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Mechanisms of nutrient limitation and nutrient acquisition in managed and unmanaged forest ecosystems

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MECHANISMS OF NUTRIENT LIMITATION AND NUTRIENT ACQUISITION IN MANAGED AND UNMANAGED FOREST ECOSYSTEMS

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ABSTRACT

MECHANISMS OF NUTRIENT LIMITATION

AND NUTRIENT ACQUISITION

IN MANAGED AND UNMANAGED FOREST ECOSYSTEMS

by

Matthew A. Vadeboncoeur

University of New Hampshire, May, 2013

Understanding the interactions between global change, human and natural disturbances, and other factors on biogeochemical processes in forests is necessary to ensure the sustainability of forest management. Here I report the results of several investigations into nutrient acquisition processes in the forests of New Hampshire. I begin with a meta-analysis of fertilization studies showing that phosphorus (P) and calcium (Ca) as well as nitrogen (N) may limit primary production in deciduous forests of the region. Because these limiting nutrients are all removed from the ecosystem when trees are harvested, I compared nutrient budgets under a range of harvesting scenarios with a variety of soil nutrient stocks across a range of forest stands. I found that depletion of even long-term P and Ca soil stocks may occur over only a few rotations if intensive harvesting occurs in inappropriate stands.

Key to successfully managing such budgets is a better understanding of the processes by which trees access limiting nutrients in primary minerals such as apatite. I conducted a greenhouse and field study examining the potential for lead isotope ratios

and rare earth elements to serve as tracers of apatite weathering by mycorrhizal fungi in soils. In the greenhouse experiment, both of these tracers showed clear effects of biological systems (mycorrhizal and non-mycorrhizal birch seedlings) on the weathering rates of trace minerals including apatite. However, there were no clear trends in these tracers when examined in mycorrhizal sporocarps collected from forest stands that we hypothesized would differ in allocation to mycorrhizal weathering.

If weathering can balance harvest losses of P and Ca, and N deposition continues its recent decline, N availability may constrain future productivity. I developed a novel tracer experiment intended to confirm earlier reports of the uptake of organic N compounds in temperate forests, which are richer in inorganic N than systems where this process is clearly demonstrated. I found low (≤16%) but significant contributions of organic compounds to the N nutrition of trees across a range of New Hampshire forest types. This research adds to our understanding of how forest ecosystems will respond to management and global change over the long term.

INTRODUCTION

Global change in temperate forest ecosystems

Ecosystems comprise biological communities and the components of their environment to which they are linked by processes transferring energy, carbon, water, and nutrients. Within such communities, organisms are linked via competition, commensalism, and trophic relationships. The species assemblage and abiotic properties of an ecosystem are a consequence of both the environment (climate, the supply of nutrients and energy, and disturbance regime), and of the interactions among organisms. In turn, communities of organisms can modify soil chemistry, water availability, decomposition rates, and other aspects of their environment (Ehrenfeld et al., 2005). As self-organized complex systems, forests and other ecosystems exhibit resistance to change or disturbance and resilience in structure and function following disturbance, but may also change over time in response to either chronic or sudden environmental changes. Understanding the complex ecological systems upon which society relies is a key component to solving environmental problems and improving the management of natural resources (Waltner-Toews et al., 2008).

Throughout the Holocene, the forests of the northeastern United States have continually changed in response to changes in climate, pedogenic development, pest outbreaks, and use by native people (Cronon, 1983; Davis and Shaw, 2001; Shuman et al., 2004). Extensive clearing for agriculture and timber production in the 18th and 19th centuries dramatically reduced forest cover in the region (Clawson, 1979; Foster, 1992;

Vadeboncoeur et al., 2010). The forests that gradually returned to the landscape over most of the 20th Century were shaped in part by the disturbances that preceded them as well as by a changing climate (Hamburg and Cogbill, 1988; Foster et al., 1998; Groffman et al., 2012; Vadeboncoeur et al., 2012b). Over the past several decades, there has been an increasing appreciation of the effects of a variety of global change drivers affecting the composition and function of ecosystems throughout the world (Vitousek, 1994), with many examples apparent here in New England. Among these are accelerated rates of climate change (Hayhoe et al., 2007; Hamburg et al., 2013), elevated atmospheric deposition of acidity and nutrients (Matson et al., 1999; Adams, 1999; Driscoll et al., 2003; Elser, 2011), increased concentrations of carbon dioxide and ozone (Dentener et al., 2006), and introduced insects and pathogens (Houston, 1987, 1994; Templer and McCann, 2010). Meanwhile, as a society we continue to look to forested areas to provide a wide range of useful services, including recreation, water quality protection, support of wildlife populations and local biodiversity, and the production of wood for timber, pulp, and fuel in a sustainable manner (Malmsheimer et al., 2008; Campbell et al., 2009; Richter et al., 2009).

Understanding the interactions between natural and human-caused inputs, removals, disturbances, and other forcings on biogeochemical processes at a range of scales will allow better management of forested landscapes. New England is a particularly interesting setting for this type of research, with regional variation in climate and atmospheric deposition, intermediate-scale variation in bedrock type and glacial geomorphology, and fine-scale variation in current and past land use and forest management. Together, these factors influence plant species composition and ecosystem

biogeochemical process rates, from biomass accumulation and soil carbon storage (Hamburg, 1984b; Hooker and Compton, 2003; McLauchlan, 2006; Reiners et al., 2012), to denitrification and loss of nitrate and base cations to surface waters (Lovett and Mitchell, 2004; Bailey et al., 2004; McLauchlan et al., 2007). The work I present here is broadly aimed at understanding to what extent nutrient cycling processes are controlled by community composition, nutrient demand, and carbon allocation, how they vary across the landscape, and how they might respond to global change. I ask four main questions about ecosystem processes in northeastern forests:

1. What nutrient or combination of nutrients (N, P, or Ca) limits primary production?
2. What is the potential for extractive forest management to deplete forest soils of production-limiting nutrients over multiple rotations?
3. Are weathering fluxes of rock-derived P and Ca controlled in part by biotic demand as induced by nutrient depletion? What geochemical tools can be used to estimate such fluxes?
4. Where N is limiting or co-limiting to production, does uptake of organic forms of N contribute substantially to overall N budgets?

Assessing nutrient limitation

Understanding long-term and short-term responses of ecosystems to changes in the input and removal rates of nutrients depends largely on knowing which nutrients limit primary production in these systems. Temperate forests are generally considered to be N-limited, and large amounts of accumulated experimental evidence show widespread N limitation across many terrestrial ecosystems (LeBauer and Treseder, 2008). On the

other hand, resource allocation theory suggests that plants and ecosystems should be expected to demonstrate simultaneous or near-simultaneous limitation by multiple resources (Chapin et al., 1985; Sterner and Elser, 2002; Davidson and Howarth, 2007; Craine, 2009), and recent global meta-analyses suggest that such co-limitation may be more common than previously realized (Elser et al., 2007; Harpole et al., 2011). These global compilations include very little data from North American temperate forests, despite a long history of intensive research. Decades of elevated acidic deposition have enriched these ecosystems with nitrogen while depleting them of important base cations such as calcium (Likens and Bormann, 1995; Likens et al., 1998).

In Chapter 1, I assess the collective strength of existing experimental data on forest production responses to N, P, and Ca fertilization in hardwood forests across the northeastern United States and southeastern Canada by with a meta-analysis. The results allowed me to critically explore the concept of ecosystem-scale nutrient limitation to forest production and to demonstrate that both P and Ca are potentially important controls of forest production in addition to N.

Modeling potential nutrient depletion by forest harvesting

Another means by which humans can interrupt the steady-state nutrient cycles in forest ecosystems is through the harvest and removal of living biomass. Mature forest ecosystems tend to cycle nutrients such as N, Ca, K, Mg, and P very tightly, with large internal fluxes (litter mineralization and root uptake) that are closely balanced over time, and relatively small inputs and outputs at an annual time scale (Yanai, 1992; Likens and Bormann, 1995; Rastetter et al., 2013). After heavy cutting and the removal of the nutrient stock contained in standing biomass, forests must go through a period where

nutrient uptake exceeds external inputs and the mineralization of recent litter, which must therefore lead to a net depletion of soil stocks of nutrients. Imbalances in nutrient budgets following harvesting, especially those of Ca and P, have raised recent and renewed concern about whether yields will be sustained over multiple rotations (Federer et al., 1989; Sverdrup et al., 2006; Thiffault et al., 2011).

Interest in forest-derived bioenergy in the United States has increased in the past decade (Kingsley, 2006; Malmsheimer et al., 2008; Richter et al., 2009), driven by fossil fuel price volatility and the goals of reducing net greenhouse gas emissions and dependence on imported energy. There are currently about 850 Mw of wood-fired electrical generating capacity in New England (about 3% of average demand), and wood biomass is used for heat in about 9% of the region's households (EIA, 2007; ISO-NE, 2011). Bioenergy that utilizes harvest residues can reduce net CO_2 emissions provided it does not lead to greater harvest intensity or frequency (Searchinger et al., 2009; Schulze et al., 2012), though soil C budgets following harvests are still somewhat uncertain (Buchholz et al., 2013). Whether the region's forests can provide locally-sourced, renewable energy depends on the ability of these ecosystems to sustain their productivity in spite of nutrient losses due to harvesting.

In New England forests that have been cut over 1-3 times, regrowth is generally vigorous and forests approach a biomass equilibrium in 80-120 years (Reiners et al., 2012; Rastetter et al., 2013). However, because forests are not typically fertilized after harvest (Bennett, 2010; Evans et al., 2010), and because total soil stocks of nutrients are finite at management-relevant time scales, it may be possible to severely deplete soil nutrients over multiple rotations. Assessing the sustainability of any management regime

requires careful accounting of nutrient inputs and outputs, including atmospheric and soil weathering inputs, as well has exports of biomass and hydrologic outputs across representative forest stands.

In Chapter 2, I examine nutrient budgets and various soil nutrient stocks across 15 sites in the White Mountains in order to assess the potential for nutrient depletion over multiple rotations under various management practices. The range of model assumptions shows that the natural "background" weathering rate of geologically-derived macronutrients (P, Ca, and K) and the fraction of primary minerals available for accelerated weathering are important determinants of long-term system behavior. Unfortunately, estimates of these rates are poorly constrained, and improving such estimates should be a high priority for future research.

Developing tools to constrain mycorrhizal weathering rates

Accelerated weathering of primary minerals, particularly apatite, in the rooting zone of nutrient-depleted forest ecosystems has been proposed as a mechanism for balancing the Ca and P demand of regenerating forests (e.g. Hamburg et al., 2003), but such fluxes have usually been inferred by subtraction of other budget terms with large and often unquantified errors (Likens et al., 1994; Yanai et al., 2010). The extent to which apatite weathering rates are upregulated in response to biotic demand is a critical question in determining the long-term sustainability of forest biomass harvesting in the regions' often nutrient-poor granitic soils. Such active upregulation of weathering in response to demand for a specific nutrient at the ecosystem scale is not typically considered in broad-scale assessments of "critical zone" soil weathering processes, (e.g. Rasmussen et al., 2011), but better understanding such processes remains a priority of

this research community (Taylor et al., 2009; Brantley et al., 2011). A reliable tracer system for the weathering of important primary minerals (apatite in particular, which weathers easily and contains both Ca and P) would provide an important independent metric to confirm whether rates of weathering in the soil profile respond to increased biotic demand.

Some varieties of ectomycorrhizal fungi can directly weather feldspar minerals and associated apatite by exuding organic acids and providing a sink for nutrient ions (Leake et al., 2008; van Scholl et al., 2008). Access to apatite when P demand is high has been show to stimulate ectomycorrhizal production (Hagerberg et al., 2003). The importance of this process to overall ecosystem budgets is vigorously debated (Van Breemen et al., 2000; Smits et al., 2005; Sverdrup, 2009), and needs to be studied more thoroughly in the context of real-world ecosystems (Rosenstock, 2009), which may require new methodological approaches.

Minerals differ widely in the ability of their crystal structures to accommodate substitutions of trace elements for their major constituent elements; these compatibility differences are commonly used in geochemical investigations. For example, apatite often contains rare earth elements (REEs) at 10^3-10^4 times their concentration in the whole rock, with greatest enrichment among the lighter-mass REEs such as lanthanum and cerium (Bea et al., 1994). Because they are biologically inactive and chemically very similar, the REEs and their elemental ratios prove to be useful tracer of the dissolution of apatite. Uranium is another element that partitions preferentially into apatite during the crystallization of igneous rocks (Bea et al., 1994). Over time, U decays to ^{206}Pb and ^{207}Pb, (radiogenic Pb) which are then be enriched in apatite relative to non-radiogenic

^{204}Pb. Both Pb isotope ratios and REE concentrations have been used to infer apatite weathering rates (Erel et al., 2004; Harlavan et al., 2009).

In Chapter 3, I present the results of two related studies: a greenhouse experiment employing mycorrhizal birch trees growing with mesh bags filled with crushed granite, and the collection of fungal sporocarp samples in forest stands differing in age and soil parent material. Together, these are intended to evaluate the potential of REE and Pb isotope tracer systems to assess apatite weathering rates in natural and experimentally manipulated ecosystems.

Quantifying the contribution of organic nitrogen uptake by mycorrhizal roots

At the other end of the spectrum from disturbed aggrading ecosystems co-limited by rock-derived nutrients (P or Ca), lie steady-state ecosystems that efficiently recycle nutrients and tend to be limited by N mineralization rates. Especially in cold environments, the N cycle may be substantially short-circuited by the direct uptake of organic N (e.g. amino acids) by plants (Chapin et al., 1993, 2003). To some extent, this process frees plants from limitation by microbial mineralization rates in environments where the process is slow. However, the importance of organic N uptake to overall N budgets in temperate systems is widely debated (Jones et al., 2005a; Näsholm et al., 2009; Hobbie and Hobbie, 2012).

One mechanism by which organic N uptake is expected to occur in temperate ecosystems is via mycorrhizal fungi. These symbiotic fungi efficiently explore the soil for nutrients and water, in exchange for carbon from the plant symbiont (Smith and Read, 2008). Some ectomycorrhizal fungi, a class which is often dominant in temperate and boreal forests, are known to exude extracellular protelytic enzymes and to have high

uptake affinities for amino acids and oligopeptides that are the immediate products of protein degradation (Chalot and Brun, 1998; Lilleskov et al., 2002; Näsholm et al., 2009).

Despite chronically elevated N deposition, forests in the northeast still tend to cycle N relatively conservatively, storing the accumulated excess either in standing biomass or in soil organic matter (Magill et al., 2004; Nadelhoffer et al., 2004). Such high retention of N indicates that the ecosystems have not yet reached "saturation" with respect to N (Aber et al., 1989, 2003), unlike European forests which received much greater rates of N deposition in the late 20th century (Mohren et al., 1986; Ågren and Bosatta, 1988). Since the 1990s, rates of N deposition have declined rapidly, as has stream output of N (Bernal et al., 2012; Likens and Buso, 2012); this change might reasonably be expected to increase the degree to which some forests rely on organic N uptake, particularly those subject to large removals of nutrient capital over time.

In Chapter 4, I describe an experiment conducted using a novel approach that is complementary to existing methods used to test the hypothesis that the uptake of organic N uptake represents a substantial fraction of total N supply to temperate forest trees. I present clear evidence of organic N uptake by mycorrhizal roots in New Hampshire forests, confirming the results of previous studies using similar methods but making fundamentally different experimental assumptions. However, the contribution of organic N to total uptake seen in this experiment is relatively small, and the question of its ecological significance is open to interpretation.

The research I present here lies at the intersection of ecosystem ecology, global change science, and soil biogeochemistry. Understanding how global change drivers and

human management of ecosystems alter biogeochemical processes and ecological relationships is critical to proper management and conservation of species diversity and ecosystem function in a changing world. Moreover, it is by studying these processes of change, in which well-adapted communities and co-evolved mutualisms are challenged, disrupted, and in some cases re-assembled, that we can better understand the mechanisms responsible for species dominance and long-term ecosystem stability (Craine, 2009), as well as the long-term interactions between geochemical and ecological systems (Brantley et al., 2011).

CHAPTER 1

META-ANALYSIS OF FERTILIZATION EXPERIMENTS INDICATES MULTIPLE LIMITING ELEMENTS IN NORTHEASTERN DECIDUOUS FORESTS*

Abstract

It is widely accepted that nitrogen limits primary production in temperate forests, although co-limitation by N and P has also been suggested, and on some soils Ca and base cations are in short supply. Deciduous forests of the northeastern US and southern Canada are well-studied from silvicultural and ecological perspectives, but poorly represented in global meta-analyses of nutrient limitation. I used a meta-analytic approach to determine the overall strength of accumulated evidence for limitation of primary production by N, P, and Ca in the northern hardwood region, using 35 fertilization experiments in deciduous forests on glaciated soils across the northeastern US and southern Canada.

Overall, there was strong evidence for N limitation (mean response ratio = 1.42 - 1.53 using two statistical methods; $p < 0.01$ for both). Forest productivity also tended to increase with additions of P (means = 1.04 - 1.15; $p = 0.49$ and 0.03 respectively) and Ca

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(mean = 1.36 - 1.24; $p = 0.13$ and $p < 0.001$ respectively). Across all treatments, 85% of response ratios were positive. Multiple-element additions had larger effects than single elements, but there was little evidence for synergistic effects in factorial experiments.

1.1 Introduction

Understanding nutrient limitations is a key goal of ecosystem science and is critical to predicting responses to environmental change. Because natural forest communities include multiple species and ages of trees, with varying nutrient requirements and acquisition strategies, nutrient limitation at the community scale is not as conceptually straightforward as it is for single organisms (Chapin et al., 1986). However, the effects of altered availability of various nutrients on net primary productivity (NPP) are relevant to a variety of ecosystem-scale questions (e.g. whether atmospheric N deposition increases rates of CO_2 sequestration, or whether repeated removals of nutrient capital as biomass will reduce productivity). Globally, soil age is a key determinant of which nutrient limits productivity (Walker and Syers, 1976; Vitousek and Farrington 1997). Rock-derived elements such as P are less available in highly weathered soils, and while most soils lack bedrock-derived N, atmospheric deposition and N_2 fixation provide exogenous N inputs to the soil. P limitation is common in tropical systems, but it is rarely documented or even investigated in temperate forests on geologically young soils.

Responses to nutrient additions can be complex. If plants are able to dynamically allocate resources towards the acquisition of their most limiting nutrient(s), a plant at equilibrium would be equally limited by all resources (Bloom et al., 1985; Chapin et al., 1987). Altered allocation to acquisition of N, P, and C under fertilization are well

documented (Treseder and Vitousek 2001; Treseder 2004). Classic "law of the minimum" limitation may occur if plants aren't completely successful at balancing allocation, but assuming some dynamic control of allocation to the acquisition of various resources, the next limitation should be encountered rather quickly after relief of primary limitation (Davidson and Howarth, 2007). Furthermore, at the community scale, species composition is driven in part by competition among species for nutrients (Tilman, 1985); long-term deficiencies in one nutrient will favor species with low requirements for it, or with mechanisms for efficiently acquiring or recycling it. Over time, nitrogen present in excess of demand is lost from ecosystems via leaching or denitrification (Vitousek and Howarth, 1991). Systems without profound shortages of either N or P may therefore tend to approach co-limitation, in which the ratios of two or more available resources nearly match biotic demand (Vitousek and Farrington, 1997; Davidson and Howarth, 2007).

