

Nutrient Co-limitation in Young and Mature Northern Hardwood Forests

Intellectual Merit - Theory suggests that ecosystem productivity should be co-limited by multiple nutrients, although temperate forests have long been thought to be primarily nitrogen limited. Experimental tests of nitrogen vs. phosphorus limitation in temperate forest systems are lacking. Our proposed research combines modeling and field studies to explore processes mediating nutrient colimitation in relation to successional change in managed hardwood forest systems. The Multi-Element Limitation (MEL) model is unique in representing co-limitation from the perspective of resource optimization theory and whole-ecosystem biogeochemistry. We have extended the model to include P as well as N, carbon, light, and water, and applied it to simulate primary and secondary succession in northern hardwood forests. Using nutrient manipulations (N, P, N+P) in replicated stands of three ages (~20, 30, and > 100 years) at the Bartlett Experimental Forest in New Hampshire, we will test the patterns of resource limitation predicted by the model and multiple mechanisms of allocation of effort to acquire N and P. Specifically, the model predicts a greater response of aboveground productivity to N+P than N or P alone. In older stands, it predicts a greater response to N than to P addition, but in younger stands, the model suggests that the supply of N from detritus should be sufficient to create P limitation.

In the MEL model, co-limitation is maintained by variable allocation of effort to obtain multiple resources. These relationships are poorly constrained by measurements at present, and the importance of many possible mechanisms is uncertain. In our field experiments, we will test whether several types of effort correspond qualitatively to theoretical predictions, and we will obtain quantitative relationships that can be used to improve the realism of the model. Specifically, under conditions of greater P limitation, we hypothesize that plants will allocate more effort to phosphatase enzyme production, apatite weathering (including deployment of mycorrhizal roots at depth), root uptake of P, and P resorption from leaves. Under conditions of greater N limitation, we hypothesize that plants allocate more to root uptake of N and to N resorption from leaves, and that plant allocation indirectly enhances mineralization of soil organic matter and nitrogen.

Our field results will improve understanding of N and P acquisition and limitation in temperate forests. Our results will also improve the MEL model, both in the representation of processes already included (biomass allocation to fine roots and mycorrhize, nutrient uptake capacity of roots, and feedback from P limitation to primary mineral weathering) and in the evaluation of processes not currently represented (feedback from N or P limitation to organic matter mineralization, different proportions of arbuscular vs ectomycorrhizal associations, and differential rooting depth to access primary minerals vs. organically cycling nutrients). A better understanding of the capacity of ecosystems to balance the acquisition of limiting resources will enable us to better direct our efforts to protect ecosystem function in future scenarios of continued elevated N deposition, increasing CO₂ availability, and changing climates.

Broader Impacts - As part of the proposed research, we will use extensive and ongoing relationships with forest managers (White Mountain National Forest), the general public (Hubbard Brook Science Links Program), and K-12 educators (Environmental Literacy Program and Forests For Every Classroom) to take the results of the proposed research to a much larger audience. Those involved in this project have a demonstrated track record of working with all of these groups and will enhance these connections as part of this project. All the principal investigators are committed to the open dissemination of data and research results and, to this end, are actively involved in a variety of information distribution systems, web sites and data synthesis workshop activities. This project will train four graduate students and involve a large number of undergraduates in the research process. We intend to maintain the nutrient manipulations as a long-term resource for future study of nutrient co-limitation during secondary succession in an important forest type.

Nutrient Co-limitation

Not long ago, productivity in temperate forests was generally believed to be nitrogen limited, whereas productivity in tropical forests was thought to be phosphorus limited. However, concepts of resource optimization (Mooney 1972, Thornley 1972, Mooney and Gulman 1979, Shuter 1979) suggest that ecosystems are normally co-limited, not only for N and P, but for other resources as well (such as water, light, and carbon). Optimization theory proposes that plants continually reallocate assets (energy, carbon, enzymes) toward the resources that are most limiting. Optimizing acquisition of multiple resources should tend toward a condition where all resources are equally limiting (Bloom et al. 1985, Chapin et al. 1987). Over a century of forest succession, this optimization tends to synchronize ecosystem resource cycles, resulting in resource supply rates that are in proportion to plant requirements (Field et al. 1995).

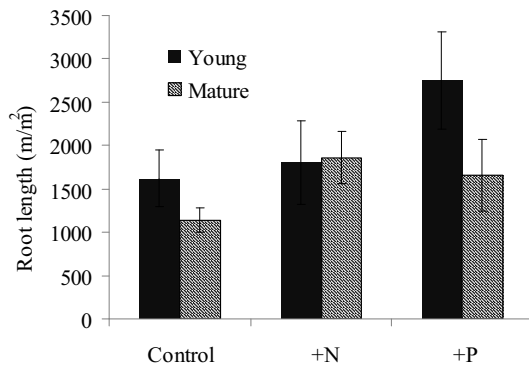


Figure 1. Root foraging in ingrowth cores (10 cm depth) amended with N and P incubated for one growing season in our research sites.

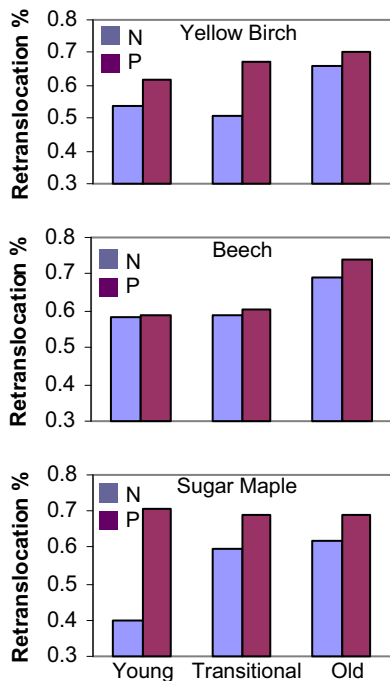


Figure 2. Foliar retranslocation of P exceeds that of N for yellow birch and sugar maple, especially in young stands (unpublished data).

Simulation models have been employed to explore the mechanisms whereby nutritional balance is maintained in changing environments. These models initially focused on the balance between foliar C fixation and root uptake of N (Rastetter et al. 1997, Herbert et al. 1999, Rastetter et al. 2001), but the latest version, described below, recognizes trade-offs among mechanisms of accessing N and P, as well as other resources. The capacity of plants and ecosystems to alter the availability and acquisition of multiple resources will be critical to maintaining ecosystem functions in a rapidly changing world.

A recent meta-analysis reported that both terrestrial and aquatic systems are most often co-limited by N and P, defined by response to experimental enrichments of N, P, and NP (Elser et al. 2007), consistent with expectations arising from optimization theory. However, of the 72 studies representing terrestrial ecosystems, only four occurred in temperate forest ecosystems: a pineland plantation in New Jersey (Gleeson and Good 2003), a southern maple-oak forest (Corbin et al. 2003), a southern pine-oak forest (Stevens and Jones 2006), and a northern conifer-hardwood forest (Tripler et al. 2002). None of these studies evaluated overstory response to the full NxP factorial design necessary to assess co-limitation. Nevertheless, evidence from other studies complements that presented by Elser et al. (2007) to argue against simple limitation by N. For example, NP co-limitation was suggested in eastern hardwood forests (Lea et al. 1980; Auchmoody 1982), and P limitation has been suggested in sugar maple forests (Paré et al. 1993; Gradowski and Thomas 2006, 2008). Preliminary results from the Bartlett Experimental Forest on fine root proliferation in nutrient-enriched patches suggest a shift from P limitation in young forests to N limitation in mature forests (Fig. 1). At the same sites, we found

greater P than N retranslocation from senescing foliage, especially in young and mid-transitional stands (Fig. 2). Phosphorus limitation in young forests is further supported by our observations of a substantial reduction in foliar N:P ratios in young stands following long-term (5 yrs) balanced-nutrient fertilization (macro- plus micronutrients, Fahey et al. 1998). We have measured greater net N mineralization and phosphatase activity in soils in young than old stands at our research sites (Fig. 3). That young forests are especially P limited is consistent with the suggestion of enhanced apatite weathering in young forests (Yanai et al 2005) suggested by an early-successional peak in calcium availability (Hamburg et al. 2003). At the Hubbard Brook Experimental Forest, 55 km from our sites at Bartlett, N:P ratios in foliage increased from 1966 to 1992-2000, suggesting recent increases in P limitation, perhaps associated with continuing atmospheric N deposition (Fig. 4).

