and a control. The form of nutrient addition is important: our P source does not contain Ca, unlike most P fertilizers. Annual additions of N and P began in spring 2011 and will be continued throughout this proposed research. All plots are 50 x 50 m, except that in the mid-aged stands at Jeffers Brook and Hubbard Brook, 30 x 30 m plots were the largest that fit

within the even-aged management unit. In all plots, we avoid the edges of the treated area and conduct our measurements on trees at least 5 or 10 m from plot boundaries.

Methods for Objective I: Test for co-limitation of aboveground and belowground productivity

Hypothesis 1: Aboveground productivity is co-limited by N and P

Tree growth. We measured trees by species and size class (>10 cm, 2-10 cm, and < 2 cm DBH) in the 30x30 m measurement area within each study plot prior to initiating treatments (2008-2011) and early post-treatment, in 2015. We will repeat these measurements using the same methods in 2020, the 10th year of treatment, to test for growth response to treatments. Tree biomass (by component part) will be estimated for all trees in the measurement area of each plot using the allometric equations developed and validated in the White Mountains for northern hardwood species in early- and late-successional forests (Whittaker *et al.* 1974, Siccama *et al.* 1994, Fahey *et al.* 1998, Arthur *et al.* 2001), supplemented by allometric equations developed for the young stands in our study (Fatemi 2007). These allow us to estimate the biomass of perennial tissues (woody biomass production).

Aboveground net primary productivity will be calculated by adding woody biomass production and litterfall production, which we will continue to monitor continuously in each stand. Litterfall collectors are deployed in each plot and collected at least three times per year. We will sort litter by species in selected years to determine changes in species dominance of the canopy (Fahey *et al.* 1998), and fruits and seeds will be sorted to help address Hypothesis 4. Seed production is the topic of a PhD dissertation, for which Adam Wild collected seed in 2018 following a mast year for beech and sugar maple.

Hypothesis 2: Belowground productivity is least under N+P limitation

Belowground production. Direct measurement of belowground production is problematic and subject to errors that are difficult to evaluate (Tierney and Fahey 2007). We use an integrated set of measurements to compare effort allocated to belowground resource acquisition across treatments, and we will repeat them in 2019-2020. As described below, we will measure fine root biomass, fine root growth, and belowground carbon allocation, estimated based on soil respiration.

Fine root biomass will be measured by soil coring at about the time of peak biomass (Fahey and Hughes 1994) in late summer 2019. Ten soil cores will be extracted from each plot to 30 cm depth using a PVC corer. Roots will be hand-sorted from cores from the 0-10 and 10-30 cm depth increments and classified by diameter (<1.0 mm, 1-2 mm, 2-5 mm). Dead roots will be separated from live roots based on morphological criteria (Bledsoe *et al.* 1999).

Root ingrowth cores (Vogt and Persson 1991, Fahey and Hughes 2004) will be used to quantify root growth responses to treatments. In fall 2019, 10 replicate soil cores will be extracted from each plot and replaced with uniform mixtures of root-free soil collected from the treated buffer zone within each plot. Ingrowth cores will be collected after one growing season following methods used successfully in these study sites (Naples and Fisk 2010, Shan *et al.* in prep). Fine root growth (< 1 mm diameter) will be quantified by measuring the biomass and length of roots colonizing cores. Although the ingrowth core technique may not give unbiased quantitative estimates of fine root production (Tierney and Fahey 2007), it will provide a reliable assessment of relative root growth responses to the treatments.

Soil respiration will be estimated in each plot as CO_2 efflux in 2019 and 2020. Seven permanent soil collars in each plot have been monitored since 2009, using a LI-COR 8100.

Standard precautions to minimize bias will be taken (Davidson *et al.* 2002, Fahey *et al.* 2005a). Measurements will be taken every three weeks throughout the snow-free period (April-November). Soil temperature and moisture will be measured adjacent to the collars at each sampling. To estimate annual soil respiration we will rely on year-round measurements from the HBEF to impute fluxes in winter (Fahey *et al.* 2005a).