Recent global meta-analyses of fertilization experiments have confirmed that most terrestrial ecosystems increase NPP following N addition (LeBauer and Treseder 2008), but that P limitation and N+P co-limitation are also common (Elser et al., 2007). Surprisingly, few northeastern deciduous forests were included in these analyses; Elser et al. (2007) used only one, and LeBauer and Treseder (2008) included two. However, many fertilization experiments (e.g. Magill et al., 2004; Moore and Ouimet, 2006; Pregitzer et al., 2008) have been conducted in this region since the pioneering work of Mitchell and Chandler (1939) at Black Rock Forest in New York.

Recently, many of the longest-run and most robust forest fertilization experiments have dealt with questions about the ecological and community effects of N saturation. Nitrogen-saturation theory predicts that forests receiving chronic N deposition will first

respond with increased NPP, and then suffer a decline as the capacity of the system to store excess N in biomass and soils is exceeded (Aber et al., 1989). In late-stage N saturation, forests may become P-limited (Mohren et al., 1986; Stevens et al., 1993), because P is commonly the next-most-limiting nutrient after N, and P availability is reduced with acidity. In the northeastern US, researchers have been concerned that the acidifying effects of chronic N and sulfur (S) deposition could lead to forest decline, due to both large leaching losses of base cations and increased mobility of toxic Al cations (Fenn et al., 2006). This raises the possibility of eventual Ca limitation, especially in managed forests where biomass removal and hydrologic export of nutrients following disturbance result in large net losses of nutrient capital, most dramatically for Ca (Federer et al., 1989).

Because of concerns about forest decline and nitrogen saturation, as well as a desire to manage forests for economic value, a large number of experiments have addressed the question of nutrient limitation in hardwood forests of the northeastern US and southeastern Canada, but their collective results have not yet been summarized statistically. Forests in the region are characterized by variation in N deposition, pH, soil mineralogy, soil depth, and forest age and composition, making it difficult to draw general conclusions about nutrient limitation from any single fertilization study. For example, young forests may be more nutrient demanding relative to mineralization, and arbuscular mycorrhizal species might be more responsive to N availability than ectomycorrhizal species (Thomas et al., 2009) due to the inability of arbuscular mycorrhizal fungi to utilize soil organic N (Chalot and Brun, 1998).

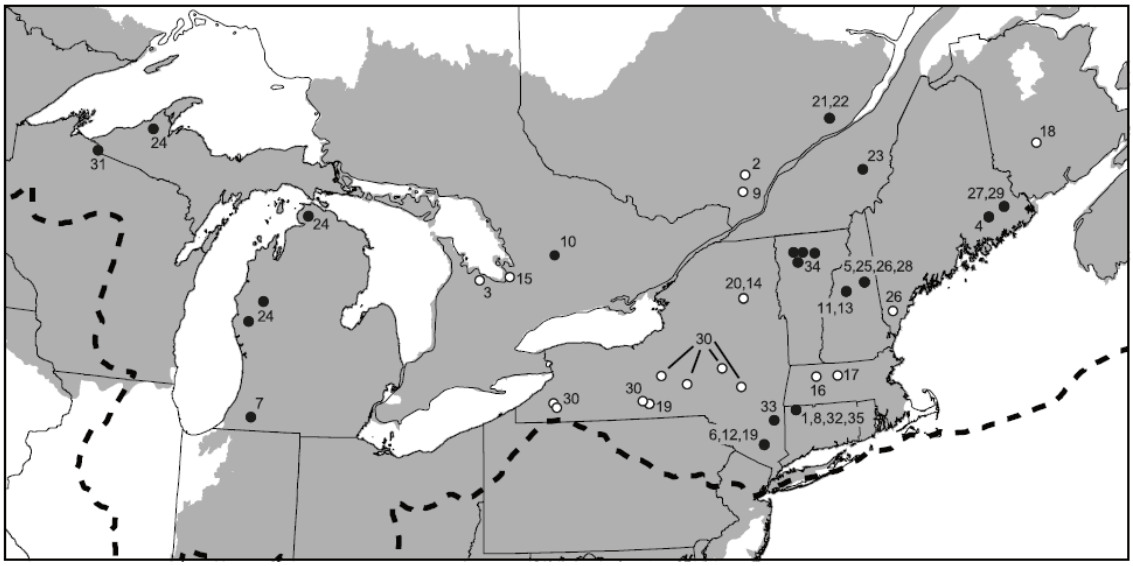
I used meta-analysis (Hedges et al., 1999) to synthesize results from 35 fertilization experiments covering a range of environmental conditions to determine the strength of existing evidence for N, P, and Ca limitation of primary production in hardwood forests. Meta-analysis is a powerful statistical tool that allows data compiled from many similar experiments to be used to infer the direction and magnitude of an effect, often with either greater statistical power or broader basis to draw general conclusions than is possible with a single experiment. I also examined whether the effects of fertilization differed significantly with the amount and form of fertilizer added, and with site characteristics such as the background rate of inorganic N deposition¸ species, and stand age. Finally, I used relevant subsets of the compiled data to determine whether there were significant synergistic effects when nutrients were added together (Elser et al., 2007; Davidson and Howarth, 2007), and whether foliar N:P indicates the nutrient status of a stand.

1.2 Methods

1.2.1 Study region

The area included in this study includes deciduous-dominated forests on glaciated soils in the northeastern United States and southeastern Canada (Fig. 1.1). Only studies from sites on glaciated soils were included, because substrate age has a strong influence on the balance between nutrients derived from the atmosphere (N) and those derived from rock (Walker and Syers, 1976; Vitousek et al., 2010). Conifer-dominated forests were not included in this synthesis.

Figure 1.1 Study region with the locations of experiments used in the meta-analysis. Sites represented by open circles were only included in the simple meta-analysis. The solid black line is the southern extent of the Wisconsin glaciation (Dyke et al., 2003), and the shaded region is the temperate deciduous and mixed forest biome (Olson et al., 2001). Studies are numbered alphabetically by author (see Appendix 1.1).



1.2.2 Dataset criteria

Only studies describing the results of nutrient manipulations involving closed-canopy, hardwood-dominated forests, plantations, or regenerating clearcuts were used. Pot and greenhouse experiments were not included, nor were studies of natural gradients in nutrient availability. All studies used included at least one fertilization with N, P, or Ca, in known quantities per area, and reported data on at least one component of aboveground production (deciduous litterfall or woody biomass increment), or a proxy measurement (diameter, basal area, or volume increment) at the tree or plot scale. In the few cases where multiple publications described results from the same experiment, only the longest-term results were included. Additions of potassium (K) and magnesium (Mg) were also tracked, but were insufficient in number to warrant inclusion in the meta-analysis.

Searches on combinations of terms including “forest,” “fertilization,” “fertilizer,” “limitation,” “growth,” “production,” “nitrogen,” “phosphorus,” “calcium,” “lime,” “dolomite,” and “NPK” were conducted using the ISI Web of Science database and Google Scholar. Relevant papers were searched for citations even when they did not include data that were usable in the meta-analysis. I found a total of 66 journal articles, gray literature reports, and theses describing fertilization studies in and near the study region, of which 35 met my criteria (Appendix 1.1). Because many studies report the results of multiple treatments (e.g. multiple rates or combinations of fertilizers), or divide their results by species without scaling to the stand level, this dataset includes 211 observations, each comprising production data from a treatment and a control. Where results were reported only as graphs, data were estimated by hand-measuring or with Engauge digitizing software (http://digitizer.sourceforge.net/).

1.2.3 Meta-analysis methods

Meta-analysis is a powerful technique for combining the results of many different studies (Arnqvist and Wooster, 1995; Hedges et al., 1999), and is now commonly used in ecology to estimate the direction and magnitude of effects resulting from similar experimental manipulations (LeBauer and Treseder, 2008; Nave et al., 2010). The first step of any meta-analysis is to standardize treatment effects as “response ratios”, which allow the comparison of data that are reported using different metrics (Hedges et al., 1999). A response ratio (R) is calculated for each treatment result reported, as the ratio of production under fertilization to production under control conditions. When possible, I used pre-treatment data to normalize for plot differences (Salonius et al., 1982). An increase in production with fertilization corresponds to response ratio $R > 1$, and a

decrease to $R < 1$. Positive and negative responses reported as statistically significant were tallied for each nutrient or nutrient combination added (N alone, N plus other nutrients, P alone, P plus other nutrients, Ca alone, and Ca plus other nutrients; note that "P alone" includes phosphate fertilizers that contain some Ca, such as superphosphate and triple superphosphate.

In 20 studies the data necessary to conduct a full, statistically valid meta-analysis (sample size and standard deviation of each measured variable) were not reported. I therefore began with an inclusive but relatively simple methodology, following Elser et al. (2007), in which all observations are given equal weight in calculating means and confidence intervals for *R*. Response ratios were *ln*-transformed to normalize the sampling distribution before averaging (Hedges et al., 1999), and confidence intervals are therefore asymmetric. This method was used to analyze all data by addition type (each element alone and in combination with others), and to calculate the mean response to each element by species. Three observations could not be included because $ln(R)$ is undefined when production data for either the control or treatment are negative (net production data are sometimes negative due to mortality). Two-sided *t*-tests on $ln(R)$ were used to compare differences between simple mean response ratios.

Fifteen studies (including 98 observations) reported data sufficient for inclusion in a more rigorous meta-analysis following the methodology outlined by Hedges et al. (1999). Briefly, *R* was *ln*-transformed and sample size and variance data were used to calculate a weight. The weighted mean *ln* response ratio and its standard error were then calculated, taking into account within- and between- experiment variances. Finally, these results were reported as response ratios by taking the antilog of the means and confidence

interval limits. Two-tailed *p*-values for each response ratio were calculated using the standard error of each weighted mean *ln*(*R*) and Student's *t*-distribution.

1.3 Regression analysis

I used single and multiple linear regression to determine whether relationships existed between the response ratio and stand age, DIN deposition rate, whether fertilization was continual or a single pulse, whether measurements were made at the scale of individual trees or whole stands, the mean annual and cumulative amount of each element added, experimental duration, interactions between DIN deposition and each nutrient addition, and 2-way interactions among the nutrient additions. Rates of N deposition are inconsistently reported in the 35 studies, so modern N deposition at each study location was estimated from a kriged interpolation (calculated in ArcGIS 9.1) of 1999-2008 mean DIN at 58 NADP sites (USA), and 12 CAPMoN sites (Canada). For earlier experiments, interpolated current DIN estimates were reduced by 2.1% for each decade they preceded the year 2000, based on the long-term trend in N deposition reconstructed by Bowen and Valiela (2001). Backward stepwise multiple regression based on Akaike's information criterion was used to arrive at the best parsimonious linear model.

1.4 Other statistical tests

One-way ANOVAs were performed on *ln*-transformed response ratios to determine whether there were significant differences in response ratios by the metric used to assess production response (n = 208 in 6 categories: diameter, basal area, volume, biomass, foliage, and total ANPP), or by the form of N fertilizer added (n = 97 in 4 categories: NH_4 only, NO_3 only, NH_4NO_3, and urea).

I tested whether strong co-limitation was indicated by synergistic effects as found by Elser et al. (2007) with a much smaller meta-analysis on the seven studies that reported factorial results. For each study, R_{NP}, R_{PCa}, and R_{NPCa} were calculated as the ratio of the combined effect to the largest single-element effect. As in the larger simple meta-analysis, response ratios were *ln*-transformed before calculating unweighted means.

To test the hypothesis that foliar N:P ratio can be used to separate N-limited from P-limited plants (Güsewell, 2004), I ran a linear regression on the P-only response ratio against the control foliage N:P (mass basis) where reported (n = 8).

1.5 Results

1.5.1 Meta-analysis results

In the simple analysis of 208 observations, all categories of additions resulted in significant increases (α = 0.05) in aboveground productivity (Fig. 1.2a). The mean response ratio for N with other nutrients (1.70) was significantly greater (p = 0.01), than for experiments that added N alone (1.42). Additions of P with other nutrients had an average response ratio of 1.61, which is significantly (p < 0.001) greater than that for P alone (1.15). The difference between mean response to additions of Ca alone (1.44) versus with other nutrients (1.68) was of marginal significance (p = 0.06).

The more statistically rigorous meta-analysis (98 observations), also showed that average responses to all nutrient additions were positive, though not all effects were significant (Fig. 1.2b). Additions of N alone significantly increased NPP on average (p < 0.01), but additions of Ca or P with or without other nutrients, or of N with other nutrients, did not have significant effects on average. This may be due in part to small sample sizes (Table 1.1), especially for P-only fertilizations (n = 3; the extremely large

confidence interval is a consequence of running the *ln*-transformed meta-analysis with so few observations). There was a significant effect of Ca addition when all observations (with and without other nutrients; $n = 31$; $p = 0.03$) were combined. Overall, 85% of response ratios observed for both single-element and multiple-element additions are > 1 (*ln* response ratio > 0), and the shapes of these curves are generally similar (Table 1.1; Fig. 1.3).

Nine of 12 species with multiple single-species observations significantly increased production in response to N alone (Table 1.2). Neither of the species with multiple P-only additions increased production significantly, and of six species in which Ca was added alone, only sugar maple (*Acer saccharum* Marsh.) increased production significantly (Table 1.2). Mixed-species stands showed significant production increases in response to N-only additions and to mixed-element additions overall, but not to P-only additions.

1.5.2 Multiple regression results

Individually, stand age and the rate of DIN deposition showed significant negative linear relationships with the response ratio (Table 1.3). In the full multiple regression model, which included 11 terms and 6 interactions, only the annual and cumulative N addition rates had significant positive effects. Backward stepwise multiple regression eliminated all terms except the annual rate of N addition and a significant negative interaction between this term and DIN deposition. While highly significant ($p < 0.001$), the reduced model explained very little of the total variance in response ratios; the adjusted R^2 was 0.09.

Table 1.1 Sample sizes and effect directions in the meta-analysis dataset.

Number of observations by added element, scale of observation (stand versus tree), direction of effect, and reported significance and variance; ‘‘variance reported’’ indicates the number of observations in each category that can be included in a formal meta-analysis.

	total *n*	positive	sig. positive	sig. negative	*n* with variance reported
Nitrogen	**154**	**132**	**63**	**4**	**73**
N only	100	84	34	3	60
trees	85	71	31	3	53
stands	15	13	3	0	7
N plus other nutrients	54	48	29	1	13
trees	32	28	19	1	6
stands	22	20	10	0	7
Phosphorus	**58**	**52**	**23**	**0**	**12**
P “only” (with Ca)	12	8	1	0	3
trees	6	4	0	0	1
stands	6	5	1	0	2
P plus other nutrients	46	44	22	0	9
trees	24	23	11	0	2
stands	22	20	11	0	7
Calcium	**74**	**62**	**37**	**3**	**32**
Ca only	31	24	14	2	18
trees	27	21	13	2	18
stands	4	3	1	0	0
Ca plus other nutrients	43	38	23	1	14
trees	22	17	12	1	7
stands	21	21	11	0	7

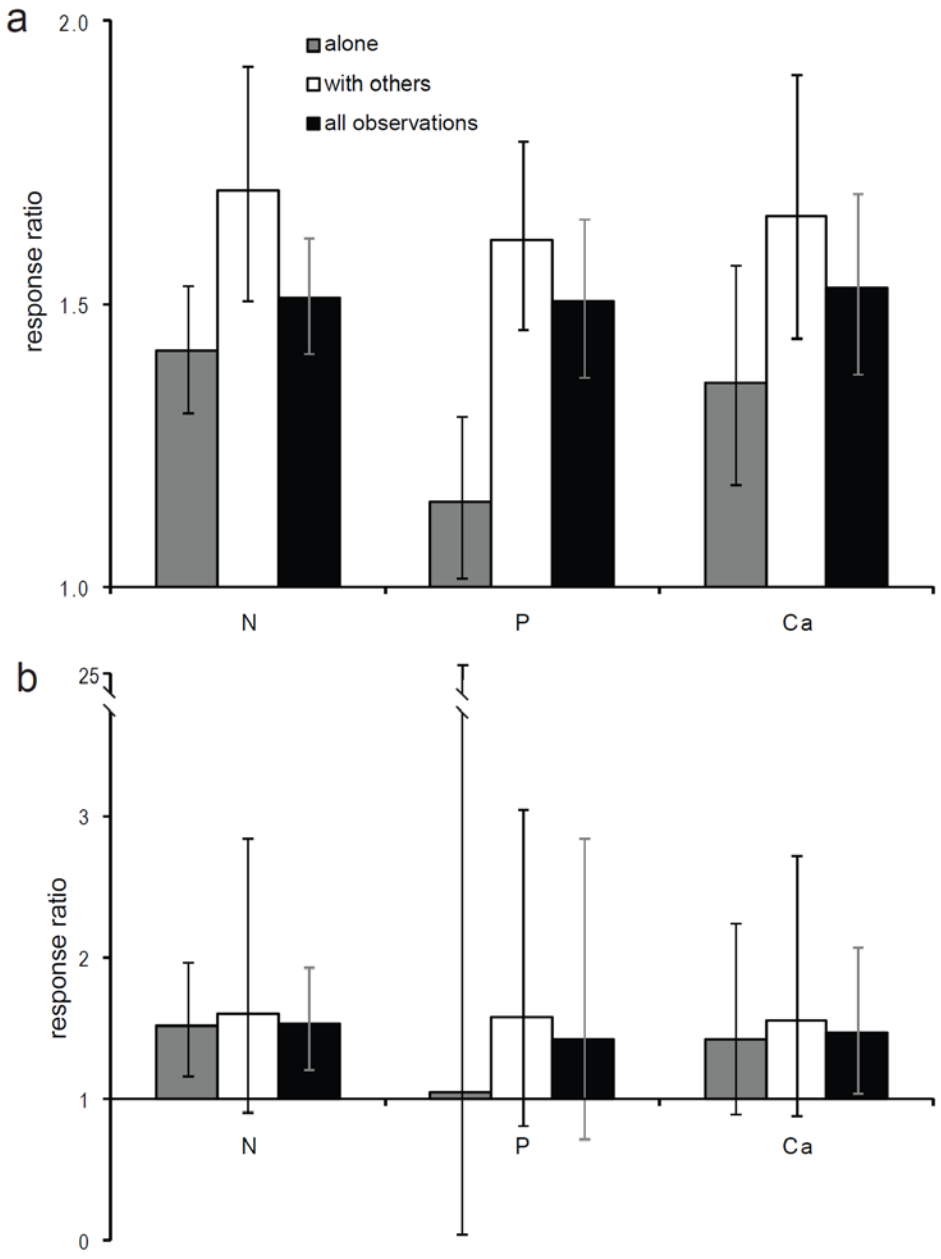


Figure 1.2 Mean response ratios by nutrient addition category.

a. Simple mean response ratios calculated across seven categories of nutrient additions, using data from all 35 studies.

b. Mean response ratios calculated using a smaller data set (21 studies) where reported sample sizes and variance data allow the use of statistical methods recommended by Hedges et al. (1999).