Clearly, more evidence is needed to test whether temperate forests, which are key global carbon sinks, fit the global patterns of N and P co-limitation established by Elser et al. (2007). Moreover, understanding the processes mediating co-limitation requires that we examine, together in a single integrating framework, mechanisms that potentially contribute to resource optimization. Forest harvest removes nutrients from recycling pools, disturbing previously formed balances between plant demand and recycling supply. These biogeochemical consequences of ecosystem disturbance and recovery, in combination with anthropogenic N deposition, may be expected to shift the relative strength of limitation by P and N over secondary succession. Using a new version of the Multi-Element Limitation model (described below), we predict that young temperate forests are already more limited by P than by N. We will assess the relative limitation by N and P by measuring productivity following N, P, and NP additions, and we will examine mechanisms of ecosystem allocation of effort to acquire P vs. N to improve understanding of these patterns of limitation over secondary succession.

A key feature of ecosystem optimization of resource acquisition under co-limitation is differential species response to changing resource availability. Most temperate forests are composed of complex tree species mixtures, and interspecific interactions can be expected to contribute to overall ecosystem acclimation to nutrient limitation. For example, the relative dominance of early- vs. late-successional species in younger forests and of ectomycorrhizal vs. arbuscular mycorrhizae in stands of any age could respond to changes in availability of N vs P, but little basis currently exists for predicting these responses and their implication for overall ecosystem resource optimization. Our experimental design, encompassing multiple species and three stand ages, is ideally suited to contribute insights into the implications of these key features of nutrient co-limitation. Testing and improving the theory and relationships represented in the MEL model will allow us to better evaluate

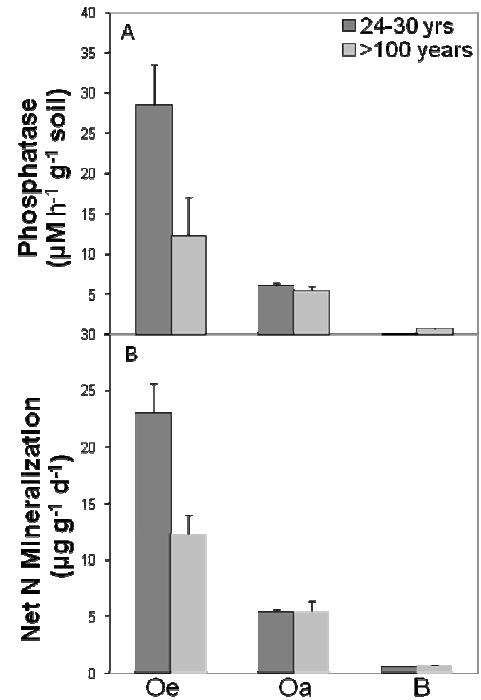


Figure 3. Phosphatase (a) and net N mineralization in a 21-day lab incubation (b) in soils from 3 young and 3 old stands.

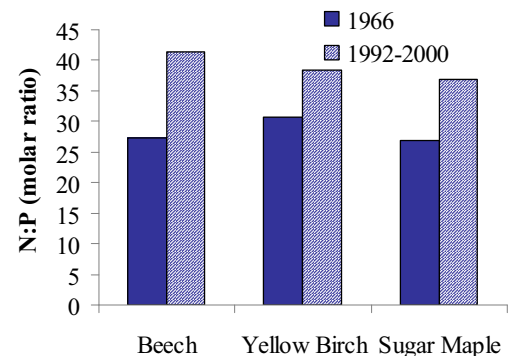


Figure 4. Foliar N:P ratios of dominant tree species at Hubbard Brook. Data from Likens and Bormann 1970 and Fahey, unpublished.

the future response of forests to continuing changes in N deposition, temperature, precipitation, and CO₂.

The Multiple Element Limitation Model, MEL

The MEL model (Rastetter and Shaver 1992, 1995; Rastetter et al. 1997, 2001, 2005) was designed explicitly to examine resource optimization and multiple resource limitation, which is the focus of the field experiments proposed here. We therefore used this model to develop testable hypotheses concerning the relative limitation of N and P in young and mature northern hardwoods (described below). The field experiments will allow us to test the ideas encoded in the model, as well as some not yet included, and thereby help improve the model structure and constrain its parameterization. We will then use the model to explore the limits of our understanding of the responses of northern hardwood forest to changes in N deposition, climate and elevated CO₂.

The heart of the MEL model is the algorithm for allocating assets toward the uptake of various resources. We lump those assets into a single abstract variable we call "effort" and assume that the total effort increases in proportion to the active biomass (leaves plus fine roots). Uptake of a resource (U_i) is then calculated as a rate constant (g_i) times the active biomass (B_A) times the fraction of effort allocated to the resource (V_i) times a function describing the availability of the resource in the environment (E_i) relative to the uptake kinetics of the vegetation (e.g., a Michaelis-Menten equation, Eqn 1). In the new version of the model, to allow for more than two resources, effort is then allocated among resources based on the ratio of resource requirement (R_i) to uptake (Eqn 2), where a is an acclimation rate constant and Φ is calculated to assure that $\sum V_i = 1$. The requirement (R_i) is assessed as the amount of the resource needed to replace losses in biomass turnover, growth, maintenance, and expenditures for the uptake of other resources (e.g., N spent on phosphatase enzymes, C spent on symbiotic fungi or bacteria). Time-averaged values of requirement and uptake are used in the acclimation equation to account for phenological differences in the timing of resource uptake and use.

$$U_i = g_i B_A V_i \frac{E_i}{k_i + E_i}$$

Equation 1

$$\frac{dV_i}{dt} = a \ln \left(\Phi \frac{\bar{R}_i}{\bar{U}_i} \right) V_i \text{ for } \Phi = \prod_{i=1}^n \left(\frac{\bar{U}_i}{\bar{R}_i} \right)^{V_i}$$

Equation 2

Application of the MEL model to northern hardwood forest

We recently extended the MEL model to include P and applied it to data from the Hubbard Brook Experimental Forest, where P cycling has been well described (Yanai 1992). To simulate secondary succession and describe differences between young and mature forests, we clearcut the living biomass, removed a fraction as forest products (based on the removals reported for a bole-only harvest, Yanai 1998), and converted the rest into coarse and fine litter. In the first 5 years, the canopy was open and the decomposition of residual soil organic matter exceeded demand by vegetation, and so effort was allocated toward C and water (Fig. 5). Allocation shifted toward N and P acquisition as growth demand for nutrients increased over the next 10 years. Most important to the

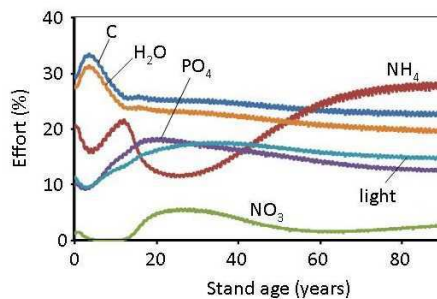


Figure 5. Effort allocated to acquisition of resources during secondary succession in northern hardwoods.