Belowground carbon allocation is calculated as soil respiration minus aboveground litter production (Nadelhoffer and Raich 1992). We recognize that this approach ignores changes in belowground carbon storage and these estimates must be used with that caution. Differences associated with treatment could reflect differences in belowground carbon flux through trees or differences in the rate of change in soil carbon storage, either of which would be of interest.

Increased effort belowground in response to nutrient limitation will be inferred by increased root biomass, ingrowth, and belowground carbon allocation.

Methods for Objective II: Mechanisms that restore balance between N and P limitation

Hypothesis 3. Plant and microbial responses to nutrient limitation act to restore co-limitation

Foliar resorption is indicated by the nutrient concentration in senesced leaves (resorption proficiency) and by the proportion of nutrients resorbed from green leaves (resorption proficiency) (Killingbeck 1986). In 2019, we will collect green leaves from the dominant species in each stand, from the same trees sampled pre-treatment and in 2015. We sample leaves from two sides of each tree using a shotgun. Samples are weighed fresh as well as after oven drying, as leaf dry matter content indicates leaf toughness (Pérez-Harguindeguy *et al.* 2013), which has been reduced by N addition in our stands. For both green and senesced leaves, leaf area will be quantified using ImageJ, which allows calculation of specific leaf area. Resorption can thus be reported on a leaf area basis, not just a concentration basis (van Heerwaarden *et al.* 2003). Increased P resorption in response to N limitation and increased N resorption in response to P limitation would provide evidence of a mechanism restoring colimitation.

Activities of the soil enzymes beta-glucosidase (C-acquiring), acid-phosphatase (P-acquiring), and leucine amino-peptidase and N-acetyl-glucosaminidase (N-acquiring) will be quantified in soils collected from organic and mineral horizons in all stands in the first year of the study. Ratios of these enzymes and vector analyses of their relative proportions will be used to indicate relative demands for C, N and P by soil organisms (Sinsabaugh *et al.* 2008, Moorhead *et al.* 2016), to test whether resource allocation to enzyme production responds to fertilization in directions that balance nutrient availability.

Nutrient availability and microbial pools. We will quantify resin-available N and P, net N mineralization, microbial respiration, and microbial C, N, and P pools as indicators of nutrient availability and to identify feedbacks to nutrient recycling processes that modify nutrient availability. Resin-available N and P responded to treatments in the first growing season (Fisk *et al.* 2014) and each nutrient continues to be elevated in plots where it is added. Suppression of resin-available N by P fertilization was detectable by the 5th year of treatment and continued measurements will reveal whether this response is sustained and whether a similar effect develops for the effect of N addition on resin-available P.

Hypothesis 4: Changing species dominance over longer time periods contributes to restoring co-limitation when N and P supply are not balanced.

Canopy structure and leaf area distribution by species will be quantified using 3D Terrestrial LiDAR scanning on a 10-m grid to create a high-density point cloud (~6 mm point

spacing) of vegetation surfaces in each plot. A map of vegetation area in 1 m^3 voxels throughout the canopy will be created, after accounting for occlusion and variation in point density with distance from the scanner (Maynard *et al.* 2013, Beland *et al.* 2014). Leaf-on and leaf-off scans will be differenced to create a map of leaf area only. Crown width will be determined for each stem and the lowest and highest leaf return points within this width around each mapped tree stem will be used to estimate canopy top and bottom height and create an estimated crown volume. Voxels will be assigned to tree crowns based on these crown volume cylinders. We will then estimate total leaf area for each tree species in 1-m vertical bands through the canopy by summing leaf area of all voxels assigned to each species in each height band. Leaf area dominance for each species in each plot will then be calculated (Fahey *et al.* 1998).

       Distribution of roots by species as a function of soil depth will be measured in the second year of the study using molecular genetic methods (Yanai *et al.* 2008, Fisk *et al.* 2010, Kennedy *et al.* 2012, Dulmer *et al.* 2014). Composite root samples will be collected from the organic and mineral soil horizons in each plot. DNA will be extracted from subsamples, the *trnL* intron will be PCR-amplified (Brunner *et al.* 2001), and the relative importance by species will be estimated using fragment size analysis, which provides clear size separation of our dominant species (Fisk *et al.* 2010, KJ Minick and S Shan, unpublished data). Together with leaf litterfall, these measurements will allow us to calculate fine root:leaf biomass ratio by species in each plot, which is a key variable in the MEL model.