All error bars show 95% confidence intervals for the mean response ratio.

					N only			P only			Ca only			Multiple		
Species	**MR type**	**total studies**	**total obs.**	**single-species obs.**	***n***	**mean *R***	**95% C.I.**	***n***	**mean *R***	**95% C.I.**	***n***	**mean *R***	**95% C.I.**	***n***	**mean *R***	**95% C.I.**
Acer rubrum L.	AM	10	33	19	8	1.25	0.86-1.81	1	1.12		3	0.93	0.42 -2.07	7	**1.72**	1.09-2.69
Acer pensylvanicum L.	AM	1	3	0												
Acer saccharum Marsh.	AM	26	65	46	15	1.28	0.99-1.64	2	0.97	0.59-1.59	15	**1.67**	1.37-2.04	14	**1.53**	1.18-1.98
Betula alleghaniensis Britton	EM	7	16	5	2	1.60	0.68-3.74				2	1.13	0.94-1.36	1	1.51	
Betula papyrifera Marsh.	EM	8	29	22	3	**1.69**	1.19-2.40	3	1.06	0.91-1.23	4	1.06	0.90-1.24	14	**1.73**	1.45-2.06
Carya glabra (Mill.) Sweet	EM	1	1	1	1	2.50										
Carya ovata (Mill.) K. Koch	EM	1	3	0												
Carya spp.	EM	1	1	0												
Fagus grandifolia Ehrh.	EM	10	26	8	5	**1.67**	1.36-2.61				2	1.10	0.88-1.37	1	1.20	
Fraxinus americana L.	AM	5	14	11	10	**1.30**	1.10-1.53							3	1.61	0.93-2.76
Liriodendron tulipifera L.	AM	2	6	6	4	**1.55**	1.29-1.87							2	1.46	0.75-2.84
Nyssa sylvatica Marsh.	AM	1	1	1	1	1.75										
Pinus strobus L. *	EM	1	1	0												
Populus grandidentata Michx.	EM	3	11	8	1	1.52		1	1.35		1	1.17		5	**2.10**	1.62-2.72
Populus tremuloides Michx.	EM	2	12	12	6	**1.42**	1.22-1.66	1	1.41		1	1.54		4	**1.94**	1.31-2.88
Prunus pensylvanica L. f.	AM	2	4	0												
Prunus serotina Ehrh.	AM	3	9	6	4	**1.26**	1.09-1.47							2	**1.37**	1.08-1.75
Quercus alba L.	EM	1	1	1	1	1.62										
Quercus prinus L.	EM	2	3	2	2	**1.62**	1.38-1.90									
Quercus rubra L.	EM	10	28	22	14	**1.59**	1.14-2.20	1	1.42		2	1.30	0.29-5.76	3	1.45	0.32-6.70
Quercus velutina Lam.	EM	1	2	0												
Tilia americana L.	EM	2	8	8	8	**1.65**	1.10-2.48									
Tsuga canadensis L. *	EM	1	3	0												
Mixed stands		9	27	--	11	**1.18**	1.07-1.30	3	1.17	0.75-1.83				12	**1.35**	1.08-1.70

Table 1.2 Number of observations and mean response ratios by species.

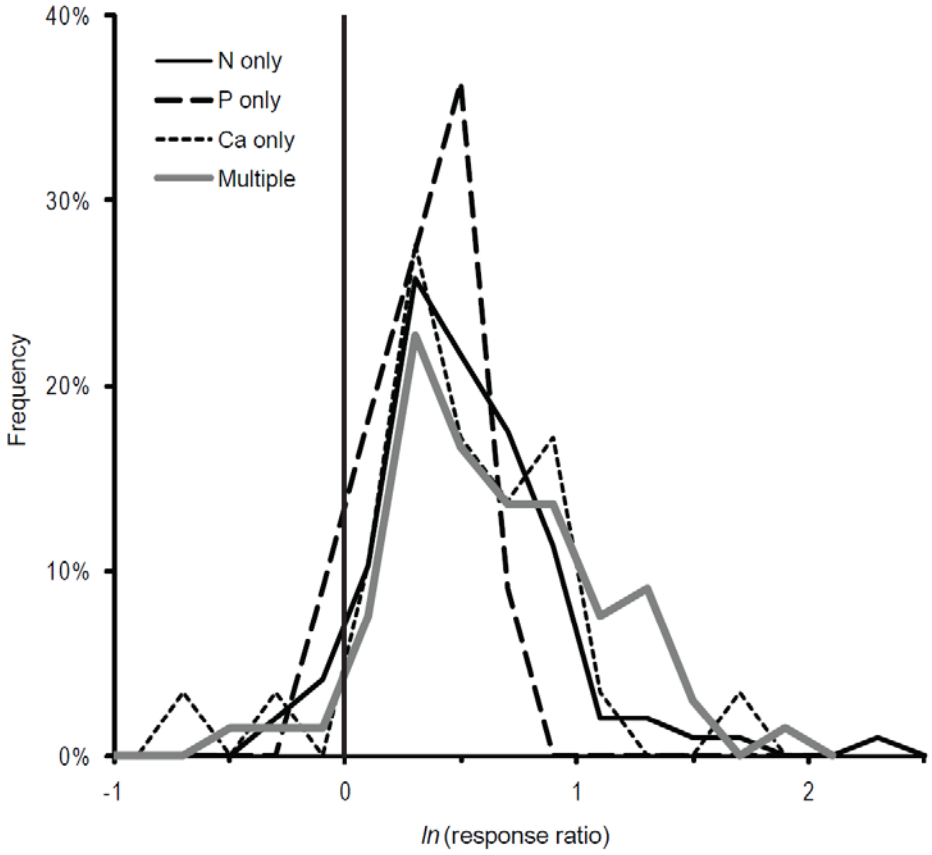


Figure 1.3 Response ratio histograms.

Histograms of the *ln*(response ratio) for four categories of manipulations. Note that three observations are not included because *ln*(response ratio) is undefined when the response variable is negative for either the control or treatment groups. The vertical line at 0 shows the expected mean and mode under the null hypothesis of no fertilizer effect on aboveground NPP. Across all 208 observations, 85% are positive.

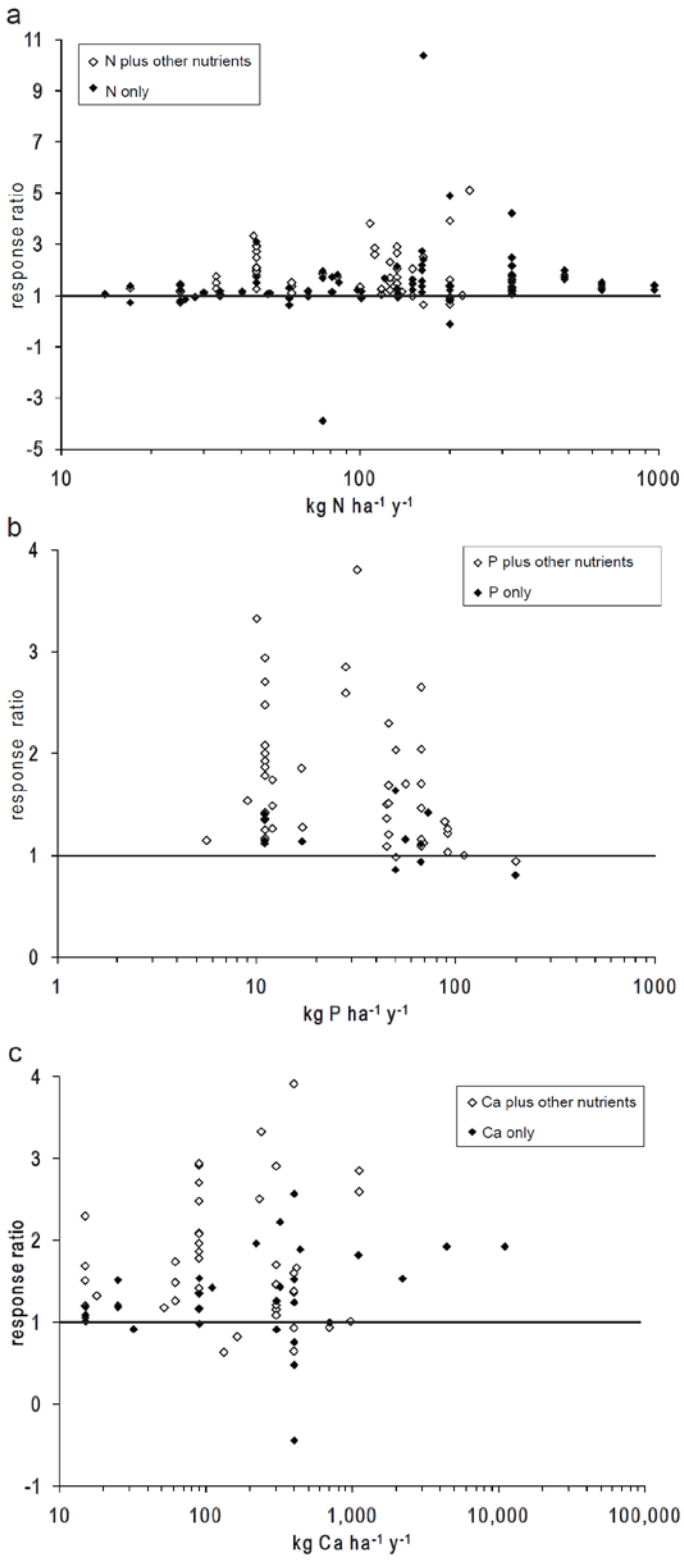


Figure 1.4 Response ratios vs. addition rate for N, P, and Ca.

Note the logarithmic scale on x-axes.

Table 1.3 Multiple regression model summary.

Summary of effect direction and significance in single-effect, full multiple, and backward-stepwise reduced multiple regression models.

	Single models		***Full multiple model***		***Best multiple model***	
	Effect	*p*	Effect	*p*	Coef-ficent	*p*
Annual N	+	ns	**+**	**0.01**	**+0.017**	**0.001**
Cumulative N	+	ns	**+**	**0.04**		
Annual P	-	ns	-	ns		
Cumulative P	-	ns	-	ns		
Annual Ca	+	ns	-	ns		
Cumulative Ca	+	ns	+	ns		
Age of stand	**-**	**0.002**	-	ns		
Experiment duration	-	ns	-	ns		
Continual	+	ns	+	0.09		
Stand	+	ns	-	ns		
N deposition	**-**	**0.006**	-	ns	-0.057	ns
N dep. x ann. N			-	ns	**-0.003**	**0.002**
N. dep x cum. N			-	ns		
N dep. x ann. P			+	ns		
N. dep x cum. P			+	ns		
N dep. x ann. Ca			-	ns		
N. dep x cum. Ca			-	ns		
Ann. N x ann. P			+	ns		
Ann. N x ann. Ca			+	ns		
Ann. P x ann. Ca			+	ns		

Note: ‘‘Continual’’ indicates that fertilization was done continually rather than as single pulse. ‘‘Stand’’ indicates that measurement is based on the stand scale rather than at the scale of individual trees. The full multiple model has an adjusted R^2 of 0.06, $p = 0.05$, and AIC = 19.5. The ‘‘best multiple’’ model has an adjusted R^2 of 0.09, $p < 0.001$, and AIC = 0.3. p values > 0.10 are shown as ‘‘ns’’ and p values < 0.05 are shown in bold. Rates of nutrient addition or deposition are in units of kg ha^{-1} yr^{-1}.

1.5.3 Results of other statistical tests

The production metric used (diameter, basal area, volume, biomass, foliage, or total ANPP) significantly affected response ratios (ANOVA $n = 208$, $F = 3.54$, $p < 0.01$). The form of N fertilizer added had no significant effect on response ratios (ANOVA $n = 97$; $F = 1.04$; $p = 0.37$). Response ratios in the multiple-nutrient treatments of factorial experiments were not significantly greater than the single-nutrient additions: $R_{NP} = 1.05$ ($n = 10$; $p = 0.83$), $R_{PCa} = 1.02$ ($n = 7$, $p = 0.82$), and $R_{NPCa} = 1.09$ ($n = 6$; $p = 0.72$). There was not a significant linear relationship between the N:P ratio of control foliage and P-only response ratio ($n = 8$; $p = 0.65$; $R^2 = 0.04$).

1.6 Discussion

1.6.1 Growth responses to nitrogen fertilization

Nitrogen limitation was tested in 100 observations, with $R > 1$ in 84. Of 34 observations with an N effect reported as significant, 31 had $R > 1$ (Table 1.1). Lebauer and Treseder (2008) found that, globally, temperate forests increased production 19% on average in response to N fertilization. Elser et al. (2007) report a similar result (~ 25%) for terrestrial ecosystems in general. In this meta-analysis, additions of N alone resulted in somewhat greater but not significantly different increases in production (42-51%; Fig. 1.2). However, in contrast with the findings of Elser et al. (2007), adding N in combination with other nutrients did not significantly increase the mean effect (Fig. 1.2).

The amount of N added annually was identified by the reduced multiple linear regression model (Table 1.3) as the only factor significantly affecting the response ratio, along with a negative interaction term that indicates the effect of N fertilization was reduced where atmospheric DIN deposition was high. Mean annual N additions varied

widely (14-970 kg N ha^{-1} y^{-1}; Fig. 1.4a), and usually greatly exceeded ambient DIN deposition (2-10 kg N ha^{-1} y^{-1}). Cumulative N addition had a significant effect independent of annual N addition rate in the full multiple regression model, but was not included in the final regression. The large cumulative N additions in some experiments (up to 2100 kg ha^{-1}) raise the question of why forests should still respond to N additions. Hydrologic and atmospheric losses of N (Vitiousek and Howarth, 1991) might remove some of the added N, or alternatively N might be a resource useful in acquiring other nutrients (e.g. N-rich phosphatase enzymes; Treseder and Vitousek, 2001).

The assembled data show that positive responses to N outnumber negative responses at all annual fertilization rates (Fig. 1.4a). This is somewhat surprising because declining production is a hypothesized consequence of late-stage N saturation (Aber et al. 1989). However, no such effects have been shown for hardwood forests in North America under current N deposition rates (Fenn et al., 2006). Magnani et al. (2007) found a strong positive relationship between N deposition and net ecosystem production at deposition rates similar to those in the study region, and Thomas et al. (2010) report that the growth of several deciduous species correlates positively with N deposition rate. In one N addition study I included, increased mortality was responsible for a decline in production (reported as live aboveground biomass increment) in stands receiving 50-100 kg N ha^{-1} y^{-1} (Wallace et al., 2007). Increased production in the remaining trees could either be due to fertilization or simply to the thinning effect of fertilizer-induced mortality.

Nitrogen was most commonly added as ammonium nitrate (NH_4NO_3), and occasionally as sodium nitrate ($NaNO_3$), Calcium nitrate ($Ca(NO_3)_2$), ammonium sulfate

($(NH_4)_2SO_4$), urea ($(NH_2)_2CO$), or as "complete NPK" fertilizer in which the form of N is not reported. Similar to the findings of Lebauer and Treseder (2008), the form of N fertilizer added did not significantly influence response ratios in this study.

1.6.2 Growth responses to phosphorus fertilization

Elser et al. (2007) report that the mean response of terrestrial ecosystems to P addition is a ~25% increase in production. In the current study, the mean effect of adding P alone was 15% (using inclusive methodology; Fig. 1.2a; $p = 0.03$), or a non-significant 4% (using more rigorous methods on a much smaller dataset; Fig. 1.2b). The smaller effects of P relative to N in the study region may be related soil mineralogy. For example, soils on granitic till contain substantial primary mineral P as apatite, which may be an important source of Ca (Blum et al., 2002). On the other hand, in unglaciated soils just south of the study region in Pennsylvania, Auchmoody (1982) found evidence for N+P co-limitation in black cherry, though Ward and Bowersox (1970) found no response to P alone in mixed oaks.

The one significant response to a P-only addition in the meta-analysis data set was in 14-year-old quaking aspen (Safford and Czapowskyj, 1986), suggesting that P limitation might occur mostly in young forests. Naples and Fisk (2010) found root ingrowth responses to P in regenerating but not mature hardwood stands in New Hampshire, and studies of birch seedlings potted in forest soil responded to N, P, and N+P fertilizations (Hoyle, 1969; Safford, 1982). St.Clair et al. (2008) commented that young sugar maples may be more susceptible than mature trees to P limitation. While Gradowski and Thomas (2008) inferred P limitation in mature sugar maples based on vector analysis and shoot extension, diameter increment (used in the meta-analysis) did

not show a significant response to P fertilization. Whether regenerating forests pass through a P-limited stage under certain conditions deserves further research.

Phosphorus is typically added as triple superphosphate ($Ca(H_2PO_4)_2$). No experiments in my data set added P without also adding Ca in this form, making it difficult to draw conclusions about the effect of P alone on ecosystem productivity. Further complicating matters, P added in soluble form can be rapidly immobilized through sorption to secondary minerals (Jiao et al., 2007). P additions varied from 6 - 200 kg P ha^{-1} yr^{-1} (Fig. 1.4b), and positive responses to P occurred at all but the highest fertilization rates, for which there are few observations. Some have reported increased foliar P concentrations with fertilization (Mercer, 1974; Safford and Filip, 1974; Ellis, 1979; Safford and Czapowskyj, 1986; Fahey et al., 1998; Gradowski and Thomas, 2008), indicating that fertilization did significantly increase P availability. However, others (Finn and White, 1966; Schmitt et al., 1981; Leech and Kim, 1990; Ouimet and Fortin, 1992) found no such increase, which is consistent either with P-sufficiency in the control trees, or with insufficient P availability relative to other added nutrients.

Among "P-only" fertilizations, two-thirds of responses are positive (Fig. 1.3; Table 1.1). Such gains in aboveground NPP might be due in part to reduced carbon allocation to mycorrhizal fungi. A meta-analysis by Treseder (2004) found that average mycorrhizal abundance decreased by 32% under P fertilization, and allocation to mycorrhizal fungi comprised up to ~20% of total NPP in field studies reviewed by Hobbie (2006).

Some have suggested that high foliar N:P ratios imply P-limitation; Güsewell (2004) reviewed these claims and found that N limitation often occurs at N:P < 10, and P

limitation at N:P > 20 (mass basis). I found little support for this idea. There was no significant relationship between foliar N:P and the response to P fertilization. While no study in this data set had a foliar N:P > 20, the only study with a significant response to P alone (Safford and Czapowskyj, 1986), actually had the lowest foliar N:P (~7).