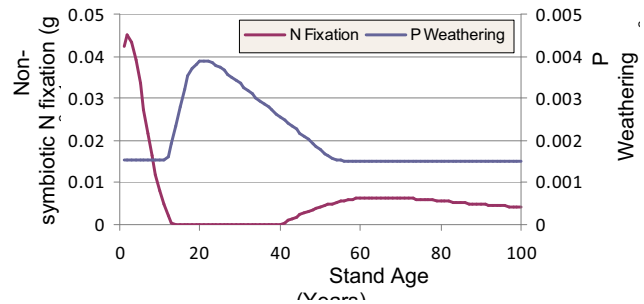


Figure 6. Rates of biologically enhanced P weathering and non-symbiotic N fixation predicted by MEL during secondary succession in northern hardwoods.

goals of our study, from 15-45 years the model predicted a period of pronounced P limitation during which effort toward P was high and the vegetation expended resources toward enhancing weathering of apatite (Fig. 6). This was followed (45-85 years) by a period in which the model predicted N limitation and effort was reallocated toward NH_4 at the expense of all other resources. Although the predicted rates of non-symbiotic N fixation (Fig. 6) are probably too low for us to measure in the field, the pattern is indicative of the tradeoff between N and P limitation in the model. Finally, in the mature forest, the internal resource cycles were synchronized and the allocation of effort stabilized (Fig. 5).

We simulated the application of N, P, or N+P (at the rate of 50 kg/ha/yr) beginning at 30 or 80 years (Fig. 7). In both cases, the model predicted a greater response to N+P than to either N or P alone, consistent with co-limitation as represented in the model. The younger forest was more P limited (responding more to P than N), while the older forest showed a greater growth response to N than to P. Coarse and fine litter pools followed the production of living biomass. Soil organic matter pools (not shown) were not very responsive to treatment in the model. Effort allocated to N vs. P acquisition (not shown) was consistent with the simulated growth responses. We propose to test the predicted growth response to N, P and N+P of replicate northern hardwood stands of different ages in our field experiments (Objective 1).

Mechanisms of N and P Acquisition

In our field experiments, we will test mechanisms of N and P acquisition to learn how they vary in relation to forest successional age and to examine their potential to contribute to development of co-limitation in northern hardwoods (Objective 2). Some of these mechanisms are already represented in the MEL model, such as belowground C allocation, biologically accelerated apatite weathering, nutrient uptake capacity, and foliar resorption. We will also address additional mechanisms and evaluate their importance for inclusion in the improved version of MEL (Objective 3), such as explicit representation of root deployment in different soil layers, the activity of phosphatase enzymes, and proportions of AM vs EM mycorrhizal fungi. We will also consider the feedbacks of N and P availability on mineralizing organic nutrients and mobilizing very slowly available forms of P, other mechanisms that may be contributing to maintaining N and P co-limitation in these forests.

Mechanisms to validate and refine as represented in MEL

The most general indicators of effort for soil nutrient acquisition are fine root production and total belowground carbon allocation (TBCA). We expect TBCA to increase in response to belowground resource limitation, but we know little about whether that effort is partitioned differentially to P or N acquisition. Thus, it will be useful to learn whether assets are allocated independently for different resources, by complementing estimates of TBCA with measures of some of the efforts that contribute to TBCA. In the MEL model, N- or P-specific mechanisms to which effort is allocated, belowground, include apatite weathering for P acquisition and fine root uptake capacity for either N or P. Foliar nutrient resorption is an aboveground mechanism for relieving N or P limitation in the model.

Apatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH},\text{F},\text{Cl})$) is a ubiquitous trace mineral that is the primary mineral source of P in virtually all soils. We have identified that apatite in the soils from our study area is an

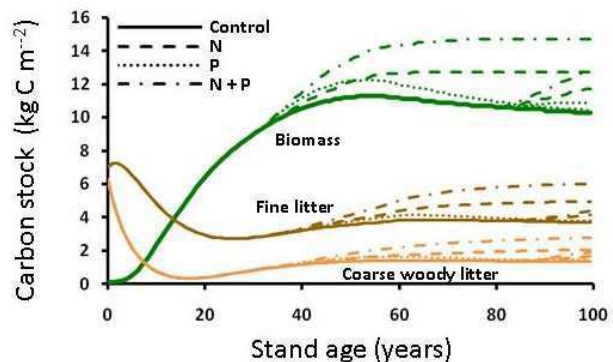


Figure 7. Simulated living and detrital C stocks following addition of N, P, or NP at rates of 50 kg/ha/yr beginning at 30 or 80 years of age.

important source of Ca to the vegetation, and pointed out that it was previously overlooked in calculations of Ca weathering (Blum et al. 2002; Nezat et al. 2008). There is growing evidence that apatite can be dissolved by bacteria and fungi when there is a high demand for P. Ectomycorrhizal hyphae in some forest systems are attracted to apatite when P availability is low, and they appear to contribute to apatite weathering (Hagerberg et al. 2003, Nilsson and Wallander 2003, Wallander et al. 2001, Rosling et al. 2004). Rare earth element (REE) patterns have been used to identify nutrient input to soils from apatite dissolution (Hagerberg et al. 2003; Hoffland et al. 2004; Harlavan et al. 2009; Hobbie and Bryce, 2008) and other minerals (Fu et al. 2001; Semhi et al. 2009). In Hubbard Brook soils, SEM images of plagioclase-feldspar mineral grains show etchings and dissolution pits (unpublished data) that are similar in morphology to patterns in other ecosystems that have been attributed to fungal weathering of feldspar to obtain apatite inclusions (Van Breemen et al. 2000). Apatite appears to be an important Ca source for ectomycorrhizal trees (Blum et al. 2002). The new version of MEL includes a biologically induced acceleration of P weathering, which will be examined in this study.

Nutrient uptake capacity of fine roots has long been used to diagnose P limitation (Harrison and Helliwell 1979); the rate of nutrient uptake per unit root, exposed to uniform conditions, indicates the "effort" allocated to nutrient transport capability. At low nutrient availability, nutrient uptake capacity is commonly higher than when roots are exposed to high nutrient availability (Lee and Rudge 1986, Siddiqi et al. 1989, Treseder and Vitousek 2001). Nitrogen deposition has been shown to increase P uptake capacity of roots (Carreiro et al. 2000). For N, we have found both nitrate and ammonium uptake capacity of Engelman spruce and subalpine fir to be higher in a low-N site than in one receiving high rates of atmospheric N loading (Yanai et al. in press).

Nutrient resorption from senescing foliage is a major nutrient-conservation mechanism in perennial plants (Chapin 1980). On a global basis, the efficiency of resorption is comparable for N and P, and nutrient resorption is only weakly controlled by nutrient availability (Aerts 1996). However, we have observed both N and P resorption to decrease significantly in response to balanced nutrient additions in young northern hardwood forests (Fahey et al. 1998); no factorial studies have been reported for this forest type. A comparison of N and P resorption in our stands at Bartlett (Fig. 2) is consistent with MEL predictions of shifting limitation with stand age. Documenting resorption in response to nutrient manipulation will help us interpret age-related patterns of nutrient limitation and also allow us to better parameterize the relationships currently relating resorption to internal plant stoichiometry in MEL.