       Colonization by EM and AM fungi will be quantified for separate subsamples using standard visual methods (Brundrett *et al.* 1994) on roots of the dominant tree species (Table 2). In addition, the composition of the ectomycorrhizal fungal community will be characterized using molecular genetic techniques by T. Horton, SUNY-ESF, under separate funding. Preliminary results from hyphal ingrowth bags show that N addition reduces the richness of EM fungal taxa, and an ascomycete genus (Genea) responded to P addition.

       Treatment effects on canopy dominance, fine root abundance by species, and EM vs AM colonization rates will be used to evaluate the hypothesis that species dominance is changing in response to nutrient availability. Quite likely, our results will support a more nuanced understanding of the C, N, and P limitations of plants and mycorrhizal fungi than the simplistic generalization that AM species are favored under P limitation and EM species under N limitation.

       The variety of mechanisms contributing to co-limitation in the first 10 years of treatment will be compared across the suite of stands to evaluate their influence on limitation responses. These results will allow us to better evaluate and improve the MEL model for more general application in terrestrial ecosystems and will contribute to our theoretical understanding of nutrient limitation.

Methods for Objective III: Predict long-term forest response to anticipated future conditions using a revised and improved MEL model

The MEL model uses a mass-balance approach to couple ecosystem C, N, P, and water fluxes (Fig. 8: Rastetter *et al.* 2013, Pearce *et al.* 2015) and operates at a plot scale with daily output using a variable time-step, 4th/5th order Runge-Kutta integrator to solve a system of first order differential equations (Press *et al.* 1986). It partitions vegetation allometrically into active (leaves plus fine roots) vs. woody biomass. The active biomass is partitioned between leaves and fine roots based on the relative limitation by canopy (CO_2 and light) vs. soil resources (nutrients and water). The model partitions detritus into Phase I and Phase II soil organic matter (SOM; Melillo *et al.* 1989) and debris (coarse woody debris and standing dead plant material). The debris is gradually converted to Phase I material where it begins to decompose. Phase I SOM represents the young, more active, organic matter and implicitly includes microbial biomass. Phase I SOM both mineralizes and immobilizes nutrients, and turns over relatively quickly. Phase II SOM does not immobilize nutrients, but continues to mineralize nutrients and release C at a slow rate.
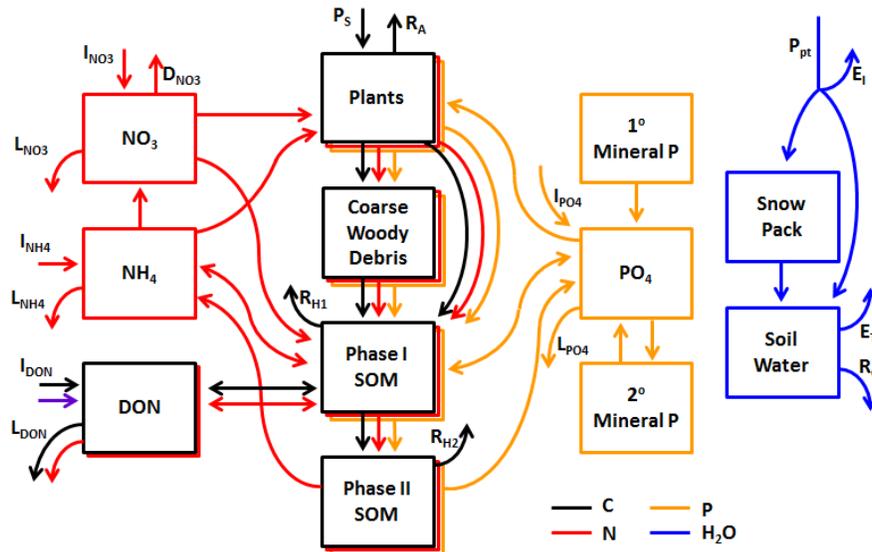