1.6.3 Growth responses to calcium fertilization

Like additions of N and P, additions of Ca had mostly positive effects (Fig. 1.3) across a wide range of fertilization rates (Fig. 1.4c). Under the rigorous meta-analysis, only the combination of all observations (Ca alone and with other elements) was significantly different from no effect (Fig. 1.2b), though this may be attributable to the larger sample size, as the mean response ratio did not differ much between the two categories. Using the more inclusive data set, additions of Ca with other nutrients tended to show a greater mean response than Ca alone, though this was not statistically significant (Fig. 1.2a).

Calcium limitation is inherently difficult to study because most forms of Ca fertilizer increase soil pH, which in turn affects the availability of other important ions, including both nutrients and potentially toxic elements. For example, the solubility of rhizotoxic Al^{3+}, sorption of PO_4^{3-}, nitrification, enzyme activities, and microbial community composition are all sensitive to pH (Sparks, 2003; Paul, 2007), and Ca availability may alter the competitive balance between roots and microbes for nitrogen (Groffman and Fisk, 2011).

In the studies reviewed here, calcium was added in various forms, most commonly as calcite ($CaCO_3$) or dolomite ($(Ca,Mg)CO_3$), but also as wollastonite ($CaSiO_3$), gypsum ($CaSO_4$), calcium nitrate, or calcium chloride ($CaCl_2$). Of these, only

$CaCl_2$ does not affect soil pH. Increases in pH of 0.1 to 0.5 units were reported following additions of 50 - 1600 kg Ca ha^{-1} (Fyles et al., 1994; Wilmot et al., 1996; Juice et al., 2006; Gradowski and Thomas, 2008), though Safford and Czapowskyj (1986) report that O horizon pH increased from 4.3 to 6.1 with the addition of ~900 kg Ca ha^{-1} as dolomite. The effects of most calcium additions on pH make it impossible to determine whether increased NPP was caused by relief of Ca-limitation in the strict sense, or indirectly by pH changes that increased the availability of other macro- and micro-nutrients, reduced Al availability, or altered the ecological relationships governing various biogeochemical process rates. The single study that added Ca as $CaCl_2$ (Kobe et al., 2002) reported increased seedling growth with fertilization, though the effect was significant for only one of three hardwood species measured.

1.6.4 Fertilization responses by species

Sugar maple was the most studied species in the data set (26 studies), reflecting concern about the apparent region-wide decline of this economically important species (Houston, 1987). The species-specific meta-analysis indicates significant positive effects of Ca fertilization and multiple-nutrient fertilization on sugar maple production, but no significant effect from N or P fertilization alone (Table 1.2). That various researchers have implicated several different nutrient deficiencies in sugar maple decline either implies multiple causes, or that region-wide phenomena such as acid deposition (Fenn et al., 2006), drought (Payette et al., 1996) or increased soil freezing (Boutin and Robitaille, 1995) can exacerbate deficiencies of nutrients already low in supply depending on local stand and soil characteristics. For example, Mader et al. (1969) recommended N fertilization, even in acidifying forms such as $(NH_4)_2SO_4$, for declining sugar maple.

Others blame the decline on cation leaching induced by acidic N and S deposition (Fyles et al., 1994; Wilmot et al., 1996; Moore and Ouimet, 2006), recommending addition of $CaCO_3$ alone or with K and Mg. Paré and Bernier (1989) and Gradowski and Thomas (2006) report P deficiency in sugar maple. Due to the influence of human land-use, sugar maple may now occur on sites to which it is poorly suited (Houston, 1999); it has increased in abundance since presettlement (Cogbill et al., 2002; Vadeboncoeur et al., 2012b).

The number of species-by-nutrient combinations with no data in Table 1.2, along with those with no confidence interval (i.e. $n = 1$) highlights the need for further work. Species in the region differ in nutrient ratios and their effects on soil cycling of nutrients (Lovett et al., 2004), which might influence their responses to chronic changes in nutrient availability, though this idea has not been extensively tested. Even among species with large numbers of observations, differences in response ratios should not be over-interpreted. For example, birch and aspen studies were mostly conducted in young stands while many sugar maple studies were conducted in declining mature stands. Thomas et al. (2010) found that arbuscular mycorrhizal species responded more strongly to chronic N deposition, but here ectomycorrhizal species showed non-significantly greater responses to N fertilization than did arbuscular mycorrhizal species (Table 1.2).

1.6.5 Effect of stand or cohort age

When analyzed in isolation, there was a significant negative effect of stand age on *R* (Table 1.3), suggesting that seedlings or young stands may tend to have greater response ratios than older stands when fertilized. In young stands, responses to fertilization may be greater because high overall nutrient demand has led to strong

nutrient limitation of production, whereas most nutrient demand in mature stands is satisfied by recycling of nutrients (Rastetter et al., 2013). However, this effect was not significant in the full multiple regression, and was dropped in the course of backwards stepwise regression (Table 1.3).

1.6.6 Influence of mensuration methods

The use of response ratios in meta-analyses is intended to minimize the effect of different metrics among studies. In this study, production in treatment and control plots was variously reported as diameter increment, basal area increment, volume increment, wood biomass increment, foliar production, and total aboveground (wood + foliage) production. While most of these metrics are based on measurement of diameter increment, different mathematical transformations could result in different reported response ratios for a similar response. An ANOVA indicated that response ratios varied significantly with the type of measurement. Specifically, basal area increment response ratios were significantly smaller than diameter and volume increment responses.

Most studies included in this meta-analysis were replicated at the tree level. Selected trees were measured before and after fertilization, or sampled with increment borers after treatment. When sample sizes are small, mortality over the treatment period is likely to be missed, and the reported aboveground production represents that of the surviving trees only. This is especially true if trees are selected at the end of the treatment period, or if vigor criteria are employed. Where fertilization increases mortality (e.g. Magill et al., 2004; Wallace et al., 2007), or in young stands undergoing thinning, mean surviving-tree production may be much greater than the stand-level live biomass increment. This effect may in part explain the greater increases in young stands

than mature stands, though it would also be expected to lead to greater increases with longer study length, which was not observed. This bias can be avoided in future studies by reporting both tree-level and stand-level production data.

1.6.7 Multiple resource limitation

At the broad scale, if a system is limited primarily by one nutrient, NPP will respond to addition of only that nutrient, but may show larger responses to combined additions once primary limitation is relieved. A system that is co-limited by two nutrients will respond modestly to additions of either nutrient, and more strongly when the two are added together, until a third limitation is encountered (Davidson and Howarth, 2007; Craine, 2009). Globally, the mean response of terrestrial ecosystems to N+P was more than twice the response to either nutrient alone (Elser et al., 2007). Positive production responses occurred with the single addition of all three elements examined here (Fig. 1.2). This pattern implies that these forests are co-limited at least in the sense that resources are allocated to obtaining these nutrients in optimal ratios, and that altered availability allows the reallocation of valuable carbon.

The observation that multiple nutrient additions result in larger NPP increases than single nutrient additions (Fig. 1.2) initially appears to support the results of Elser et al. (2007). However, the single- and multiple-element experiments are not necessarily from the same locations or even in the same forms or amounts. The small meta-analysis I ran on the factorial experiments yielded no significant effects, so the synergistic pattern observed globally by Elser et al. (2007) is not strongly supported by this regional data set. Still, the small but generally positive effects of P and Ca fertilization without N indicate some degree of co-limitation with N in these forests (Figs. 1.2 and 1.3). These forests

can be interpreted as being strongly limited by N, and more weakly limited by P and Ca (or other factors that change with soil pH) even in the absence of synergistic effects.

The law of the minimum is a description of resource limitation at a moment in time. For example, regardless of nutrient availability, a tree is always limited by light at night, and may sometimes be limited by water or temperature. In the short term, any of several factors may limit NPP, but experiments conducted at annual or longer time scales will only identify nutrients that become limiting when conditions are otherwise optimal for photosynthesis. Furthermore there can be complex interactions among limitations. Hypothetically one nutrient might increase the maximum photosynthesis rate, while another improves cold tolerance, both leading to increased production on an annual time step. Alternatively, carbon allocated to acquisition of one nutrient can be re-allocated toward another (e.g. changing rooting distribution), or one nutrient may itself be a resource for acquiring another (e.g. N allocated to phosphatase enzymes). Lags in such re-optimization of resources further complicate the matter. Understanding the interactions among these factors and other limiting resources is a vital challenge for ecosystem ecology, particularly given the rapid anthropogenic alterations of biogeochemical cycles.

1.6.8 Potential biases

The "file drawer problem," a bias against publishing non-significant results, is a drawback of most meta-analyses, which can't include inaccessible or hidden data (Rosenthal, 1979). The effect of unpublished non-significant results is reduced when, as done here, the search for relevant data includes conference proceedings, experiment station bulletins, and unpublished graduate theses, rather than limiting the scope to peer-

reviewed journals. This problem is also mitigated when multiple results (e.g. different fertilization rates or combinations) are reported in a single paper, as in 27 of 35 studies included here. Moreover, if the effect of fertilization truly were zero, there would be as many significantly negative responses as significantly positive ones, and it seems unlikely that data showing significantly reduced production with fertilization would go unpublished.

As with any meta-analysis, conclusions can only be drawn for the ecosystems collectively sampled. Clearly, sampling is non-random, both geographically (Fig. 1.1) and by species (Table 1.2). Differences in the magnitude and direction of response are likely the result of real differences in species, stand age, disturbance history, hydrologic inputs and outputs, soil chemistry, as well as interannual variability, sampling error, and measurement error. Still, this is the best that can be done to synthesize nutrient limitation data regionally: a thorough search for results both published and unpublished within a well-defined biogeographic region.

1.7 Conclusions and recommendations

The results presented here strongly support the hypothesis that nitrogen limits production in deciduous forests of the northeast, but suggest it is not the only limiting nutrient. Rather, the forests studied appear to increase productivity in response to all studied nutrients (Figs. 1.2 and 1.3). Fertilizations with multiple elements generally show larger results than those of single elements (Fig. 1.2), though factorial studies show no evidence of synergistic relief of co-limitation. Responses to P additions are relatively modest, and there is no evidence in these data that they can be predicted using foliar N:P

ratios. Responses to Ca were generally positive. However, the data were insufficient to separate the effects of increased Ca availability from concomitant increases in soil pH.

Future studies should, as resources allow, be designed in such a way that the mechanisms behind the results can be better understood and compared among research sites. Specifically:

- Studies intended to determine which nutrient limits production should ideally have a full factorial design (e.g. control, +N, +P, and +N+P).
- Phosphorus should be added in a form that does not contain calcium, such as NaH_2PO_4.
- To separate the effects of Ca fertilization from soil pH changes, a pH-only manipulation should be added as a reference, for example using Na_2CO_3. Alternatively, Ca can be added as $CaCl_2$ without affecting soil pH.
- To separate the effects of N additions from pH shifts, N could be added in forms that differ in the magnitude of their pH effects, such as $(NH_4)_2SO_4$ and urea. Nitrogen-free acidification treatments could also prove useful in disentangling the effects of pH and N.
- To account for mortality and changing competition dynamics resulting from treatment, effects on NPP should be expressed both on an individual tree and a whole-stand level. All trees should be tagged and measured before and after treatment. Study plots should be as large as is practical, and randomized-block layouts should be employed.
- Means, sample sizes and variance data should be reported for all treatments, even when not significantly different from the control, to allow inclusion in future meta-analyses.

- While convenient, metrics such as radial increment or basal area increment are difficult to compare among stands and trees of different ages. Allometric equations can be used to express these results in more biogeochemically relevant terms, such as biomass increment.

CHAPTER 2

LONG-TERM SUSTAINABILITY OF FOREST HARVESTING IN CENTRAL NEW HAMPSHIRE

Abstract

Removals of forest biomass in the northeastern US may intensify over the coming decades due to increased demand for renewable energy. For forests to regenerate successfully following intensified harvests, the nutrients removed from the ecosystem in the harvested wood (including N, P, Ca, Mg, and K) must be replenished, through a combination of plant available nutrients in the soil rooting zone, atmospheric inputs, weathering of primary minerals, biological N fixation, and fertilizer additions. To estimate how many times the forest can be harvested without diminishing productivity or requiring fertilization, we constructed nutrient budgets for three harvest intensities and compared these with detailed soil data from15 northern hardwood stands on granitic till in the White Mountain region of New Hampshire. These budgets indicate that accelerated soil weathering is required to meet nutrient requirements for biomass accumulation. Sites differed widely in the nutrient capital available to support additional removals before encountering limitations (e.g. a fivefold difference in available Ca, and a tenfold difference in weatherable Ca). Intensive short-rotation biomass removal could rapidly deplete soil nutrient capital, but traditional long rotations, even under intensive harvesting, should not induce nutrient depletion in the 21st Century. Weatherable P may

represent the ultimate limitation to continued biomass removal (in as few as 6 rotations) on granitic bedrock. Understanding how and whether soil weathering rates respond to nutrient demand will be critical to determining long-term sustainability of repeated intensive harvesting over centuries.

2.1 Introduction

2.1.1 Nutrient cycles and sustainable forestry

Deciduous forests in the northeastern United States have a long history of exploitation as a source of fuel and timber. New harvesting methods emerged in the 1970s, in which branches and low-value trees were chipped and sold as fuel rather than left on site. Studies of the increased nutrient removal associated with such harvests raised concern about the potential depletion of important nutrients, especially Ca, from forest soils (White, 1974; Johnson et al., 1988; Federer et al., 1989; Hornbeck et al., 1990; Adams et al., 2000). Interest in forest bioenergy has increased again recently (e.g. Malmsheimer et al., 2008, Richter et al., 2009), driven by energy price volatility and the goals of reducing net greenhouse gas emissions and dependence on imported energy.

Sustainable forestry comprises management practices that do not reduce the capacity of the forest to provide important ecosystem services in the future, including consideration of management effects on water quality, biodiversity, species composition, and forest productivity (Janowiak and Webster, 2010; Walker et al., 2010; Berger et al., 2013). Here we address potential productivity declines due to nutrient removal in stands harvested repeatedly. From this perspective, sustainability requires that removals of nutrients from ecosystems be balanced by inputs to plant-available pools (Worrell and Hampson, 1997; Sverdrup and Svensson, 2002; Flueck, 2009). Though many forests in

the northeastern USA have already been harvested and regrown two to three times, continued harvest removals and associated hydrologic losses of nutrients will eventually reduce net primary productivity unless ecosystem inputs increase above current estimates. Observations of nutrient availability and productivity in whole-tree harvested stands have yielded mixed results, at least for the relatively short time scales examined thus far (Thiffault et al., 2011). Though analogous forest systems elsewhere in the world are often fertilized to replace nutrients where biomass removals are high (e.g. northern Europe; Stupak et al., 2008), fertilization is not currently common practice in the northeastern USA.

Exchangeable nutrients have historically been considered the nutrient pool most available to plants and of greatest relevance in assessing productivity (Marschner, 1995). However, exchangeable pools contain only a small fraction of the additional nutrients required by a regrowing forest (e.g. Likens et al., 1994; 1998). More relevant to longer-term productivity is the rate of supply of these nutrients from less-available pools or sources external to the ecosystem, relative to the rate needed to support regrowth (Rastetter and Shaver, 1992; Craine, 2009). Indeed, at decadal time scales, even vigorous biomass accumulation seems not to deplete exchangeable soil nutrient pools (Johnson et al., 1991, 1997; Bélanger et al., 2004).

Nutrients enter the ecosystem via atmospheric deposition and the weathering of geologic substrates; N may also be fixed microbially. Base cations (Ca, Mg, K) are primarily weathered from silicate minerals, while the most important source of P is the accessory mineral apatite ($Ca_5(PO_4)_3(F,Cl,OH)$). Apatite is also an important source of Ca in granitic soils (Blum et al., 2002; Nezat et al., 2004), because it weathers more

rapidly than the silicate minerals (Allen and Hajek, 1989). Weathering rate estimates are inherently variable and difficult to compare across methods and locations (Klaminder et al., 2011; Futter et al., 2012). Long-term weathering rates have been estimated from soil profiles in the White Mountain region (Nezat et al., 2004; Schaller et al., 2010). However, base cation weathering rates needed to close ecosystem budgets (Likens et al., 1996, 1998; Hyman et al., 1998) are sometimes an order of magnitude greater than these long-term means. This discrepancy is a common finding among studies of similar soils (Table 2.1), despite the long-term decline in weathering rate that should occur as soils age (Taylor and Blum, 1995). This discrepancy has been attributed to elevated acid deposition (Langan et al., 1995), but hydrological Ca losses cannot be fully explained by observed acidic inputs (Hamburg et al., 2003). Rooting-zone soil weathering rates are difficult to assess at the watershed scale, where fluxes are small relative to the large dynamic stocks, uncertainties are often large (Likens and Bormann, 1995; Yanai et al., 2012), soils vary over short spatial scales, and significant chemical contributions to streamflow may occur below the rooting zone (Zimmer et al., 2012).

Another potential driver of high apparent weathering rates is that soil weathering may be accelerated when there is increased biotic demand (Hamburg et al., 2003). The removal of large amounts of biomass over the past ~150 years is a novel disturbance in the region's forests. Wind, ice damage, and infrequent fires have been the dominant forms of disturbance over the past 10,000 years and generally leave most nutrient capital on site. Regrowing forests may shift resource allocation towards the acquisition of nutrients other than N, such as P (Rastetter et al., 2013). Ectomycorrhizal fungi are

Table 2.1 Long-term soil weathering rates and watershed-scale denudation rates. Rates are in kg ha^{-1} y^{-1} and are for granitic soil in the study region and elsewhere. Long-term weathering rates are derived from the depletion of each element relative to an immobile reference element, assuming that the C horizon represents unweathered parent material. Denudation rates are estimated from watershed budgets in which major fluxes are measured and weathering is assumed to account for the missing term required to achieve mass balance.

a) Long-term soil profile weathering rates	**Soil age (ka)**	**Ca**	**K**	**Mg**	**P**
in study region:					
Schaller et al. (2010) regional mean	14	0.59	2.11	0.33	0.043
range of 13 site rates used in this study		0.11-1.14	0.42-4.23	0.06-0.91	0.017-0.083
Nezat et al. (2004), HBEF W1	14	1.46	4.18	0.51	0.114
studies also reporting "denudation" rates (see below):					
April et al. (1986), New York	14	2.0-3.6	5.0-5.9	1.0-1.5	
Kirkwood and Nesbitt (1991), Ontario	12	2.6	2.8	1.0	
Bain et al. (2001), Scotland		0.04-0.10	0.11-0.45	0.006-0.035	
other rates for reference:					
Taylor and Blum (1995), Wyoming	11-21	1.1-1.7	1.0-1.9	0.015	
Egli et al. (2008), Switzerland and Italy	12-16	0.0-4.3	0.04-3.7	0.11-4.7	
Olsson and Melkerund (2000), Sweden and Finland	9	1.4-1.6	0.6-1.6	1.6-2.4	~0.01
Newman (1995), New Zealand	6-12				0.1-0.3

b) Denudation rates from watershed budgets	**Ca**	**K**	**Mg**	**P**
in study region:				
Bailey et al. (1996), Cone Pond	1.2-3.3			
Hyman et al. (1998), Cone Pond	2.18	1.08	1.09	
Likens and Bormann (1995), HBEF *	21.1	7.1	3.5	
Likens et al. (1998), HBEF	2.00-3.12			
Wood et al. (1984), HBEF *				1.5-1.8
studies also reporting long-term weathering rates (above):				
April et al. (1986), New York	3.3-23.0	0-1.3	0.3-3.6	
Kirkwood and Nesbitt (1991), Ontario	10.8	0.2	2.6	
Bain et al. (2001), Scotland	1.6		2.6	
other rates for reference:				
Marchand (1971), California	17	1.1	1.8	0.03
Lelong et al. (1990), France	2.7-11.2	2.8-6.5	2.4-5.5	
Clayton and Megahan (1986), Idaho	13.6	1.63	1.43	

* Probably greatly overestimated due to budget error (Likens et al., 1994; Likens and Bormann, 1995).