Mechanisms to be evaluated for inclusion in the model

There are many other possible mechanisms of N and P acquisition that could be included in our concepts of resource optimization that are not yet represented in MEL due to a lack of information. Our field experiments will both determine the importance of these processes and also provide the relationships necessary to represent them in the model.

Mineralization of organic matter and nutrients are influenced by plant C supply and by feedbacks from nutrient availability. Like plant processes that can optimize resource use, these plant-soil interactions have the potential to moderate availability of any one nutrient. Added N tends to suppress decomposition of lignin-rich substrates and metabolism of low C:N material compared to more labile, low-N organic matter (Carreiro et al. 2000,

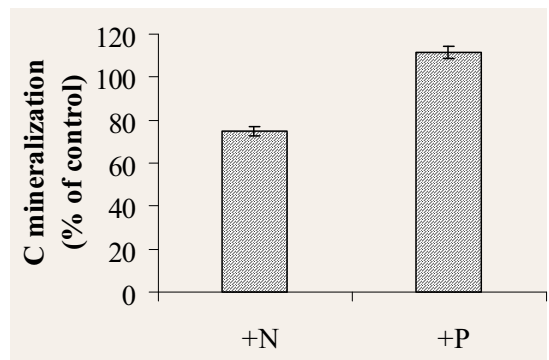


Figure 8. Carbon mineralization from Oe horizon, incubated in the laboratory with added leaf litter, expressed as a percent of controls (no N or P added). Soils originated from 9 mid-aged and mature forests in central NH.

Hagedorn et al. 2003, Sinsabaugh et al. 2005), whereas added P can enhance mineralization of organic matter (Cleveland et al. 2002, Craine et al. 2007). However, the consequences of microbial and enzymatic responses to nutrients can vary among organic matter fractions and are not predictable across different ecosystems (Grandy et al. 2008). We found that organic matter mineralization was suppressed by added N and enhanced by added P in soils from Bartlett (Fig. 8), suggesting that direct feedbacks of nutrient availability on mineralization processes contribute to patterns of nutrient limitation in this ecosystem. Plant C allocation belowground in response to nutrient demand should also indirectly influence nutrient availability through regulation of microbial activity (Högberg et al. 2001, 2008). These feedbacks can easily be added to MEL using parameters measured as part of the proposed research.

Investment in phosphatase enzymes can increase P availability, at least over the short term when labile organic P supplies are sufficient. However, the production of enzymes may be constrained by scarcity of N. The increase in phosphatase activity when N is added to both N- and P-limited forest ecosystems (Treseder and Vitousek 2001, Sinsabaugh 2002, Saiya-Cork et al. 2002) is evidence that available N enhances phosphatase production under widely varying nutrient environments. In contrast, the mycorrhizal associations that improve plant competitiveness for available P are probably constrained more by C than by N availability. A reduction of mycorrhizal associations can result from N addition to P- as well as N-limited ecosystems, and appears to be compensated for by increased phosphatase activity (Treseder and Vitousek 2001). Hence, the role of N in phosphatase production may provide a mechanism for maintaining balance in N and P supply, depending on the availability of labile organic P. This tradeoff is not currently in the model but will be added as part of the proposed research. Currently MEL has a C cost associated with increased weathering of apatite; the N cost of phosphatase could be handled in similar fashion.

Vertical pattern in the soil profile presents another potential tradeoff in allocation resulting from differential effort toward N vs P acquisition, if recycling processes in surface horizons are not sufficient to meet demands for P. Limitation by N is likely to constrain plant allocation to acquisition of mineral sources of nutrients, which are likely to be located at depth. Apatite is depleted in surface soils (A and upper B horizons) relative to lower B and C horizons in hardwood forest soils at Hubbard Brook (Blum et al. 2002, Hamburg et al. 2003, Nezat et al. 2008). Acquisition of P from sources in the lower B and C horizon would require plants to invest in roots and mycorrhizae that are vertically separated from those involved in uptake of nutrients recycled from surface organic pools, where N is concentrated. Fine roots do occur in the lower B and C horizons at our study sites, with 12% of root biomass occurring below 30 cm depth (Yanai et al. 2006). Vertical patterns of rooting are known to influence the movement of mineral nutrients to surface soil horizons and can be especially important where N is not highly limiting (Jobbágy and Jackson 2004, Kennedy et al. 2002). In MEL, surface and deep roots could be represented as accessing substitutable resources, as recently implemented for NH_4 , NO_3 , and DON.

Mycorrhizal symbiosis obviously plays a key role in plant nutrient acquisition and its role in nutrient co-limitation is largely unknown. Many temperate deciduous forests are dominated by mixtures of tree species colonized by either ectomycorrhizal (EM) or arbuscular mycorrhizal (AM) fungi. The structure and function of these two types of mycorrhizae are highly distinct. On a global basis, AM are more abundant in areas that are more P-limited, whereas EM are more abundant where N is more limiting (Smith and Read 1997). Some evidence suggests that AM contribute more to P than to N uptake (George et al. 1995). In general, mycorrhizal abundance decreases with increasing soil nutrient availability, but a meta-analysis of fertilization studies detected no difference in the responses of EM vs. AM fungi nor between N vs. P addition (Treseder 2004). A variety of alternative scenarios can be envisioned regarding the relative response of EM and AM abundance to changing N and P availability. For example, increased N is expected to increase effort for P acquisition, which could stimulate relative abundance of AM in the organic horizons where most P is acquired or of EM

in the mineral horizons where mining of P by EM is expected. Conversely, P addition can be expected to reduce AM abundance based on the well-known centrality of P to AM function (Smith and Read 1997). However, N fertilization in sugar maple reduced AM abundance (Van Diepen et al. 2007). Because both AM and EM fungal communities consist of many taxa with differing nutrient-related traits, the response of AM and EM abundance can vary in complex or nuanced ways to changes in nutrient availability (Treseder and Allen 2002, Lilleskov et al. 2002). Nevertheless, these responses are critical to understanding the problem of nutrient co-limitation and demand our attention.

Objective 1: Test for co-limitation by N and P of aboveground production in young and old northern hardwood stands

Hypothesis: Aboveground growth and productivity will respond more to NP addition than to either N or P alone.

Hypothesis: Aboveground growth and productivity will respond more to added P than N in young stands, and more to added N than P in older stands.

Alternate hypothesis: N has long been assumed to be limiting in temperate forests and we could find this to be the case.

Alternate hypothesis: Both young and old forests could now be more limited by P than N, even if they were not so prior to decades of anthropogenic N deposition.

In addition to measuring forest production, which we plan to continue over the long term, we will use vector analysis of foliar response as a more rapid indicator of ecosystem response to nutrient addition treatments. We will assess soil conditions to quantify N and P availability. Differences in allocation of effort, tested under Objective 2, will also provide evidence of differences in nutrient limitation. By studying stands of different ages we can take advantage of the disruption of resource supply and demand post-disturbance (Field 1995), such as the P limitation induced in young stands when N mineralization is high, to learn how optimization processes develop over time in this ecosystem. The young stand ages chosen for our experimental work span the interval during which the model indicates greater P than N limitation in northern hardwoods (Fig 5).