Figure 8: The Multiple Element Limitation (MEL) model. C, N, P, and water stocks and fluxes are distinguished respectively by black, red, orange, and blue lines. P_S - photosynthesis; R_A - autotrophic respiration; R_{H1}, & R_{H2} - heterotrophic respiration from Phase I and Phase II SOM; I_i - inputs of i; L_i - leaching loss of i; D_{NO3} - denitrification; DON - dissolved organic N; P_{pt} - precipitation; E_I - evaporation of intercepted water; E_T - evapotranspiration; R_O - runoff and deep percolation.

The heart of the MEL model is a dynamic algorithm that allocates plant assets (such as biomass, enzymes, carbohydrates, etc.) toward the uptake of resources from the environment (light, CO_2, H_2O, NH_4, NO_3, DON, N-fixation, and PO_4). These assets are implicitly represented as an aggregate uptake potential that is assumed to increase in proportion to the active vegetation biomass. The allocation algorithm calculates changes in the fraction of these uptake assets (effort) allocated toward acquiring each resource based on the resource optimization hypothesis (Bloom *et al.* 1985, Chapin *et al.* 1987):

$$\frac{dV_i}{dt} = a \, \ln\left(\Omega_i \, \Phi\right) V_i \; ; \qquad \Phi = \prod_{j=1}^{8}\left(\Omega_j\right)^{V_j}$$

where V_i is the effort allocated toward the acquisition of resource i, t is time, a is an acclimation rate parameter, Ω_i increases monotonically with the current ratio of requirement to acquisition for resource i, and Φ is calculated so that the dV_i/dt sum to zero (and hence the sum of the V_i remains one). This algorithm will drive the model toward a state where the ratio of acquisition to requirement is the same for all resources and in that sense toward a state where all resources are equally limiting (Bloom *et al.* 1985, Chapin *et al.* 1987). We have already calibrated and used the model to assess the synchronization of N and P cycles in northern hardwood forests (Fig.1: Rastetter *et al.* 2013).

Recent applications of MEL include assessing the synchronization of N and P cycles in hardwood forest through secondary succession (Rastetter *et al.* 2013), the recovery of tundra from thermokarst (Pearce *et al.* 2015) and from fire (Jiang *et al.* 2015), and a comparison of recovery from disturbance among HJ Andrews Forest, Hubbard Brook Forest, and tundra near Toolik Lake (Kranabetter *et al.* 2016).

We will use the improved model to explore the importance of ecosystem acclimation to changing resource limitation under various scenarios of N deposition, elevated CO_2, and climate change. Understanding the limits to resource optimization, as tested in our field experiments, will direct the future development of ecosystem theory as well as improve management of forest ecosystems under changing environmental limitations.

Project Management and Timeline

Table 3. Schedule of activities for the preceding 7 years and the proposed 3 years of the project. Pretreatment soil pools were characterized in 2004 and 2010. Fertilization began in 2011.
X – measurements in all stands; x – measurements in selected stands.

Task	Prior Activities									Proposed Activities		
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Fertilization, site maintenance	X	X	X	X	X	X	X	X	X	X	X	X
Tree inventory	X	X			x	X					X	
Canopy dominance (LiDAR, AM vs EM)											X	
Litterfall mass	X	X	X	X	X	X	X	X	X	X	X	X
Litterfall mass by species (AM vs EM)	X				x	x				X		
Litter nutrient concentrations	X		X		x	x	X			X		
Foliar nutrients by species	X				x	x	x			X		
Soil respiration	X	X	X	X	X	x	X	X	x	X	X	
Root biomass cores	X					x	x			X		
Root ingrowth cores											X	
Root species composition (AM vs EM)							x				X	
Resin-available N and P		X	X		X	X		X		X		X
N mineralization	X		X		X			X		X		X
soil enzyme activity											X	
MEL Modeling	X	X									X	X

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