HBEF = Hubbard Brook Experimental Forest

known to directly weather primary minerals (such as apatite) by etching mineral surfaces with organic acid exudates under conditions where the weathering products (such as P) are limiting (Landeweert et al., 2001; Hoffland et al., 2004; van Scholl et al., 2008). Greatly elevated rates of apparent mineral weathering have been observed in aggrading pine mesocosms (Bormann et al., 1998; Balogh-Brunstad et al., 2008), and may occur in rapidly aggrading forest stands as well (Hamburg et al., 2003; Bélanger et al., 2004).

2.1.2 Research approach and objectives

Analyses of forest management sustainability typically compare management-induced nutrient losses to nutrient inputs via atmospheric deposition and weathering (e.g. Sverdrup and Svensson, 2002; Duchesne and Houle, 2008). Building specifically on work by Federer et al. (1989), we extend this approach by comparing net nutrient loss per rotation to *stocks* that are, or might reasonably be considered, available over multiple rotations, under a range of assumptions about harvest intensity and nutrient availability. Specifically, we ask:

1. What is the net nutrient balance per rotation under various harvesting scenarios?
2. How much variation exists in nutrient stocks (exchangeable, organically bound, and apatite) among stands that are ostensibly similar in species composition and soil type?
3. Assuming that exchangeable and organically bound nutrients can be depleted over multiple rotations, which nutrient eventually becomes limiting (i.e. is exhausted first) under each harvesting scenario?
4. If apatite in the rooting zone can be rapidly made available how many additional rotations would be possible?

The first question relates directly to "strong" definitions of sustainability, whereby resource stocks must be maintained at current levels over time (e.g. Goodland and Daly, 1996; Flueck, 2009). The second and third questions stem from the observation that ecosystems may continue to function normally despite some level of stock depletion. Finally, to avoid depleting ecosystems beyond critical thresholds, it is necessary to understand the variation in soil nutrient stocks at spatial scales relevant to management decisions.

Our approach necessarily involves many assumptions about the magnitude of fluxes that are difficult to estimate across a variable landscape and about how they will change over time with increasing nutrient stress. When simplifying assumptions must be made, we choose those that likely lead to an overestimation bias of the number of rotations that can be sustainably harvested in the northern hardwood region.

2.2 Methods

2.2.1 Study sites

We sampled soils in 15 deciduous forest stands of varying age in the White Mountain region of central New Hampshire (Fig. 2.1; Table 2.2). Dominant species included American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), and yellow birch (*Betula alleghaniensis* Britton) in mature stands, and white birch (*Betula papyrifera* Marsh.), red maple (*Acer rubrum* L.), and pin cherry (*Prunus pensylvanica* L. f.) in younger stands. One site (B1) was a former pasture where red spruce (*Picea rubens* Sarg.) was mixed with northern hardwoods, and the area sampled at the Hubbard Brook Experimental Forest (HBEF) has red spruce and balsam fir (*Abies balsamea* L.) at higher elevations.

Table 2.2 Description of the 15 study stands used in the rotation analysis. "Cuts" indicates the number of times a site had been harvested as of 2004. Sites are ordered geographically from southwest to northeast (Fig. 2.1 below).

	Bedrock	Elev. (m)	FF (cm)	Depth to C (cm)	Rock vol. (%)	Age (yrs)	Cuts
BW	Concord granite	570	12	30	26	> 100	~ 1
B1	Concord granite	490	5	36	19	~70	1
HBEF	Rangeley schist	600	7	50	18	70-100	~ 1
M6	Conway granite	540	5	66	34	23	2
C1	Mt Osceola granite	570	2	74	36	14	2
C2	Conway granite	340	4	73	26	16	2
H6	Conway granite	330	13	61	17	19	2
C4	Conway granite	410	5	78	15	26	2
C6	Conway granite	460	6	38	15	28	2
H4	Conway granite	350	4	73	25	64	2
H1	Conway granite	320	5	68	14	68	2
C8	Mt Osceola granite	330	3	74	31	~ 120	1
C9	Conway granite	440	8	85	33	~ 120	1
T30	Rangeley schist	550	6	48	23	55	2
M5	Rangeley schist	630	7	48	36	26	2

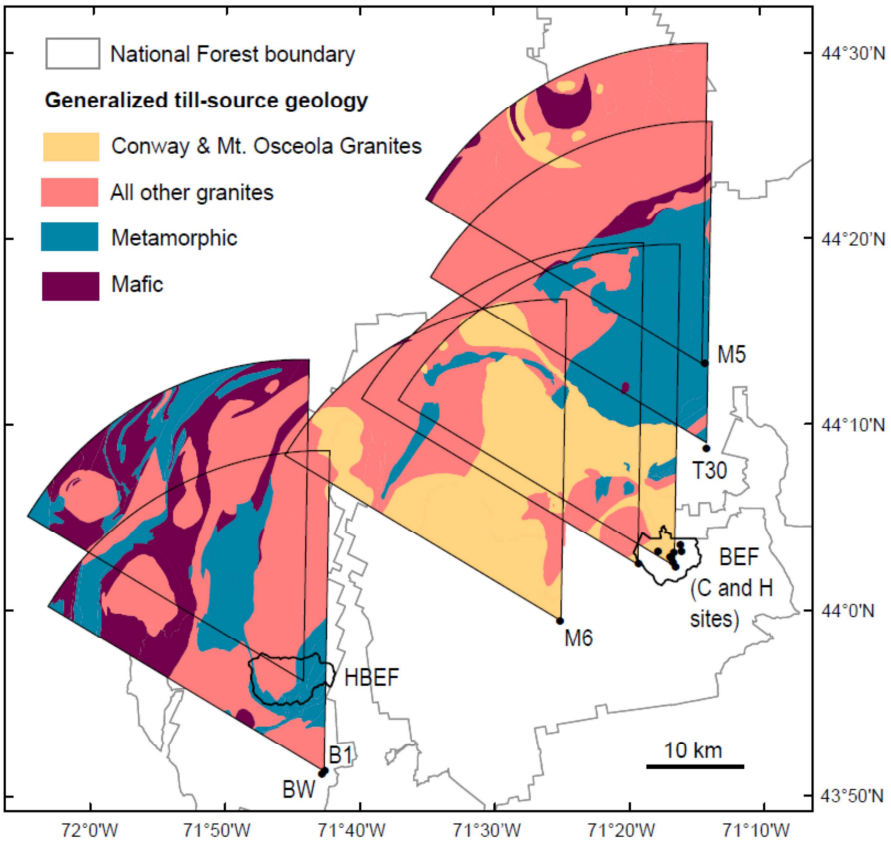


Figure 2.1 Location of the 15 study stands used in the rotation analysis. The wedge-shaped areas to the northwest of each site outline the approximate till source area for each site (Hornbeck et al., 1997). Geologic data are simplified from Lyons et al., (1997).

Soils were primarily well-to-moderately drained, coarse-loamy, mixed-frigid typic Haplorthods developed on granitic glacial till.

2.2.2 Sample collection

Three 0.5 m^2 quantitative soil pits were excavated at each of 14 study sites (excluding HBEF) in 2003-4, following methods described in detail by Vadeboncoeur et al. (2012a). The Oie and Oa horizons were collected in their entirety. Mineral soil samples were quantitatively excavated in several depth increments to the top of the C horizon, sieved to 12 mm in the field, weighed, homogenized, and subsampled. The top 25 cm of the C horizon was also quantitatively excavated in at least one pit per stand.

Soil data for HBEF were assembled from multiple data sets collected in three first-order watersheds on the same south-facing slope. Forest floor samples (60 pin-block samples) were collected at Watershed 6 in 2002 and analyzed for total elemental content (Yanai et al., in review). Mineral soils were sampled in 59 quantitative soil pits in the adjacent Watershed 5 in 1983; one from each of four elevation zones was randomly chosen for analysis (Hamburg et al., 2003). C horizon samples from Watershed 1, approximately 1 km to the east, were analyzed by Nezat et al. (2004), but sampling was not quantitative; C horizon mass in the top 25 cm was estimated as the mean of that measured in the other 14 stands.

2.2.3 Laboratory analysis

Organic horizon samples were air-dried, subsampled, and dried to constant mass at 60 ºC. Oa samples were sieved to 6 mm and Oie samples were milled. Mineral soil samples were air-dried and sieved to 2 mm; subsamples were oven-dried at 105 ºC. Total N concentrations were measured on a CE Instruments Model NC2100 elemental

analyzer. Oa and mineral soil samples were subjected to a sequential leach procedure adapted from Nezat et al. (2007) to measure exchangeable, organic, and weathering-accessible apatite fractions of each mineral nutrient. Each leaching step was conducted for 24 hours at 20 ºC. First, exchangeable cations were extracted with 1 M NH_4Cl. Then, soil organic matter was extracted in 30% H_2O_2. Finally, each sample was leached with 1 M HNO_3, which has been shown to congruently dissolve apatite in contact with the solution, though ~30% of total apatite may be shielded by more resistant minerals (Nezat et al., 2007). Oa samples were also subjected to a final leach in concentrated HNO_3 for 3 hours in a microwave digester. Oie samples were microwave-digested in concentrated HNO_3 rather than sequentially leached, because they had little mineral matter. Concentrations of Ca, Mg, K, and P in all soil extracts were measured on an Optima 3300 DV ICP-Optical Emission Spectrometer. Mineral soil samples from HBEF were not subjected to the H_2O_2 leach; P is the only element for which this leach extracts a substantial amount relative to the first (exchangeable) leach in mineral soils. We estimated H_2O_2-extractable P at HBEF using the mean ratio of total mineral soil $C:P_{H2O2}$ across the other 14 stands.

2.2.4 Scenario description

We predicted ecosystem nutrient depletion over multiple rotations based on a range of assumptions about nutrient inputs and outputs (scenarios I and II), harvest intensity (scenarios a, b, and c), and the stocks of nutrients to count as "available" to the ecosystem over multiple rotations (scenarios 1, 2, and 3). We used combinations of scenarios to address our specific research questions, and report summarized results across the 15 stands.

The net depletion or enrichment of each nutrient was calculated as the difference between the nutrient removal per rotation and the ecosystem inputs (atmospheric deposition and soil weathering) during the rotation length. We conducted this calculation under two sets of assumptions about ecosystem inputs and outputs: I) using geologic long-term average weathering inputs and assuming zero baseline streamflow output, or II) using weathering rates estimated from ecosystem budgets at HBEF and nearby watersheds and associated hydrologic outputs from the reference watershed at HBEF.

2.2.4.1 Ecosystem input and output data

Bulk atmospheric deposition and streamflow fluxes of all macronutrients have been monitored at HBEF since the 1960s (Likens, 2012a; b; c); we used mean inputs and outputs for the period 1985-2004. We did not include dry deposition as an N input, due to high landscape-scale variability (Lovett et al., 1997) and its small magnitude (e.g. 3-6% of total N inputs; Weathers et al., 2006). We include total dissolved P analyzed in bulk collector solutions, which may somewhat overstate ecosystem inputs due to the mineralization of locally derived particulate P (e.g. pollen), despite quality-control standards excluding visibly contaminated samples (Stelzer et al., 2002).

We calculated harvest-induced leaching, which we included in all scenarios, as the cumulative 22-year difference between streamwater nutrient flux from HBEF Watershed 5 (clearcut by whole-tree harvest in 1983) and that of the adjacent reference watershed after accounting for the small pre-treatment difference between these streams (Yanai et al., 2005; Likens, 2012b,c). Increases in export over the reference baseline

were similar in magnitude to those measured by Hornbeck et al. (1990) throughout New England for 3 years after clearcutting.

Scenario I: We used stand-specific weathering inputs of Ca, K, Mg, and P calculated by Nezat et al. (2004) and Schaller et al. (2010), based on profile depletion relative to titanium. One stand (M5) lacked a C horizon, making it unsuitable for this approach, so we used mean weathering rates from the other 13 stands. Two others (C1 and H6) failed to meet the assumptions of the profile method for one or more nutrients; for these elements we also used mean values. We conservatively assumed zero baseline (non-harvest-related) leaching of nutrients under this scenario.

Scenario II: We included 20-year observed streamflow losses of nutrients (Likens et al,. 2012c), and also included recent budget-based weathering estimates from HBEF Watershed 6 for Ca (Likens et al., 1998) and nearby Cone Pond for K and Mg (Hyman et al., 1998). Phosphorus weathering is highly uncertain (Appendix 2.1); we estimated P weathering from the Likens et al. (1998) estimate of Ca weathering, assuming that 17% of long-term Ca weathering was in the form of apatite (midpoint of the 12-22% range estimated by Nezat et al., 2004). This estimate of current P weathering is in the middle of the range of other estimates we considered (Appendix 2.1).

2.2.4.2 Biomass removal scenario data

To estimate net nutrient balances per rotation, we paired nutrient budgets under scenarios I and II with estimates of total nutrient export per harvest (in scenarios a, b, and c, below).

Scenario a: Stem-only removal on a ~100-year rotation is a common forest management practice in which merchantable saw and pulp logs are removed from a site,

while branches, poor quality trees, and smaller trees are left on site, either standing or as slash. This is approximately equivalent to shorter return intervals with less intensive harvesting each time (e.g. 30% of basal area every 30 years). To estimate the nutrient capital removed in this type of harvest, we used the 2007 vegetation inventory from 550-745 m elevation at HBEF Watershed 6. Wood and bark contents (Siccama, 2007) were summed for all trees > 12.7 cm DBH to estimate nutrient removals for a heavy timber and pulpwood harvest. Basal area for this stand was 25 m^2 ha^{-1}, and estimated biomass removal was 125 dry metric tons per hectare. Biomass and nutrient content may be somewhat lower than is typical for the region (Fahey et al., 2005), but the allometry and nutrient stocks are uniquely well validated (Arthur et al., 2001).

Scenario b: A more intensive scenario is whole-tree harvesting on the same 100-year rotation. This is the same as the previous scenario, except that non-merchantable parts of trees are also removed for bioenergy use, rather than being left on site. We assumed winter harvesting of deciduous trees, with no removal of foliage. We used the same vegetation inventory as in the previous scenario to calculate the biomass stock of all trees > 2 cm DBH, subtracting leaves and the small amount of slash estimated by Arthur et al. (2001). Biomass removal in this scenario is 187 dry metric tons per hectare, a 50% increase over the stem-only scenario.

Scenario c: The most intensive scenario we modeled was whole-tree harvesting on a shorter, 35-year rotation, which would theoretically maximize the biomass harvest rate, at least over the first few harvests. We used biomass and nutrient content from four stands in the Bartlett Experimental Forest (BEF) that were clearcut between 1975 and 1980, and inventoried in 2011. Nutrient concentrations (Fatemi, 2007) and allometric

equations (Fatemi et al., 2011) were specific to these stands. Basal area averaged 32 m^2 ha^{-1}. Allowing for 40% of branch biomass to be left on site due to typical harvest inefficiency (Briedis et al., 2011; somewhat more than in the very thorough W5 harvest), this harvest would yield 156 dry metric tons per hectare, 240% as much biomass over 100 years as the 100-year whole-tree scenario.

2.2.4.3 Soil nutrient availability

For each study stand, we calculated available (i.e. ultimately depletable) stocks of N, Ca, K, Mg, and P in each of three "nutrient availability" scenarios:

Scenario 1: We first assumed that only exchangeable and organically bound and complexed nutrient pools would become available over one to several harvest rotations. For the Oa organic nutrient content, we used the leach that correlated best with organic matter content for each nutrient (the 20 ºC HNO_3 leach for K and Mg; and a microwave HNO_3 leach for Ca and P), to avoid including the mineral content of the Oa horizon.

Scenario 2: Because P is the least abundant geologically derived nutrient in the soil parent material relative to biotic demand, and because apatite may be an important source of Ca to forest ecosystems in the region (Blum et al., 2002; Hamburg et al., 2003; Yanai et al., 2005) our second scenario adds apatite in the B horizon to the "available" stock of Ca and P.

Scenario 3: In the most optimistic scenario, we assumed that apatite in the top 25 cm of the C horizon was also biologically available. Federer et al. (1989) assumed that "unweathered" parent material only became available to biological uptake at a rate equal to the physical denudation rate. However, 5-7% of total fine root biomass mass in the 14 studied stands was found in the C horizon (Yanai et al., 2006; Park et al., 2007), which

appears to be typical for the region (Donahue, 1940). It is not known to what extent these roots provide access to C-horizon nutrients, but it is conceivable that rooting depth and carbon allocation to mycorrhizae and deep roots might increase when weathering-derived nutrients are limiting (Chapin et al., 1985; George et al., 1997; Bever et al., 2009; Smits et al., 2012).

2.2.5 Soil stock depletion calculations

The number of supportable rotations (N) was calculated as the ratio of the available nutrient stock (S), to nutrient removal per rotation (R), accounting for other ecosystem-scale input (F_{in}) and output (F_{out}) fluxes over the rotation length (T_R):

$$N = \frac{S}{R + T_R(F_{out} - F_{in})}$$

We used only the more conservative net nutrient budget scenario (I) for these estimates. Supportable rotations under varying harvest intensities were compared under scenarios Ia1, Ib1, and Ic1. We examined variability of depletable nutrient stocks among stands by calculating the range in number of 100-year whole-tree harvest rotations required to deplete exchangeable plus organic nutrient stocks, and exchangeable plus organic plus apatite stocks (scenarios Ib2 and Ib3).

2.3 Results

Assembling stand-level budgets for various types of rotations shows that N inputs exceed outputs in all except the most intensive harvesting scenario (c). On the other hand, nutrient balances were negative (net ecosystem depletion) for Ca under all management and nutrient input-output scenarios (Table 2.3). Magnesium and K showed net depletion under all scenarios except Ia and Ib, and P showed net depletion in all

Table 2.3 Net nutrient stock changes per rotation.
These calculations are conducted under three scenarios of harvest intensity and two nutrient budget scenarios. These are combined to calculate net stock changes per rotation, which are applied to each site under various scenarios of nutrient availability.