Objective 2: Evaluate mechanisms for maintaining co-limitation of N and P

Hypothesis: Total belowground C allocation will be reduced by addition of limiting nutrients (with limitation defined by aboveground production response, measured under Objective 1).

Hypothesis: Total mycorrhizal colonization, similarly, will be lowest where nutrients are least limiting.

Hypothesis: Under conditions of greater P limitation, plants will allocate more effort to phosphatase enzyme production, apatite weathering (including deployment of ectomycorrhizal roots at depth), root uptake of P, and P resorption from leaves, compared to conditions of less P limitation.

Hypothesis: Under conditions of greater N limitation, plants will allocate more effort to surface roots and mycorrhizae, root uptake of N, and N resorption from leaves, and mineralization of organic N will be higher compared to conditions of less N limitation.

Hypothesis: Higher N availability feeds back to slow enzymatic breakdown of complex organic matter substrates and mineralization of N, whereas higher P availability enhances organic matter breakdown and mineralization of N.

In the MEL model, co-limitation is maintained by variable allocation of effort to obtain multiple resources. These relationships are poorly constrained by measurements, at present, and the importance of many possible mechanisms is uncertain. In our experiments, we will test whether several types of effort correspond to theoretical predictions (e.g. weathering, depth distribution of effort) and we will obtain response data that can be used to improve the representation of effort in the model (e.g. fine root biomass, mycorrhizae, root uptake capacity, foliar resorption, and phosphatase activity).

Objective 3: Predict long-term forest response to anticipated future conditions using a revised and improved MEL model

The modeled mechanisms of nutrient mobilization and acquisition will be refined and augmented by the measurements made under Objective 2, and theory may be improved as well. Using an improved MEL model, we will explore the importance of resource limitation to future forest responses to continued anthropogenic N deposition, increasing CO₂, and changing precipitation and temperature scenarios.

Improvements in Response to Earlier Reviews

Five versions of this proposal (including early versions by Fisk, Yanai, Blum, and Hamburg) have been reviewed by the NSF Ecosystem Science program since 2004, and we have made many substantial changes in response to reviewer and panel comments. The most significant improvement since the last review is the field assays for heterotrophic soil respiration. We have also included additional preliminary results, as the PIs and their graduate students are sufficiently excited about the proposed work to be studying pre-treatment patterns until the treatments can be funded.

The previous revision included Rastetter and the MEL model for the first time and focused attention on the mechanisms of P vs. N acquisition during secondary succession. Goodale replaced Hamburg for soil and deep root sampling, using power-coring technology. Fahey, previously in an advisory role, took on expanded responsibilities. We dropped some of complicated experiments directed at fungal community response and the extensive measurements required for budgeting nutrient uptake, and we added other measures needed to test allocation of effort, such as total belowground carbon allocation (Fahey) and nutrient uptake capacity of roots (Yanai). Previous improvements to the proposal included monitoring soil N and P availability and adding P as NaH₂PO₄ (other P fertilizers contain Ca). We extended the timeline to 5 years to allow greater development of treatment effects, leaving some years in the middle with little activity beyond maintaining the treatments.

Research Plan

Study Sites

This study will be carried out at the Bartlett Experimental Forest in Bartlett, NH, where we have established a series of experimental plots in replicated stands of different ages in northern hardwood forest. 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 sites. Pin cherry (*Prunus pensylvanica*), white birch (*Betula papyrifera*), and red maple (*Acer rubrum*) dominate our young sites. Soils are well drained Spodosols (Typic Haplorthods) with a forest floor averaging 4.9 cm in depth (Park et al. 2007). We will study three replicate early (19-24 yr) successional stands, three young transitional (31-34 yr) and three mature (>100 yr) stands at elevations of 250-400 m. Extensive characterization of soils and vegetation at these sites by a previous NSF-funded project on the role of forest age on Ca biogeochemistry reduces the effort and expense required to carry out the proposed project. We will have access to comprehensive characterizations of the soil for 6 of our 9 study sites. Three 0.5 m² quantitative soil pits were excavated in these stands (summer 2004) as part of the Ca biogeochemistry study (Park et al. 2007). Soils were analyzed by depth for root distribution (Yanai et al. 2006), nutrient characteristics, soil mass and mineralogy. Soil chemistry was characterized by sequential extractions with 1 M NH₄Cl, 1 M cold HNO₃, and hot HNO₃, which allows quantification of apatite pools (Nezat et al. 2007, 2008; Blum et al. 2008).

Nutrient Manipulations

Within each of our nine stands, four 50 x 50 m plots were established in summer 2004, with all measurements to be made in the inner 30 x 30 m of each plot. These will be randomly assigned to

control, N, P, and NP treatments. Some pre-treatment measurements have already been made; pre-treatment measurements will be completed in all plots in 2010. Nutrient treatments will begin in fall 2010.

Nitrogen will be added as NH_4NO_3 for a total of 50 kg N/ha/yr, or roughly half the annual rate of N mineralization. Phosphorus will be added as NaH_2PO_4 for a total of 50 kg P/ha/yr, to account for the quantity that will be rapidly adsorbed and relatively unavailable to biota (Wood 1980). This rate may be adjusted in future years if we find that the readily accessible P sorption capacity has been satisfied.

The nutrient amendments will be applied in four parts distributed evenly throughout the year to provide a relatively uniform supply over time. Treatments will continue throughout the proposed study and we will seek future funding to maintain them thereafter, perhaps through the Hubbard Brook LTER. We regard this chronosequence as a long-term resource for future research endeavors.

Methods for Objective 1: N, P, or co-limitation as a function of stand age

Soil monitoring. To verify that we have altered N and P availability, we will measure resin-available N and P using ion-exchange resins incubated for two months in mid-growing season 2010 (pretreatment), 2012, and 2014. Bags will be placed between the Oe and Oa/A horizons and at 10 cm depth in the B horizon (6 replicate bags/horizon/plot). There is increased recognition that labile pools may not adequately represent P availability, and that transfers from more slowly cycling P pools must also be considered, especially in P dynamics over secondary successional development (Richter et al. 2006). Therefore we will detect possible shifts among pools by measuring occluded, non-labile organic, labile organic, and labile inorganic P pools by a modified Hedley extraction procedure, followed by standard P analysis (Tiessen and Moir 1993, Lajtha et al. 1999). Net N mineralization potential will be assayed in the same years, as described below.

Aboveground Growth and Productivity. We inventoried forest composition in the 30x30 m measurement area within each study plot (2 ages x 3 blocks x 4 treatments) in July 2004. We measured all live and dead trees >10 cm dbh and tagged all live trees. Stems 2-10 cm dbh were measured in 5x5 m subplots; saplings 50 cm tall to 2 cm dbh were measured in 2x2 m plots, and <50 cm seedlings were measured in 1x1 m plots. These measurements will be repeated in the first and final years of this study, in July 2010 and 2014. 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, Hamburg et al. 1997, Fahey et al. 1998, Arthur et al. 2001), supplemented by allometric equations developed for the young stands in our study (Fatemi 2007). Diameter growth of trees will be measured annually at a marked location on each stem, a standard approach that provides precision of <1% (Kloeppel et al. 2007) and successfully detected growth responses to fertilization in northern hardwoods (Fahey et al. 1998). Aboveground net primary productivity will be calculated by adding litterfall production, described below, to the aboveground biomass increment from 2010 to 2014.