	Ca	K	Mg	P	N
	Stock removed per harvest (kg ha^{-1})				
a) 100-year stem-only harvest	296	99	26	17	222
b) 100-year whole-tree harvest	456	189	40	28	395
c) 35-year whole-tree harvest	359	150	30	19	349
all: harvest-induced leaching	74	47	16	0	50
	Other fluxes (kg ha^{-1} y^{-1})				
all: observed precip input at HBEF	1.12	0.64	1.09	0.18	8
I: mean baseline weathering*	0.59	2.2	0.25	0.043	0
II: budget-inferred weathering at HBEF	2.28	1.08	1.09	0.18	0
II: observed stream output at HBEF	7.24	1.76	2.09	0.011	2.6
	Net stock change per harvest rotation (kg ha^{-1})				
Ia*	-198	137	15	-6	528
Ib*	-359	48	2	-18	355
Ic*	-373	-98	-26	-15	-119
IIa	-754	-150	-109	6	268
IIb	-914	-240	-123	-5	95
IIc	-567	-198	-69	-11	-210

* For calculations based on budget scenario I, site-specific weathering rates were used rather than the means shown here.

Table 2.4 Soil nutrient stocks measured at each site (kg ha^{-1}).

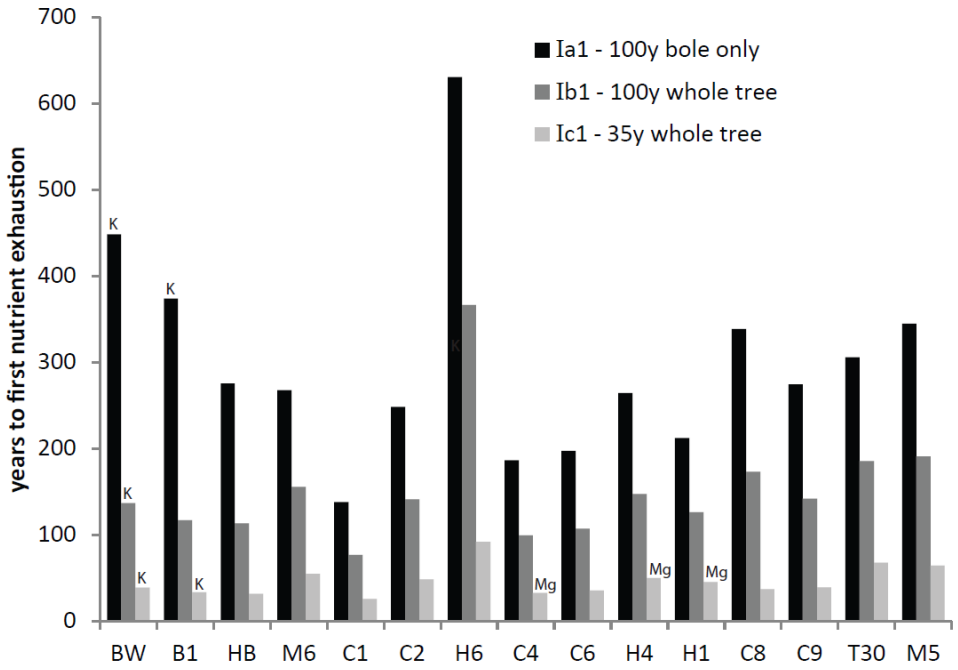
	exchangeable and organic thru B					apatite in B horizon		apatite in C horizon to 25 cm	
	Ca	K	Mg	P	N	Ca	P	Ca	P
BW	1125	176	153	178	5298	405	222	4985	1927
B1	546	152	80	125	5322	1114	674	5937	2581
HBEF	308	162	61	194	8608	647	534	1408	913
M6	594	199	45	109	7752	69	91	32	116
C1	274	260	28	38	3688	212	109	188	130
C2	523	214	43	56	4265	148	59	247	114
H6	1403	262	81	170	7734	625	493	697	387
C4	341	198	37	58	5051	73	61	194	150
C6	373	144	35	73	5277	136	64	405	313
H4	533	220	43	72	6895	411	365	110	68
H1	499	224	40	100	6338	179	151	262	152
C8	567	157	34	62	4143	586	390	1054	454
C9	471	174	32	104	6590	1266	850	1238	585
T30	755	278	74	116	5047	562	591	1269	757
M5	684	196	98	113	5673	657	483	0	0

scenarios except IIa. Though the most intensive (c) harvesting scenarios may appear to have less negative nutrient balances than others in Table 2.3, note that these values are per rotation. Normalized to the same time scale, these imbalances are far more severe than the other two cases.

The nutrients examined differed in patterns of variation among stands when we examined stocks in the exchangeable fraction plus organic matter (Table 2.4). Nitrogen and K, which varied about twofold among stands (with CVs of 24% and 21% respectively) showed considerably less variation than Ca, Mg, and P, which varied at least fivefold (CV of 45%, 47%, and 41% respectively). Variation among stands in apatite stocks in the B horizon was substantially less than in the C horizon (Table 2.4).

The number of rotations that could be supported by the complete mineralization and uptake of all organic and exchangeable nutrients in the O and B horizons varied widely among stands and especially among harvest scenarios (Fig. 2.2). Calcium was the most common nutrient to be depleted first in the bole-only scenario, though K limitation was encountered first at the Bald Mountain stands. Bole-only harvesting could be supported for one to six additional rotations by these stocks. This range would be one to four rotations if we omit stand H6, where nutrient stocks are probably overestimated; our three soil pits randomly sampled areas with much deeper Oa horizons than the mean sampled in 50 forest floor blocks in the same year (Vadeboncoeur et al., 2012a). In the whole-tree harvest scenarios, Ca, K, or Mg limited production, depending on the stand, before two additional rotations were completed. In all cases, calculations based on input-output budget II (assuming observed hydrologic losses and weathering rates calculated by difference) indicated more rapid depletion of soil nutrients than the calculations presented

Figure 2.2 Times to nutrient depletion in three harvest scenarios across 15 stands. These estimates assume that weathering proceeds at the long-term baseline value (Budget scenario I), and that only exchangeable and organically bound nutrients are available on the time scale of multiple rotations (Availability scenario 1). All three harvest scenarios are shown. Calcium is the first nutrient exhausted in all scenarios except those indicated for K and Mg. Note that our data for site H6 may overestimate Oa horizon nutrient stocks due to random sampling error.



here under budget I (assuming zero baseline hydrologic output and long-term mean weathering rates from profile depletion).

Including B-horizon apatite as a Ca and P stock that could be made available via accelerated mycorrhizal weathering dramatically increased estimates of potential future production (Table 2.5). This limit ranges widely from about six to over 40 rotations under a 100-year whole-tree harvesting rotation. However, unless the weathering of other Ca-bearing minerals also accelerates, Ca supply may present a more immediate constraint; B-horizon apatite stocks of Ca supply only an additional one to five rotations.

If roots and mycorrhizal fungi were able to efficiently utilize the much larger stocks of apatite in the C horizon (an uncertain proposition, given the low density of roots at this depth), supportable 100-year whole-tree harvests would double in some cases, though by very little where the C horizon is shallow or poor in apatite (Table 2.5).

Table 2.5 Number of whole-tree rotations required to exhaust apatite P and Ca. Weathering of non-apatite Ca was assumed constant at long-term rates (Table 2.1).

	exch + org + apatite thru B (Scenario **I2b**)		exch + org + apatite thru C25cm (Scenario **I3b**)	
	Ca	**P**	**Ca**	**P**
BW	4.2	21	18	108
B1	5.1	37	23	154
HBEF	3.2	33	8.0	75
M6	1.7	10	1.8	15
C1	1.3	7	1.8	13
C2	1.8	6	2.4	11
H6	5.1	33	6.9	50
C4	1.2	6	1.7	13
C6	1.4	7	2.6	21
H4	2.5	20	2.8	23
H1	1.7	13	2.3	20
C8	3.4	21	6.4	42
C9	5.0	45	8.6	72
T30	3.2	33	6.3	67
M5	3.6	29	3.6	29

2.4 Discussion

2.4.1 Validity of assumptions

Our estimates likely represent an upper bound to the number of harvests that each stand would be able to sustain without additional nutrient inputs. This is because we intentionally made a number of assumptions that bias the result in this direction. For example, our use of HBEF Watershed 6 for harvestable nutrient content estimates may understate regional standing biomass nutrient stocks in mature stands, due to the biomass

of this stand being on the low side of regional variation (Leak and Smith, 1996; Fahey et al., 2005; van Doorn et al., 2011; Reiners et al., 2012; Rastetter et al., 2013). On the other hand, errors are potentially quite large when applying allometric equations and nutrient contents beyond the sites to which they apply. Biomass at stands C8 and C9 at the Bartlett Experimental Forest was 35% and 26% greater than at Watershed 6, respectively, and estimated removals under WTH range from 20% greater for P to 90% higher for Ca, indicating the potential for more rapid depletion of nutrients if these sites were cut and regained their current biomass in 100 years. Measurements of standing biomass base cation content at a similar site in Québec (Tremblay et al., 2012) fall between the HBEF and Bartlett ranges, while biomass and nutrient removals estimated for a whole-tree harvested stand of unreported age in northern NH (Hornbeck et al., 1990) were somewhat lower than the HBEF Watershed 5 estimates.

We also assumed constant atmospheric inputs in the future, which is more likely for some nutrients than for others. Widespread declines in base cation deposition (Hedin et al., 1994) generally precede the 20-year period we used. The data are unclear whether P deposition has also decreased in the region, but this would be expected given that mineral aerosols are the dominant atmospheric source of both base cations and of P (Newman, 1995). N deposition has recently declined markedly in the region (Bernal et al., 2012). Balancing removals under scenarios Ia and Ib would require deposition of 2.7 and 4.5 kg ha^{-1} y^{-1} respectively, plus enough to balance any hydrologic N losses that continue under reduced atmospheric loading. The depletion of N accumulated in SOM over elevated N deposition in the 20th century would reduce the impact of this potential

future imbalance, as would biological N fixation, which has been inferred in aggrading ecosystems on N-poor substrates (Bormann et al., 2002).

We assumed that the entire organic pool of nutrients was available over the relevant time scale, though the mineralization of organically bound N and P may be limited by overall OM decomposition rates. Much of this material can be fairly recalcitrant, though mycorrhizal fungi under nutrient-limited conditions can be expected to allocate C to enzymes that may liberate these nutrients from complex organic substrates, even at a net energy cost (Orwin et al., 2011). Furthermore, we assumed that forest production and nutrient uptake would continue until available stocks of nutrients were fully depleted, though in reality uptake and growth would slow in approaching this limit.

One effect that may compensate for our multiple "liberal" assumptions is that we assume constant nutrient content of successive tree rotations. Nutrient concentrations tend to decrease with nutrient stress in foliage, and likely also in wood and bark (DeWalle et al., 1991), though this has not been extensively studied. Species differ widely in overall wood nutrient concentrations, and also in the ability to remobilize nutrients from heartwood (Meerts, 2002). To the extent that some current nutrient uptake represents "luxury" uptake, i.e. uptake beyond an amount that affects production, such decreases would increase the number of potential rotations, as future nutrient exports in the biomass would be smaller than assumed in our analyses. However, for limiting nutrients, large decreases in uptake would necessarily be met by decreases in production (Craine, 2009).

Another possible area of underestimation is our use of the top 25 cm of C horizon nutrient stocks, despite C horizons which extended deeper than this at most sites (Vadeboncoeur et al., 2012a). However, making efficient use of nutrients deeper than this would likely entail a large increase in root and mycorrhizal density and activity at these depths which are not traditionally considered part of the rooting zone.

2.4.2 Weathering

In scenario I, our calculations conservatively assumed only the long-term, pedogenic time, mean weathering rate from observed profile depletion relative to titanium. Current rates should theoretically be lower than long-term means, due to a reduction in weatherable mineral surfaces and depletion of the more rapidly weatherable minerals as soils age (Taylor and Blum, 1995). However, current watershed budgets (Table 2.1) require a rate of soil weathering greater than the long-term mean, assuming that soil organic and exchangeable pools are in equilibrium, to explain the large observed difference between outputs of base cations in streamwater and inputs in atmospheric deposition in both aggrading and steady-state stands (Likens and Bormann, 1995; Romanowicz et al., 1996).

It is difficult to explain how forests are regenerating and accumulating biomass Ca while also losing Ca and other cations in streamflow unless weathering rates are elevated far above their long-term means (Hamburg et al., 2003; Yanai et al., 2005) or the available pool below the rooting zone (which has not been monitored over time) is becoming depleted. Current biotic demand for P and base cations might exceed the long-term steady state due to prior harvest removals, early stages of ecosystem N saturation, a warming climate, and increased atmospheric CO_2 concentrations (Peñuelas et al., 2012).

The degree to which mycorrhizal weathering of apatite in the B and C horizons can mitigate nutrient depletion and subsequent declines in productivity remains to be determined, and deserves further research. Other minerals may be subject to similar processes; fungal weathering of biotite may be an important source of K and Mg to ecosystems (Wallander and Wickman, 1999; Rosling et al., 2004), which may be important in stands where these nutrients are predicted to be depleted before Ca (Table 2.2). Feldspar minerals contain the majority of total Ca and K in granite-derived soils (Nezat et al., 2007) but less work has been done to determine whether the slower process of feldspar weathering might be influenced by biotic demand for these elements.

If apatite can be made available at an accelerated rate when demand is increased, many questions remain unanswered. If Ca deficiency drives apatite weathering in excess

Table 2.6 Total biomass (dry metric tons) harvestable before nutrient exhaustion. Data are shown by site and assuming budget scenario I, availability scenario 1, and each of the three harvest intensity scenarios.

site	100 y bole only	100 y whole tree	35 y whole tree
BW	578	256	172
B1	482	218	148
HBEF	355	212	140
M6	345	291	243
C1	178	143	115
C2	320	264	214
H6	813	685	410
C4	241	186	145
C6	255	200	158
H4	341	276	222
H1	274	236	202
C8	437	323	164
C9	354	265	174
T30	395	347	302
M5	445	357	286
mean	**388**	**284**	**206**

of P demand, excess P may become “occluded”, associated with Al and Fe secondary minerals. Alternatively, if apatite is weathered at an increased rate due to biotic demand for P, Ca may leach out of the system in stream water; this process could be one explanation of sustained elevated streamwater of Ca from Watershed 5 at HBEF for at least 20 years following whole-tree harvesting (Yanai et al., 2005). Allocation of carbon to deeper roots and associated mycorrhizal fungi providing greater returns of limiting nutrients (Chapin et al., 1985; Bever et al., 2009; Kiers et al., 2011) may represent a significant carbon cost to the trees, which might have implications for productivity. Allocation to mycorrhizal fungi may account for ~20% of primary production (Hobbie, 2006), and appears to vary with the availability of N and P (Treseder, 2004; Vadeboncoeur, 2010; Vicca et al., 2012).

2.4.3 Implications for management and policy

Shorter rotations would yield more biomass in the short term, but much less in the long run (Table 2.6) due to the higher nutrient concentrations of biomass removed, as well as fewer years of atmospheric and weathering inputs between harvests. For this reason, at any given harvest intensity, longer rotation lengths would be more sustainable than short ones.

While we use a single value for harvest-induced leaching of nutrients across all harvest scenarios, more moderate harvesting scenarios (patch cutting, strip cutting, single-tree selection, diameter-limit cutting) may reduce these losses, even if they are more frequent (Hornbeck and Leak, 1992). However, harvest-induced leaching accounts for only about 20% of total rotation Ca losses under the whole-tree harvesting scenario; the bulk of nutrient capital exported each rotation is in the biomass.

It is also possible, depending in part on harvest conditions, that changes in site nutrient status will affect the species composition of the regenerating forest, with consequent effects on timber value and the wildlife habitat quality. These effects might be expected to precede declines in overall forest productivity, as species more tolerant of low-nutrient conditions become more competitive. For example, if oaks are more Ca-efficient than sugar maple (Meerts, 2002) and replace this species on Ca-depleted sites, ecosystem-scale Ca limitation would occur more slowly. Such species shifts over multiple rotations will be determined not only on nutrient availability, but also on climate change, dispersal mechanics, and silvicultural practice. These effects are typically considered aspects of sustainability (Worrell and Hampson, 1997), and may reasonably affect the decisions of land owners and foresters regarding the intensity of future management.

2.4.3.1 Landscape-scale variation

Our data show a high degree of variability in soil nutrient stocks at the landscape scale. All stands included in this analysis are upland sites representative of the type harvested in the region; half had been clearcut since 1970. Variability can be dramatic even at small spatial scales. For example, the mean coefficient of variance (CV) among the three 0.5 m^2 pits at each stand in B-horizon apatite Ca was 67%. Nezat et al. (2004) found similar variation in nutrient stocks and weathering rates across HBEF Watershed 1 (12 hectares). Much of this variation is due to the amount of soil, though total soil nutrient contents also vary with the depth of the O horizon and parent material. Thus, nutrient content increases with the depth of these horizons and decreases with soil rockiness.

Variation is greatest across stands separated by tens of kilometers, which is probably attributable to variation in parent material mineralogy (Fig. 2.1). Stands with the lowest stocks of nutrients tended to be located on Conway granite, while those on other types of granitic bedrock (particularly Concord granite at Bald Mountain) had dramatically greater C-horizon Ca and P capital (Table 2.2). These differences reflect documented differences in apatite abundance between these lithologies (Billings and Wilson, 1965). Also of relevance in glaciated landscapes is the parent material in the source region "upstream" of each stand, which may have contributed significantly to the local glacial till; in glaciated areas the till source area may be more important than underlying bedrock in predicting potential nutrient supply (Fig. 2.1; Hornbeck et al., 1997).

2.4.3.2 Regional-scale variation

The stands in our study do not represent the full range of soil types in the region. Much wider variation would probably be apparent if we had included sites with a variety of sedimentary parent materials. Nezat et al. (2008) characterized HNO_3-extractable Ca stocks (including both apatite and carbonates) across the northeastern United States from New York to Maine, including three of our study stands, which were generally low, especially relative to sites on carbonate-bearing sedimentary parent material. Both the HBEF and Cone Pond watersheds have lower-than-average streamwater Ca export compared with other small watersheds in northern and western New England (Hornbeck et al., 1997), suggesting generally higher Ca availability outside the White Mountains.

Unfortunately, systematic data on soil mass and mineral content do not exist at a regional scale. NRCS soil classifications focus on physical and limited chemical

characteristics of the soils, which provide some important information (texture, rockiness, organic concentrations) but are insufficient to address questions of long-term nutrient supply. These classifications, along with site-index guidelines relating soil texture and slope position to species composition and production (e.g. Leak, 1978), are a reasonable starting place for estimating long-term production, but ultimately such assessments will require information on soil mineralogy. Existing regional analyses classifying ecosystem sensitivity to acid deposition (e.g. Robinson, 1997) give a rough sense of parent material controls on base cation supply, but do not address soil primary P stocks. New continental-scale soil chemistry datasets (Smith et al., 2011) provide coarse but potentially useful data on regional variation in soil P and other nutrients.

2.4.3.3 Fertilization

Fertilization could mitigate or reverse nutrient depletion from harvest removals. However, the possibility of short-term nutrient pulses in runoff has raised concern, especially because the most cost-effective time to fertilize is during harvesting (Stupak et al., 2008). Fertilizers and the labor required to apply them may be quite costly relative to the marginal time-discounted value of forest products removed, especially if the return interval is long. Over the long term, hardened (slow-release) wood ash might be an economical alternative to mined mineral fertilizers, especially as global exploitable phosphate reserves become depleted (Smil, 2000). From an ecosystem perspective, the application of locally sourced wood ash to regenerating forests is an appealing solution, as it closes the nutrient cycle. However, care must be taken in determining heavy metal concentrations, appropriate application rate, and timing (Karltun et al., 2008). While gains in productivity in the current rotation may not be substantial without also adding N

(Pitman, 2006), our analysis indicates that returning mineral nutrients may be critical to sustaining future rotations.

2.4.3.4 Policy implications

Forest harvest guidelines generally recommend against whole-tree harvesting at sites with wet or thin soils, steep slopes, or rare species (Stupak et al., 2008; Evans et al., 2010). Coarse sandy soils or those with a history of fire or intensive agriculture have also been suggested as indicators of vulnerability to nutrient loss (Hallett and Hornbeck, 2000). Our analysis shows that such guidelines might not identify some sites that are vulnerable in the long-term to nutrient depletion; some sites that appear to be most vulnerable to nutrient depletion were clearcut by the USFS in the past 30 years.

Across much of the Northeast, particularly in areas where bedrock is more mafic or contains carbonates, nutrient stocks are probably adequate to support one to several whole-tree rotations at about a 100-year interval without substantial ecosystem consequences. However, short-rotation clearcuts have a high risk of depleting nutrient capital due to greater total biomass removal rates and shorter recovery time, and should not be considered without additional intensive research into mineral soil weathering rates and nutrient stocks at a range of spatial and temporal scales. Currently, woody biomass prices are too low for such intensive management to be economically viable, but this situation could change rapidly if policies favoring bioenergy were adopted at the state or federal level, so it is important to ensure that best-practices guidelines recognize this risk. Biomass accumulation in stands that our analysis indicates are vulnerable to nutrient depletion are similar to that in stands throughout the region (Fatemi et al., 2011; Reiners et al., 2012), and if 100-year rotation lengths are utilized there should be little concern

that whole-tree harvesting might lead to a net depletion in exchangeable base cations (Johnson et al., 1991; Bélanger et al., 2004) for the foreseeable future. However, more research is needed to determine which forests might face nutrient depletion with future harvesting and whether bioenergy can be derived from these forests into the 22nd century.

CHAPTER 3

DEVELOPING BIOGEOCHEMICAL TRACERS OF APATITE WEATHERING BY ECTOMYCORRHIZAL FUNGI

Abstract

Chronic acid deposition has depleted calcium (Ca) from many New England forest soils, and intensive harvesting may reduce phosphorus (P) available to future rotations. Granitic glacial till soils contain trace amounts of apatite, a primary calcium phosphate mineral, which is an important long-term source of both P and Ca to ecosystems. The extent to which ectomycorrhizal fungi enhance the weathering rate of primary minerals in soil remains poorly quantified, in part because within-plant processes mask signals from biogeochemical tracers. Rare earth elements (REEs) and Pb isotope ratios show some potential for revealing differences in soil apatite weathering rates across forest stands and silvicultural treatments. To test the utility of these tracers, we grew mycorrhizal and non-mycorrhizal birch seedlings under controlled P-limited conditions, supplemented with mesh bags containing granite chips. Granite chips incubated with seedlings showed elevated exchangeable REE concentrations and significantly more radiogenic Pb isotope signatures relative to those without, supporting enhanced apatite dissolution. REE concentrations in roots were greatly elevated in treatments with granite relative to those without granite. Among roots grown with granite, radiogenic Pb

isotopes correlated with REEs, demonstrating uptake of apatite weathering products. However, in both roots and leachates, some non-mycorrhizal birches induced as much weathering as those with mycorrhizae. Ca/Sr and Ca/Ba ratios in roots appeared to be less sensitive indicators of apatite weathering. Ectomycorrhizal sporocarps collected in six New Hampshire forest stands showed some variation among sites and taxa in these tracers, but did not lead to clear conclusions about stand-scale soil weathering processes. In summary, these tracer systems show promise as tracers of biological weathering processes, but may be difficult to use in real-world ecosystems.

3.1 Introduction

3.1.1 Soil weathering in the context of forest ecosystem nutrient budgets

Chronic acid deposition and intensive harvesting have prompted debate about the potential for the depletion of nutrient stocks from forest ecosystems to reduce primary production throughout the northeastern United States (Federer et al., 1989; Likens et al., 1996; Adams et al., 2000), particularly in areas with thin, acidic soils derived from granitic bedrock, such as the White Mountains of New Hampshire and the Adirondack region of New York. Recently, Naples and Fisk (2010) used fertilized root-ingrowth cores to infer some degree of calcium (Ca) and phosphorus (P) limitation in regenerating (post-clearcut) New Hampshire forests. Despite the strong evidence that forests are usually primarily N-limited (LeBauer and Treseder, 2008), fertilization experiments suggest that N+P co-limitation may also occur in some stands in the region (Vadeboncoeur, 2010; Crowley et al., 2012), while Ca limitation in the strict sense cannot be inferred from the design of most Ca addition experiments. Phosphorus may temporarily limit regenerating hardwood stands as stoichiometry of accumulating

aboveground biomass changes asynchronously with N and P mineralization rates from litter and soil organic matter (Rastetter et al., 2013). Such model predictions are sensitive to the assumed soil weathering rates of P and other nutrients (Chapter 2).

As soils age, the ecosystems they support generally shift from limitation by nitrogen, which is fixed or deposited from the atmosphere and practically absent in igneous minerals, to limitation by phosphorus, which is relatively rapidly weathered from primary minerals in soils, and eventually becomes unavailable in insoluble secondary minerals (Walker and Syers, 1976; Vitousek et al., 2010). However, a wide range of ecosystems may be close to balanced co-limitation between N and P (Chapter 1, Elser et al., 2007). In New England's granitic bedrock and the soils derived from them, the majority of weatherable P is found in the accessory mineral apatite, a calcium phosphate that weathers more readily than the bulk rock (Lindsay and Vlek, 1977; Guidry and Mackenzie, 2003; Neaman et al., 2006). Rooting-zone apatite weathering is a major source of Ca and the dominant input of P to forest ecosystems in this region (Blum et al., 2002; Nezat et al., 2008).

In managed forest ecosystems, harvested biomass removed from the site contains large stocks of P (~30-40 kg P ha^{-1}; Arthur et al., 2001) that in the absence of biomass removal would recycle conservatively through the ecosystem (Yanai, 1992). Exchangeable and organically bound stocks of these nutrients in forest soils are only 1-3 times the stock removed by the whole-tree harvest of a mature stand (Chapter 2), though aggrading forests may not measurably deplete these stocks (Johnson et al., 1995, 1997; Thiffault et al., 2011). At current rates of acidic deposition, base cations leach from the soil profile at greater rates than their deposition from the atmosphere, with leaching

temporarily increasing upon harvesting (Hornbeck and Kropelin, 1982; Federer et al., 1989; Likens and Bormann, 1995). Imbalances in nutrient budgets, especially those of Ca and P, following harvesting have raised recent and renewed concern about whether yields will be sustained over multiple rotations (Sverdrup et al., 2006; Thiffault et al., 2011, Chapter 2). These concerns are particularly relevant as forests are being widely touted as a source of renewable and sustainable bioenergy. While the fertilization of forests is common practice in many high-intensity forestry systems worldwide, it is not currently economical in the Northeast, and questions about the long-term sufficiency of global P supplies for agricultural uses (Cordell et al., 2009; Elser and Bennett, 2011) make its continuing use in forestry uncertain over multiple rotations.

The remaining ecosystem input that can balance forest ecosystem budgets, and the one typically calculated by subtraction of other measured fluxes and stock changes, is soil weathering. Chemical weathering is the gradual process by which primary minerals in the soil parent material are either dissolved, entering the ecosystem and becoming subject to hydrologic loss, or converted to thermodynamically stable secondary minerals. However, baseline weathering rates of primary P inferred from soil profile depletion (Nezat et al., 2004; Schaller et al., 2010) are often more than an order of magnitude too low to replace the large nutrient export represented by harvest removals at a ~100-year rotation (Chapter 2). Accelerated weathering of primary minerals in the rooting zone of nutrient-depleted forest ecosystems has been proposed as a mechanism for balancing the Ca and P demand of regenerating forests (e.g. Hamburg et al., 2003), but these fluxes have again been inferred mainly by subtraction of other budget terms with large and often unquantified errors. Such calculations are unlikely to reveal differences in weathering

rate among sites with the required precision (Klaminder et al., 2011; Futter et al., 2012). A reliable tracer system for the weathering rates of important primary minerals (apatite in particular, which weathers easily and contains both Ca and P) would provide an important independent metric to confirm whether rates of weathering in the soil profile respond to increased biotic demand.

3.1.2 Mycorrhizal fungi as soil weathering agents in forest ecosystems

The extent to which biotic demand for nutrients determines patterns of chemical weathering is an important unanswered question in critical zone science (Brantley et al., 2011). Ectomycorrhizal fungi form symbiotic relationships with plant roots in which nutrients acquired by exploratory fungal hyphae in the soil are exchanged at the root for carbohydrates fixed by the plant. Some taxa of ectomycorrhizal fungi can directly weather feldspar minerals (Leake et al., 2008), and presumably also associated apatite (Fig. 1). Organic acids released by fungal hyphae in close physical contact with mineral grains can greatly accelerate mineral dissolution, while chelating acid anions and the absorptive surfaces of the fungal hyphae reduce free ion concentrations in solution (Landeweert et al., 2001; Welch et al., 2002; Rosling and Rosenstock, 2008; van Scholl et al., 2008; Bonneville et al., 2011; Gazzè et al., 2012). Plants may depend upon ectomycorrhizal fungi to liberate phosphorus from primary minerals in soil and rock, specifically from apatite (Wallander, 2000; Hagerberg et al., 2003; Adeyemi and Gadd, 2005). Mycorrhizal fungi forage for exploitable nutrient patches in the soil (Rosling et al., 2004; Leake et al., 2008), while plants in general allocate more carbon to mycorrhizal fungi when nutrient limited (Rosenstock, 2009), and apatite in particular appears to stimulate ectomycorrhizal production (Hagerberg et al., 2003). As the importance of this

process to overall ecosystem budgets is vigorously debated (Van Breemen et al., 2000; Smits et al., 2005; Sverdrup, 2009), additional study of these processes is needed both in realistic culture conditions and in the context of real-world ecosystems.

Ectomycorrhizal fungi vary widely in their enzymatic capabilities, depth of soil exploration, and production of organic acids that can enhance nutrient acquisition (Agerer, 2006; Van Scholl et al., 2008). The ectomycorrhizal community changes significantly under chronic N deposition (Lilleskov et al., 2008, 2011), presumably due to decreased competitive advantage of fungi that efficiently utilize organic forms of N and a general down-regulation of plant C allocation to mycorrhizal fungi. The interactive effects of losses of P and base cations coupled with chronic deposition of N and acidity on forest biogeochemical cycles may hinge on the response of mycorrhizal fungi to deposition and other global change factors.

3.1.3 Geochemical tracers of apatite weathering

3.1.3.1 Alkali Earth metals

Sr and Ba are chemically similar to Ca and have both been used as biogeochemical proxies to determine the relative contributions of various sources of Ca to ecosystems (Blum et al., 2002; Bullen and Bailey, 2005; Drouet and Herbauts, 2008). Apatite has a much greater Ca/Sr and Ca/Ba ratios than feldspars, which contain the majority of Ca in granitic rocks. However, significant biological fractionation of Ca from Sr and Ba occurs in trees, and these discrimination factors must be independently determined by species (e.g., Dasch et al., 2006) and also may be affected by mycorrhizal colonization (Hoff, 2009). Strontium isotopes can be used to distinguish between atmospheric and mineral sources of Sr (and Ca by inference) to ecosystems, but since Sr

and Ca are decoupled by biological processes, the fidelity of this proxy is somewhat questionable. In testing hypotheses that include changes in fungal C allocation, the lack of data on Ca/Sr and Ca/Ba fractionation within mycorrhizal fungi makes the use of such proxies to distinguish among sources of Ca within the soil particularly problematic.

3.1.3.2 Rare Earth elements

Minerals differ widely in the ability of their crystal structures to accommodate substitutions of trace elements for their major constituent elements; these compatibility differences are commonly used in geochemical investigations. The crystal structure of apatite accommodates relatively high concentrations of metals with 3+ charge and large ionic radii, including U, Th, and REEs (Hughes et al., 1991; Fleet and Pan, 1997), as a substitute for the calcium cation. Apatite sequesters REEs at concentrations up to 10^4 times their overall concentration in the whole rock, with greatest enrichment among the lighter-mass REEs such as lanthanum (La) and cerium (Ce) (Nagasawa, 1970; Watson and Green, 1981; Gromet and Silver, 1983; Bea et al., 1994). Europium (Eu) is enriched in apatite to a much lesser degree than the other REEs, due to its variable charge under reducing conditions (Duchesne, 1983). Because they are biologically inactive and chemically almost identical, the REEs and their elemental ratios prove to be useful tracer of the dissolution of apatite (Harlavan and Erel, 2002; Harlavan et al., 2009; Calvaruso et al., 2013). However, even when taken up by roots, these elements are not very mobile within plants, and may accordingly be fractionated within the plant (Kabata-Pendias et al., 1992; Tyler, 2004; Stille et al., 2006; Semhi et al., 2009).

Sporocarps of ectomycorrhizal fungi provide a convenient way of sampling soil-free fungal tissue, with the added benefit of easily distinguishing among functional

groups of ectomycorrhizae. To the extent that REEs accumulate in the tissue of the fungal symbiont (Borovička et al., 2011), they may trace the mineralogic sources of nutrients accessed by ectomycorrhizal fungi. Differences in REE concentrations and elemental ratios among fungal species may be useful in determining which fungal taxa are important agents of apatite weathering. Moreover, the measurement of these tracers in a given species of sporocarp across study sites may provide insights into ectomycorrhizal weathering of apatite at the stand and landscape scales.

3.1.3.3 Pb isotope ratios

Uranium and thorium also partition preferentially into apatite and other accessory phases during the crystallization of igneous rocks, while Pb distributes more uniformly among minerals (Bea et al., 1994). In rocks that are sufficiently aged (on the order of 10^8 years) for substantial decay of ^{232}Th, ^{235}U and ^{238}U to have occurred, accessory mineral phases that are initially enriched with Th or U will become enriched in the stable Pb daughter isotopes of their decay chains: ^{208}Pb, ^{207}Pb, and ^{206}Pb, respectively. In contrast, ^{204}Pb will reflect its initial distribution among mineral phases, as it is not a product of radioactive decay. Pb isotopic ratios in soil profiles have been used to examine sources of atmospheric Pb deposition (Sturges and Barrie, 1989; Hansmann and Köppel, 2000; Kaste et al., 2003) and also together with REE concentrations to infer the weathering of accessory minerals including apatite (Erel et al., 2004; Harlavan et al., 2009).

3.1.3.4 Application of REE and Pb isotope tracer systems

Because REEs are biologically inactive, have a 3+ charge, and are similar in hydrated radius to Ca and Sr, REEs released to in soil solution are likely to be held on the soil exchange complex and diffuse slowly in soils (Land et al., 1999; Sparks, 2003).

Passive uptake of these elements is likely to be low except when initially released at high concentration in close proximity to an absorptive fungal hypha. Lead is also biologically inactive, and since its isotopes are chemically identical and very similar (within 2%) in mass, are not substantially fractionated in biological systems. Together these tracer systems may be able to provide strong evidence of immediate fungal uptake of apatite weathering products.

3.1.4 Study design

Here we present the results of two studies intended to examine the utility of REEs and Pb isotopes as tracers of apatite weathering in forest ecosystems on granitic parent material. First, we conducted a greenhouse experiment in which mycorrhizal and non-mycorrhizal birch seedlings were grown semi-hydroponically with and without mesh bags containing unweathered granite chips. Second, we collected ectomycorrhizal sporocarps in six forest stands on granitic till soils and compared REE and Pb isotope ratios in sporocarps and soil extracts from each site.

We postulate a coherent "signature" of apatite dissolution consisting of 1) elevated concentrations of REEs, 2) elevated LREE/HREE ratios, and 3) more radiogenic Pb isotope ratios, and 4) elevated Ba/Ca and Sr/Ca ratios. In the greenhouse experiment, we predict that this signature will be discernible in mycorrhizal birch roots with access to granite, relative to non-mycorrhizal roots and those grown without access to granite. We hypothesize that similar signatures will be apparent in rooting zone soil extracts from young regenerating forests, which have greater net demand for P, relative to nearby mature forests, and in sporocarps of long-distance functional types relative to those of short-distance types.

3.2 Methods

3.2.1 Greenhouse experiment methods

3.2.1.1 Study organisms

Yellow birch (*Betula alleghaniensis* Britton) is an important tree species in northern hardwood forests at all successional stages. Yellow birch seeds are very small (averaging 10 mg), providing little nutrient capital to the young seedling. Because germinants establish successfully on exposed mineral soil or on decomposing wood (Burns and Honkala, 1990), where nutrient mineralization rates are low, young seedlings of yellow birch may rely heavily on mycorrhizal fungi to provide nutrients.

Leccinum snellii is a mycorrhizal associate of birches. Like many members of the Boletaceae and Suillinaceae families, *L. snellii* has long-distance hydrophobic rhizomorphs that allow it to efficiently explore large volumes of soil (Agerer, 2006); some fungi in this group are suspected of having strong mineral weathering capacities (Wallander and Wickman, 1999; Wallander, 2000). *Cortinarius violaceus* is a medium-fringe associate of many tree species (Agerer, 2006). *Cortinarius* mycorrhizae are noted for enzymatic capacities (Lilleskov et al., 2011), but weathering abilities of this group are not well characterized.