Litter production. Litterfall was collected in fall 2004 and 2005 and will be collected again in 2010 (pre-treatment), 2012 (1 year post-treatment) and 2014 (3 years post-treatment), using 10 0.15 m² baskets per plot. Litterfall will be collected immediately after peak litterfall at the end of October each year, in May, and in September of each year. Leaf litter will be sorted by species, dried and weighed. Litter material for chemistry will be collected from netting suspended above the ground during rain-free periods during peak litterfall. These and other tissue samples will be analyzed for P, Ca, Mg, K, and Na concentrations using ICP-OES (Perkin-Elmer Optima 5000) following microwave digestion in nitric acid, and for total N using a modified Dumas Combustion analyzer (CE Instruments NC2100 elemental analyzer). Woody litterfall is also being monitored in these plots (Acker 2006), but will not be included in short-term estimates of aboveground productivity because of high interannual variability.

Vector analysis. We will use vector analysis to interpret changes in foliar nutrition in response to N, P, and NP additions (Fig. 9; Ouoreshi and Timmer 2000, Haase and Rose 1995). Nutrient deficiency is indicated when concentrations increase, but not by as much as mass and content (vector C). At optimal nutrition, concentrations remain constant while mass and content increase (vector B). Luxury consumption means that concentrations increase without changing mass (vector D). Toxicity is indicated if mass decreases (E, F). Dilution (vector A) could occur when growth limitation has been relieved by addition of another nutrient.

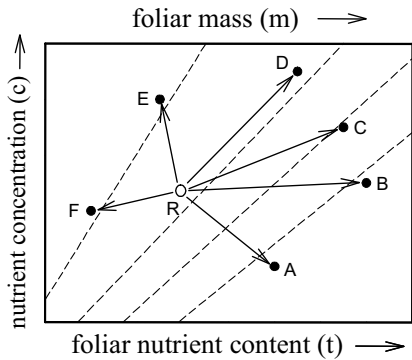


Figure 9. Vector analysis of response to nutrient treatment. Dashed lines connect points of equal foliar mass. Foliage or litter from fertilized plots can be compared to a reference, either pretreatment or untreated plots. See text for interpretation of the resulting vectors.

Samples will be collected from sun leaves of five trees of the three dominant species in each plot using a 12-gauge shot gun, both pretreatment (2010) and 1-year post treatment (2011). We will apply vector analysis to the dominant species in each age class of stands, using controls as the reference and N- P-, or NP-amended plots as the treatment. We can also compare foliar nutrient status before and after treatment. We will collect foliage from the same trees before and after treatment. This approach may reveal that different species are differentially limited by particular nutrients. For an integrated measure of ecosystem response, we will also apply vector analysis to the stand-level average foliar concentrations based on the relative contributions of each species to total litterfall mass. We expect this approach to be more robust in comparing pre- and post-treatment nutrient concentrations than in comparing treated plots to controls, because of the spatial variation in species composition.

Methods for Objective 2: Measuring mechanisms of N and P acquisition

Belowground production. Direct measurement of fine root production and TBCA is problematic and subject to error and bias that are difficult to evaluate (Tierney and Fahey 2007). We propose an integrated set of measurements that will provide reliable indications of these key belowground responses as indicators of effort in nutrient acquisition. First, we will quantify fine root biomass at the known time of peak root biomass (late summer; Fahey and Hughes 1994) in pretreatment and in the third year post-treatment. Second, we will measure fine root growth using the ingrowth core method; this method has been shown to provide reliable estimates of relative root growth in response to experimental treatments (Tierney and Fahey 2007). Third, we will estimate TBCA as the difference between TSR and aboveground litterfall (Nadelhoffer and Raich 1992). We recognize that changes in TSR can result from either or both root-associated respiration and heterotrophic respiration. To use TBCA as an index of effort requires estimation of the response of heterotrophic respiration to our treatments, and we will use laboratory assays and field measurements in trenched plots. In sum, we will use a combination of three complementary approaches to provide indication of the overall response of effort to belowground resource acquisition and its response to the treatments.

Total soil respiration (TSR) will be estimated in each plot as CO_2 efflux from soil (F_{CO_2}) pretreatment (2010) and in 2012, 2013, and 2014. Ten permanent soil collars will be installed in each plot to minimize disruption of forest floor during measurements. A LI-COR soil respiration chamber will be used in conjunction with a LI-COR infra-red gas analyzer to measure F_{CO_2} . 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 will be measured adjacent to the collars at each sampling. To allow estimation of annual TSR, we will also measure diurnal variation and winter soil respiration rates (Fahey 2006).

Heterotrophic respiration will be measured as soil CO₂ efflux from 4 trenched plots (Lee et al. 2003) 2 m in diameter in the buffer zone of each treatment plot. Plots will be trenched in 2010 and monitored along with TSR in 2012, 2013, and 2014. In addition, we will incubate Oe, Oa/A, and B horizon (top 10 cm) soil in the laboratory in 2010 (pretreatment), 2012, 2013, and 2014. We will capture seasonal variation by sampling in early, mid, and late growing season. Multiple soil cores will be collected from each of 4 quadrants per plot, separated into horizons, and composited within quadrants for 4 samples/horizon/plot. Subsamples will be incubated in sealed Mason jars for 3 weeks and CO₂ accumulation in jar headspace will be quantified once a week using a LI-COR 820 CO₂ analyzer. These soil samples will also be subsampled for use in other measurements, described below.

Fine root biomass will be measured by soil coring at about the time of peak biomass in late summer pre-treatment (2010) and in 2013. Six soil cores will be extracted from each plot to 50 cm depth using a 10-cm diameter gas-powered diamond-bit corer. Roots will be hand-sorted from cores from the forest floor and from 10-cm depth increments in the mineral soil, and classified by diameter (<0.5 mm, 0.5-1.0 mm, 1-2 mm, > 2 mm). Dead roots will be separated from live roots based on morphological criteria (Bledsoe et al. 1999).

Fine root growth will be measured using in-growth core techniques (Vogt and Persson 1991, Fahey and Hughes 1994) in each plot in 2012 and 2014. Eight soil cores (5 cm diameter, 15 cm depth) will be extracted from each plot in October. A uniform mixture of root-free soil from the upper B horizon will be used to fill the core holes at about the field bulk density. The following September, cores will be extracted from each ingrowth core using a slightly smaller diameter corer. Roots will be hand sorted from ingrowth cores and those < 1.0 mm diameter will be separated, scanned using WinRhizo software (Regent Instrument Inc. Quebec, Canada), dried, and weighed. Although the accuracy of the ingrowth core technique is questionable for quantitative assessment of fine root production (Tierney and Fahey 2007), it will provide a reliable comparison of relative root growth responses to the treatments.

Mycorrhizae will be quantified with 2 complementary methods that, together, should best assess allocation in relation to the potential for nutrient uptake in soil. First, a subsample of < 0.5mm roots from each root core collected for biomass will be examined microscopically to quantify the frequency of EM vs AM root tips. Viewed at 50X magnification, EM root tips can be readily distinguished using visual criteria from tips that are not EM, the latter including both arbuscular and non-mycorrhizal tips. After screening several hundred root tips from each core, the subset of non-EM infected roots will be cleared and stained and examined for AM structures using standard methods (Johnson et al. 1999). This approach will allow us to quantify the frequency of EM, AM (arbuscules and vesicles), and non-mycorrhizal root tips in each plot and thereby determine whether allocation of effort to mycorrhize is responsive to N or P limitation, which would warrant inclusion in MEL.

Second, we will use a combination of hyphal lengths and phospholipid-fatty acid (PLFA) analyses to assess patterns of fungal hyphae in soil, using subsamples of soils collected for heterotrophic respiration potential (pretreatment (2010) and 2013; mid-season only). Total hyphal length will be quantified for individual subsamples by standard direct microscopy methods with fluorescein diacetate staining (Söderström 1977, Ingham and Klein 1984), which we have modified to maximize extraction efficiency in our soils. PLFA analysis will be used to distinguish AM fungi from ECM and saprotrophic fungi in subsamples composited within plots. Composite samples will be immediately frozen (-80 °C), and then lyophilized prior to PLFA analysis. We will use the signature fatty acids 16:1 ω 5 for AM fungi and 18:2 ω 6,9c for ECM and saprotrophic fungi (Olsson 1999). Potential uncertainties arising from our inability to distinguish saprotrophic from ECM hyphae should have minimal effect in mineral soil (Lindahl et al. 2007), and in organic horizons can be partially addressed in comparison with root-tip colonization patterns.

Foliar resorption. We will compare N and P concentrations and content in foliage and litter (measured under Objective 1) to estimate N and P resorption, expressed on a leaf-area basis (Fahey and Birk 1990).

Mineral weathering. We will use chemical tracers such as the rare earth element concentrations and ratios in roots and fungal hyphae to indicate the uptake of nutrients from apatite (Hagerberg et al. 2003). Ingrowth cores for fine root growth (above) will be used as controls and will be paired with apatite-amended ingrowth cores installed at the same time. All ingrowth cores will receive the same fertilization treatment as the entire plot (none, N, P, or NP). Upper B horizon soil used in ingrowth cores does not contain apatite (Blum et al. 2002), and apatite-amended ingrowth cores will receive 5% apatite by weight. To test for a foraging response, root length and mass in apatite-amended cores will be compared to controls, which will be amended with 5% quartz to control for any possible texture effects of apatite treatments. Roots recovered from the 8 replicate cores per plot will be pooled by plot, sonicated with DI water, and inspected by SEM-EDX x-ray mapping to ensure that they are free of adhering particles. If particles are observed, cycles of sonication and rinsing in DI water will be repeated until roots are free of particles. Roots will be microwave digested in concentrated nitric acid and analyzed for major and trace elements, including REEs, by ICP-OES and ICP-MS. The proportion of P obtained from primary mineral sources will be used to test model predictions of accelerated mineral weathering under P limitation.

We expect that apatite weathering and uptake into roots is mediated by mycorrhizal fungi. To evaluate this idea, we will quantify mycorrhizal colonization of root tips and fungal hyphal length within cores, and test the relationships between apatite uptake and total mycorrhizae or fungal hyphal length.

Nutrient mineralization potential. Given the likelihood that availability of N and P influence processes that recycle these nutrients, from organic as well as mineral-associated pools, measuring these feedbacks is essential for interpretation of plant responses to treatments as well as for refining the MEL model. We will measure net N mineralization potential and the activity of P and N mineralizing enzymes in all plots in summer 2010 (pre-treatment), 2012, and 2014, using the soil samples collected for heterotrophic respiration potential, described above. Subsamples will be extracted in acetate buffer and reacted with the appropriate substrates to measure activity of acid phosphatase, aminopeptidase, chitinase, glucosidase, polyphenol oxidase, and peroxidase enzymes (Sinsabaugh and Foreman 2001, Saiya-Cork et al. 2002). We will also test for transfers of P from more slowly cycling to more labile pools, as described above under soil monitoring.

Nutrient uptake capacity. We will measure N and P uptake capacity of fine roots in the final year of the project, using intact root branches in the field (Lucash et al. 2005, 2007). We have used this method in mixed species forests by tracing each root back to a tree or to a root coarse enough to have distinguishing bark characteristics. We will assess nitrate, ammonium, and phosphate uptake capacity of the three dominant species in each stand to assess the allocation of effort to N and P acquisition as a function of the relative limitation of P and N, both as induced by treatments and as a function of stand age, as determined under Objective 1. These observations will provide MEL with parameters describing uptake kinetics as a function of resource limitation.

Methods for Objective 3: Improvements to MEL and understanding of forest ecosystems

We have implemented an eight-resource version of MEL: CO₂, PO₄, H₂O, light, NH₄, NO₃, DON, and N₂-fixation and converted the model to a daily time step to allow for plant phenology, seasonality in resource availability, and tie losses of dissolved materials to a daily soil-water budget. Preliminary results of MEL simulations for the northern hardwood forest were shown above (Fig. 5, 6, and 7). To give the best possible predictions of forest response to future conditions, this new model must be carefully calibrated to reflect our best current understanding. For example, we have

revised expectations of ecosystem biomass accumulation (Fahey et al. 2005) since MEL was parameterized for C and N (Rastetter et al. 1997, 2001, 2005). We also have new information on fine root mass (Park et al. 2007) and leaf area (unpublished data) as a function of ecosystem biomass, collected in part at our Bartlett sites. We plan a publication on the new model that will make use of this improved calibration for Northeastern forests.

In the last year of the project, we will compare the results obtained from the N and P treatments with the model predictions (Fig. 5-7). The data collected as part of this project will help to refine the model and our concepts of resource optimization. Our results will be used to parameterize the relationships in the model that describe the effects of allocation of effort towards various resources. In particular, we anticipate being able to improve the model calibration of belowground C allocation, biologically enhanced weathering of primary minerals, and uptake capacity of PO₄, NH₄, and NO₃. Processes that could be added to the model structure include the deployment of roots at different depths to obtain substitutable resources, the role of mycorrhizal type, and the feedback of N and P availability on organic matter decomposition, including phosphatase enzymes. We will use the improved model to explore the importance of ecosystem acclimation to changing resource limitation under various scenarios of N deposition, elevated CO₂, 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.

Timeline – Project Team and Schedule of Tasks

An interdisciplinary approach is required for a study of nutrient co-limitation by a forested ecosystem. We have brought together a team that includes expertise in ecosystem modeling, forest structure, forest soils, root and litter production, microorganisms, geochemistry and mineral weathering. The perspectives and skills represented in this collaboration allow us to address issues fundamental to ecosystem nutrient cycling. The assembled group of PIs have worked together previously, meet quarterly at Hubbard Brook LTER meetings, and hold regular conference calls. We

Table 1. Timeline of activities. Nutrient treatments will be applied beginning at the end of 2010 and will continue indefinitely.

| Year | 2010 | 2011 | 2012 | 2013 | 2014 |
|--|------|---------|---------|---------|---------|
| Soil nutrient availability | X | | X | | X |
| Soil respiration | X | | X | X | X |
| Microbial respiration, enzyme activity | X | | X | | X |
| Root cores, mycorrhizal colonization, hyphae, PLFA | X | | | X | |
| Ingrowth cores: root and fungal growth, mineral weathering | | Install | Collect | Install | Collect |
| Tree inventory, growth response | X | | | | X |
| Foliage, leaf litter, retranslocation | X | | X | | X |
| Nutrient uptake capacity | | | | | X |
| Vector Analysis | | X | | | |
| MEL model development | X | | | | X |

have an excellent working relationship and are well poised to complete this research. Primary responsibility for each of the PIs are as follows: Yanai – foliage and leaf litter, including vector analysis and estimates of nutrient retranslocation, and nutrient uptake capacity; Fahey – soil respiration, total belowground carbon allocation, and fine root biomass; Goodale – sampling soil, coarse roots, and roots at depth; Fisk - fungal, enzyme, and soil nutrient work; Blum – mineral weathering assessed by uptake of rare earth elements; Rastetter – simulation modeling. In order to facilitate coordination, a technician from SUNY-ESF will oversee field work and data management,

and we will plan our time in the field to optimize our interactions.

Broader Impacts

The broader impacts of the proposed research are described according to the five components defined by the NSF Grant Proposal Guide.

- **How well does the activity advance discovery and understanding while promoting teaching, training and learning?**

Student learning is enhanced by use of the MEL model as an important component of the Ecological Modeling class in the Semester in Environmental Sciences Program at MBL

(<http://courses.mbl.edu/SES/>). Science education will also be enhanced through the training of four graduate students and numerous undergraduates. Students from SUNY-ESF, Cornell, and Miami University will be housed together during the summer, which allows for a shared experience among students with differing levels of training. In previous projects we have had great success using joint housing and shared projects to facilitate a wider research experience for a diversity of students. In addition, our project will participate actively in the Environmental Literacy Program, a cooperative effort between the HB LTER project and the USDA Forest Service. This program supports secondary school teachers through a combination of Teaching Guides, teacher training events and school partnerships with six regional high schools. We have found that by involving the primary researchers in the training exercises, the teachers develop a higher level of comfort with the material and a greater enthusiasm for teaching it. We will evaluate the educational tools we create in collaboration with the institutions listed above using formative evaluative mechanisms already integrated into the work of the FFEC: baseline focus groups, first phase field-testing of prototype materials, and second phase field-testing of near final tools. The required expertise to carry out these evaluations exists within the participating organizations including the HBRF.

- ***How well does the proposed activity broaden the participation of underrepresented groups (e.g., gender, ethnicity, disability, geographic, etc.)?***

Half of our 6 Principal Investigators are women, and all of us are committed to promoting the participation of underrepresented groups. We hope to help fill the pipeline with a broader group of new graduate and undergraduate students, including those supported through the Plymouth State REU program and the MBL SES, which have active recruiting programs for minority students, as do our respective universities. There are few ethnic minorities currently trained in ecosystem science, so recruiting young people is important, as is outreach to the broader community, described below.

- ***To what extent will it enhance the infrastructure for research and education, such as facilities, instrumentation, networks and partnerships?***

The establishment of a long-term nutrient manipulation experiment will be a resource for decades for researchers interested in N and P and forest succession. The inclusion of the youngest cohort of stands in our experimental design ensures that early successional processes can be studied even after our mid-age stands transition to mature structure and nutrient status. The partnership between the Bartlett and Hubbard Brook Experimental Forests is still developing, and the link between this project and the HB LTER will further that relationship (we plan to apply N and P treatments to a young and mature stand at HB). The addition of Rastetter to the HB Committee of Scientists is an addition to intellectual infrastructure that we hope will persist beyond this project.

- ***Will the results be disseminated broadly to enhance scientific and technological understanding?***

Our results will be disseminated primarily in high-impact peer-reviewed journal articles. Public outreach will be accomplished through activities coordinated by the Hubbard Brook Research Foundation (HBRF), including our nationally prominent Science Links program (http://www.hubbardbrookfoundation.org/science_links_public_policy/). Data and results from this project will be posted at the Hubbard Brook web site (<http://www.hubbardbrook.org>).

- ***What may be the benefits of the proposed activity to society?***

An improved understanding of ecosystem optimization of resource use, including the maintenance of co-limitation, is important to forest management, environmental protection, and basic scientific knowledge. The improved MEL model will help pose questions about the future importance of P limitation under continuing N deposition, the implications for C sequestration with forest development, and the supply of N and P to downstream ecosystems threatened by eutrophication. Balanced nutrient cycling is essential to maintaining forest sustainability, and we will work with scientists and managers at the White Mountain National Forest to evaluate

silvicultural practices in light of results from the proposed research. For example, their forest management plans require justifying intensive harvest removals in terms of the forests ability to acquire nutrients for regrowth. Feedbacks such as the biological enhancement of mineral weathering when nutrients are limiting are clearly relevant to this appraisal.

Results of Prior NSF Support

R.D. Yanai: DEB-0235650, with J.D. Blum, S.P. Hamburg, and M.A. Arthur. \$593,000, 2003-2007. *Biotic Control of Calcium Supply: Distinguishing Sources to Regrowing Forests*. This project established the replicated chronosequence of stands to be used in the proposed research. Including REU and ROA supplements, this project has involved two additional PIs, 9 graduate students, 34 undergraduates, and 7 technicians. Eleven papers have been published, and 9 more are in preparation.

E.B. Rastetter: DEB-0108960, with G.R. Shaver. \$417,946, 2001-2004. *Species-, Community-, and Ecosystem Level Consequences of the Interactions among Multiple Resources*. We continued development of MEL model resulting in five papers. **Rastetter et al. 2001** and **Vitousek et al. 2002:** We assessed strategies of symbiotic N fixation vs. N uptake from soils. A single equation in model explains the CO₂ stimulation of N fixation, the loss of N fixation when legumes are fertilized, and the relegation of N fixation to early succession even though N limitation persists into late succession. **Herbert et al. 2003:** We examined secondary growth in Amazon Forests, assessing shifts in limitation from N to P during re-growth after pasture abandonment. **Herbert et al. 2004:** We assessed the effects of resource optimization on community structure along fertility gradients. The highest diversity was found at intermediate ratios of community leaf area to root length, indicating a condition where the community was co-limited by light and nutrients. **Rastetter et al. 2005:** We examined NH₄ and NO₃ losses versus recalcitrant organic N losses and found that long-term C sequestration was severely curtailed if N loss from ecosystems is as recalcitrant organic N. Funds from this grant supported one postdoctoral fellow. Models developed under this grant are published on our web site: <http://ecosystems.mbl.edu/>. The model we are now using was developed under funding from this project.

J. D. Blum: NSF-EAR 0433772, \$219,210, 9/1/04-8/31/08. *Collaborative Research: Mercury isotope fractionation during microbial and abiotic redox transformations*. This grant supported research on development of methods for high precision Hg isotope measurements, studies of the microbial fractionation of Hg, studies of the photochemical fractionation of Hg, and applications of Hg isotope and concentration analyses to Hg biogeochemical problems. This project has produced 8 abstracts and 10 peer-reviewed publications, supported 5 undergraduate research assistants and 2 PhD students, and partially supported 1 postdoctoral fellow.

M.C. Fisk: DEB-0542446, with P.M. Groffman (PI), T.J. Fahey, J. Yavitt, J.M. Maerz, and P.J. Bohlen. \$149,942, 2006-2010. *Invasion of North Temperate Forest Soils by Exotic Earthworms*. This project is a renewal of a study of the biogeochemical consequences of earthworm activity in forest soils, which resulted in 12 publications and 1 PhD and 2 MS theses and supported numerous undergraduates.

C.L. Goodale: DEB-0614099, with R.A. Houghton (PI), J.C. Jenkins, and M.J. Ducey. \$67,998, 2006 – 2009. *The Northeastern Carbon Sink: Enhanced Growth, Regrowth, or Both?* This project uses Forest Inventory & Analysis data to test whether forest growth rates have changed in the northeastern U.S as predicted by the PnET-CN forest ecosystem model in response to changes in climate, CO₂, and N deposition. The first publication will show that N deposition has changed rates of growth and survivorship in some northeastern tree species.

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