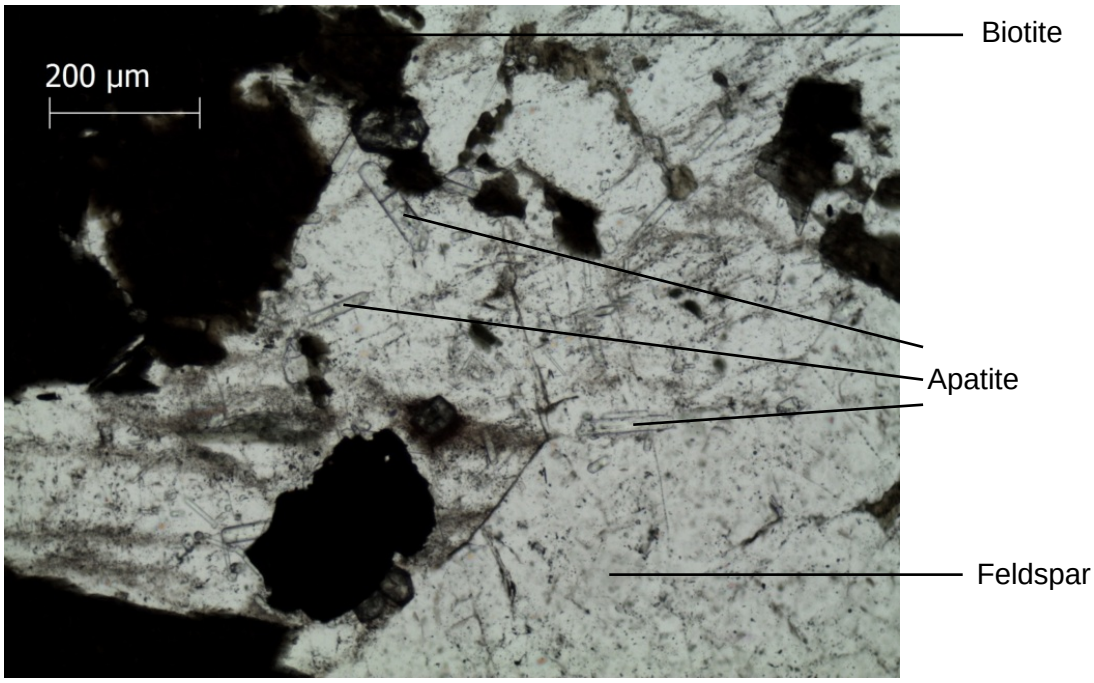
3.2.1.2 Culture methods

Sporocarps of the fungal study species were collected prior to the experiment. An inoculum was prepared by making a slurry from the hymenium of each sample, filtering to 50 microns, and storing at 4 ºC for up to 14 days until use.

Granite was collected from the abandoned Redstone Quarry (44.0184 ºN, 71.0978 ºW) and surface washed before grinding. The Conway granite (Fig. 3.1) is a

biotite granite approximately 180 Ma in age (Eby et al., 1992) containing 0.04% to 0.6% apatite (estimated from Billings and Wilson, 1965; Fig. 3.1). Ground rock was passed through a series of stainless steel sieves, and the fraction between 250 and 500 μm retained and thoroughly rinsed. We then filled 50 μm nylon mesh bags with 10.00 ± 0.05 g of granite chips (Appendix 3.1.1).

Figure 3.1 Apatite in a sample of Conway granite from Redstone, NH. Apatite occurs primarily as inclusions in feldspar and along grain boundaries. Thin-section photomicrograph by Ian Honsberger.



Commercially obtained yellow birch seeds were cold-stratified for 30 days at 4 ºC. Seeds were surface-sterilized with 30% hydrogen peroxide and 99% isopropanol, and germinated in sealed dishes on sterilized filter paper. Thirty-two uniform germinants were selected, assigned randomly to 4 treatments (Table 3.1) and planted in 10 cm polypropylene pots filled with rinsed coarse perlite. Mesh bags containing granite had been placed horizontally in the perlite at a depth of 3-4 cm in each pot (Appendix 3.1.2). Pots were rinsed thoroughly with deionized water and allowed to drain. The root of each germinant in the mycorrhizal treatments was dipped in the appropriate inoculum slurry before planting.

Pots were moved to the greenhouse and misted automatically with tapwater for 14 days to mitigate transplant shock (Appendix 3.1.3). Thirteen germinants died during this period and were replaced; the seven that died after this time were not replaced. Photoperiod was constant at 14 hours. We employed a randomized-block design, in which each block (a single greenhouse tray) contained one replicate of each treatment. Pots were ordered randomly within each block, and trays were rotated weekly to randomize the effects of light and temperature gradients. The abiotic control was included only in every second block due to lower expected variance and the high likelihood that all treatments would be analyzed, unlike the biotic treatments where the potential for mortality required greater replication.

Each pot was watered twice daily with 1-5 ml of hydroponic nutrient solution (the amount needed varied with evaporative demand and as plants grew). We modified an existing hydroponic solution formulation (Hobbie and Colpaert, 2004) to induce P-limitation (solution N:P ratio = 33 on a mass basis; Table 3.2). Solution pH was 4.5,

Table 3.1 The number of replicates in greenhouse experiment.
"Init." indicates the number in the initial design, and "surv." indicates those surviving to harvest. Not all samples were analyzed for Pb isotopes and REE, Ba, Ca, and Sr concentrations. All 21 surviving birches were analyzed for stable C and N isotopes.

plant	**fungus**	***pots with granite***						***pots without granite***			
			birch root samples			granite exch. leachates		birch root samples			
		init.	surv.	Pb	REE	Pb	REE	init.	surv.	Pb	REE
Birch	*Cortinarius*	8	6	5	6	5	6	--	--	--	--
Birch	*Leccinum*	8	5	3	3	3	4	8	5	4	4
Birch	None (non-mycorrhizal)	8	5	3	3	5	4	--	--	--	--
None	None (abiotic control)	4	--	--	--	3	4	--	--	--	--

Table 3.2 Concentrations (μM) of nutrient ions in the hydroponic solution. Formula is modified from Hobbie and Colpaert (2004).

Ion	Concentration (μM)
NH_4^+	585
NO_3^-	899
K^+	330
PO_4^{3-}	20
SO_4^{2+}	75
Mg^{2+}	49
Ca^{2+}	29
BO_3^{3-}	4.0
Fe^{3+}	2.5
Mn^{2+}	1.5
Zn^{2+}	0.10
Cu^{2+}	0.10
MoO_4^{2-}	0.01

typical for a spodosol B horizon derived from granitic till under northern hardwoods. Altogether, each pot received approximately 600 ml of nutrient solution during the growth period. Plants were occasionally automatically misted with tapwater when humidity was low.

Pots were harvested 132 days after planting. Twenty-one birches remained alive at this time. Foliage and stems were separated with stainless steel blades and placed into labeled, pre-weighed, acid-washed polyethylene vials. Roots were gently rinsed in deionized water and worked free of the perlite (Appendix 3.1.4). Plant tissue samples were dried for 48 hours at 50 ºC before weighing. Granite from each mesh bag was split into two samples and then frozen; one was freeze-dried to determine water content and the other was reserved for analysis.

3.2.2 Field experiment methods

3.2.2.1 Study sites

We sampled soils and ectomycorrhizal sporocarps in six well-characterized forest stands at the Bartlett and Hubbard Brook Experimental Forests in New Hampshire (Table 3.3). Stands represent two age classes: aggrading (~ 35 years since clearcut), and mature (> 100 years since last harvest). Study stands are currently being used in a long-term multiple nutrient fertilization experiment (MELNHE), but here we analyze only samples collected from control plots or from > 10 m outside the fertilized plots.

All sites are hardwood stands growing on well-drained glacial till at least 1 meter deep. The bedrock, from which most till is derived, is Conway granite at Bartlett, and Rangeley Formation schist at Hubbard Brook, (Lyons et al., 1997). The aggrading

Table 3.3 Description of the study stands where soils and sporocarps were collected.

site	last harvest	elev. (m)	aspect	slope	major species [1]	bedrock	apatite [2]
Bartlett - C4	1978 (aggrading)	410	NE	20-25%	BEPA, POGR, PRPE	Conway granite	0.07
Bartlett - C6	1975 (aggrading)	460	NNW	13-20%	BEAL, BEPA, ACRU	Conway granite	0.12
Bartlett - C8	1883 (mature)	330	NE	5-35%	FAGR, ACSA	Conway granite	0.18
Bartlett - C9	~1890 (mature)	440	NE	10-35%	FAGR, ACSA	Conway granite	0.27
Hubbard Brk M	1970 (aggrading)	500	S	10-25%	BEAL, PRPE, ACSA	Rangeley schist	0.57
Hubbard Brk O	~1910 (mature)	500	S	25-35%	FAGR, BEAL	Rangeley schist	0.60

1. ACRU= *Acer rubrum*; ACSA= *A. saccharum*; BEAL= *Betula alleghaniensis*; BEPA= *B. papyrifera*; FAGR =*Fagus grandifolia*; POGR= *Populus grandidenta*; PRPE= *Prunus pensylvanica*. Species listed are > 15% importance value.
2. Apatite P is reported as acid-leachable P (mg/g) in the C horizon; see Chapter 2.

Bartlett sites (C4 and C6) were recently identified as potentially P-limited (Naples and Fisk, 2010; Rastetter et al., 2013).

3.2.2.2 Sample collection

Samples of ectomycorrhizal sporocarps in the Boletaceae, Cortinariaceae, Russulaceae, and Amanitaceae were collected in the summer and fall of 2011 and 2012 and keyed to species. Most samples comprise a single sporocarp, but in some cases, multiple individuals growing in close proximity on a single visit were composted to provide sufficient sample. We selected three taxa with sufficient representation across sites for analysis: *Cortinarius violaceus, Lactarius camphoratus* plus a morphologicaly

similar unidentified *Lactarius*, and at least three *Leccinum* species that could not be identified based on sequences cataloged in GenBank (see section 3.2.3.3, below). These taxa represent a range of ectomycorrhizal functional types (Agerer, 2006; Hobbie and Agerer, 2010), from those with long-distance hydrophobic extraradical mycelium (*Leccinum*) to those with only short-distance hydrophilic extraradical hyphae (*Lactarius*). Where possible, two separate samples of each taxon from each site were selected for analysis. Sporocarp samples were dried in paper bags at 50 ºC under high air flow.

Soils from the Bartlett sites were collected at multiple depths in three quantitative pits per site in 2004 (Vadeboncoeur et al., 2012; Chapter 2), air-dried, and archived in polypropylene jars. For this analysis, samples were composited by site for each analyzed depth increment (Oa, 0-10 cm, 30-50 cm, and C horizon). The two stands analyzed at Hubbard Brook (in the Watershed 101 clearcut, and just outside the cut to the west) are adjacent to each other; sampled areas all lie within 100 m of the stand boundary. In 2012, we sampled three soil profiles near the boundary, in depth increments corresponding to those used at Bartlett, and composited the samples by depth increment.

3.2.3 Sample processing

3.2.3.1 Preparation of samples from greenhouse experiment

Root samples from the greenhouse experiment were subsampled and digested in triple-distilled concentrated nitric acid in Teflon vials. Samples of hydroponic solution and greenhouse tapwater were reserved for REE and isotopic analyses. Of the 21 birch seedlings that survived to harvest, some samples were consumed in preliminary analyses of insufficient data quality; in all we present the data for 15 birch root samples for Pb isotopes, and 16 for REEs, Ba, Ca, and Sr (Table 3.1).

Samples of greenhouse tapwater and hydroponic solution were set aside for elemental concentration and stable isotope analysis.

3.2.3.2 Granite leachates and soil preparation

Exchangeable cations were extracted from the greenhouse experiment granite samples in a 1 M ammonium chloride solution at pH 7.0. An unweathered sample of granite chips was leached with 1 N nitric acid at 20 ºC for 24 hours to extract apatite and other easily weathered accessory minerals (Erel et al., 2004; Nezat et al., 2007). A separate sample was and analyzed for total elemental concentrations by X-ray fluorescence (XRF). We analyzed 16 granite leachates for Pb and 18 for REEs (Table 3.1).

Exchangeable cations were leached from soil samples as described above, followed in a subset of samples by an apatite extraction in 1 N nitric acid.

3.2.3.3 Sporocarp sample preparation

Surface tissue was removed from the pileus of each field-collected sporocarp selected for analysis, to avoid contamination with soil, and 50-100 mg of clean tissue was excised with a stainless steel blade. Samples were digested in triple-distilled concentrated nitric acid in Teflon vials. Digestion blanks were run in parallel with each batch.

A small sample of the hymenium of each analyzed sporocarp was reserved for species confirmation by genetic sequencing. DNA was extracted following a modified glassmilk procedure (Hayward and Horton, 2012). We amplified the internal transcribed spacer region using forward primer NSI1 and reverse primer NLB4 (Martin and Rygiewicz, 2005). We confirmed amplicon presence through gel electrophoresis using a

1% agarose gel, then sequenced amplicons in the forward direction using PCR primers on an ABI 3730xl sequencer (Applied Biosystems, Carlsbad, CA).

3.2.4 Analysis of major and trace element concentrations

Concentrations of Ca, Ba, Sr, REEs, and Pb were measured on aqueous samples diluted in 2% nitric acid with an attoM high resolution single collector ICP mass spectrometer. Ca and Sr were analyzed separately from the heavier and less abundant elements. Eight Ca and Sr standards were run from 0.5 to 500 ppb. A monitor containing 10 ppb of each element was run every 8 samples. Replicate analysis precision averaged 3% for Ca and 6% for Sr.

For the heavy element run, the 8-point standard curve ranged from 7 ppt to 2.2 ppb for Ba, La, Ce, Pr, Nd, Sm Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, and Pb. Blanks were ~10 ppt for Ba, below 5 ppt for LREEs and Pb, and below 1 ppt for HREEs. Samples were rediluted as necessary to allow analysis of all target elements in the calibrated concentration range. Tl concentrations in most samples were too high for a Tl spike correction to be useful. Separate calibration standards were prepared with the addition of 10 ppm Fe, and 1 ppm of each K, Ca, Mg, and Na, to screen for signal suppression in natural-matrix samples. A monitor sample with this matrix and 50 ppt of Ba, REEs, and Pb was run every 3-5 samples, and used to correct for linear detector drift during the run. Pb concentrations were determined from mass 208; for the nitric acid leach of fresh granite, $^{208}Pb/^{204}Pb$ ratios (see section 3.2.5, below) were used to correct the total Pb concentration. Analytic precision (expressed as the mean coefficient of variation of raw count data) was < 5% for Ba, Pb, and most REEs for roots and granite leachates, and < 8% for Eu, Tm, Yb, Lu. For sporocarps, analytic precision was < 3% for

Ba and Pb, and < 8% for La and Ce, but ranged from 14-47% for the less abundant REEs Pr through Lu.

3.2.5 Analysis of Pb isotope ratios

Pb was purified from aliquots of each sample digest in 1 N hydrobromic acid run through columns containing Bio-Rad AG1x8 anion exchange resin, and eluted with 6 N hydrochloric acid (Bryce and DePaolo, 2004). Isotopic measurements of Pb were conducted on a multicollector ICP mass spectrometer. Sample analyses were bracketed between analyses of SRM981. Standard deviations for SRM981 were 0.009 for $^{208}Pb/^{204}Pb$, and 0.003 for $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$.

3.2.6 Analysis of C and N stable isotope ratios

Root, stem, and foliage samples from the greenhouse experiment were analyzed for C and N stable isotope ratios on a DeltaPlus XP mass spectrometer interfaced to a Costech Elemental Analyzer. Sporocarp samples and an evaporated aliquot of reserved hydroponic solution were analyzed in the same way. Roots, stems, and leaves from all 21 surviving birch seedlings were analyzed for stable C and N isotopes.

3.2.7 Statistics

In birch roots, leachates, and mushrooms, elemental concentrations and their ratios were approximately log-normally distributed, and so were log-transformed before testing the significance of differences among groups. Stable isotope ratios of C, N, and Pb were not transformed.

For birch roots, we used one sided *t*-tests to test the hypotheses that there was greater radiogenic Pb, greater REE concentrations, and greater ratios of light to heavy REEs in:

1) roots from pots with granite vs. those without;

2) mycorrhizal vs. non-mycorrhizal roots grown with granite, and

3) *Leccinum* vs. *Cortinarius* inoculated roots grown with granite.

For granite leachates, we used the same approach to examine the same expected differences between:

1) granite from pots with trees vs. those without trees,

2) granite from pots with mycorrhizal trees vs. non-mycorrhizal trees, and

3) granite from pots with birches inoculated with *Leccinum* vs. *Cortinarius*.

The contribution of apatite to the Pb analyzed in root and granite leach samples was evaluated with a three-part mixing model (Phillips, 2001), based on $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$, and using the nitric acid leach of fresh granite, the hydroponic solution, and tapwater as isotopic endmembers.

We also tested for linear correlations between Pb isotope ratios (represented by the apatite contribution estimated from the mixing model) and non-transformed Ca, Sr, Ba, and REE concentrations and elemental ratios in roots and granite leachates, as well as C and N stable isotope values in roots.

Differences among sporocarp analyses by site (Hubbard Brook vs. Bartlett, representing different parent material mineralogy), stand age class (aggrading vs. mature), and fungal taxon (*Cortinarus* vs. *Lactarius* vs. *Leccinum*) were analyzed with stepwise multiple linear models.

3.3 Results and discussion

3.3.1 The apatite signature

3.3.1.1 Acid leachable REEs in unweathered granite

The 1 N nitric acid leach was intended to remove easily weatherable minerals from unweathered rock, which we predicted would primarily comprise apatite (Nezat et al., 2007). The high La/Ba ratio (~23) relative to whole rock (~0.37) indicates little contribution of feldspar minerals to this leach. As with nearly all samples examined, abundances of REEs followed the Oddo-Harkins rule, by which odd-numbered elements are less abundant than even-numbered elements (Koljonen and Rosenberg, 1974). Acid-leachable concentrations of REEs ranged from 110 ng Eu g^{-1} granite to 173 μg Ce g^{-1} granite (Table 3.4). In some cases, acid-leachable REE concentrations exceeded whole-rock REE concentrations estimated by XRF, indicating imperfect homogenization and subsampling of analytic cuts.

The nitric acid leach shows high ratios of light REES (LREEs, e.g. La, Ce) to heavy REEs (HREEs, e.g. Yb, La) compared with bulk Conway granite and some literature values for apatite (Table 3.4), which appears to be due to the high concentrations of the LREEs La through Nd. The La/Eu and Sm/Eu ratios are greater than in bulk rock, due to the wider range of mineral compatibilities for Eu compared to the other REEs (Gromet and Silver, 1983; Bea et al., 1994), but are within the range of other reported apatites (Table 3.4).

Assuming that the whole rock is 0.094% apatite, based on the XRF whole-rock P_2O_5 concentration of 0.04%, and assuming that REEs all came from apatite, estimated concentrations of REEs in apatite range from 120 ppm Eu to 18% Ce. Relative to other

Table 3.4 Acid-leachable and whole rock Ba, REE, and Pb concentrations. Concentrations in apatite are estimated from the acid leach; literature values for apatite samples from other granites are listed for comparison. See also Hughes et al. (1991).

	μg g^{-1} granite		μg g^{-1} apatite					
	acid leachable	XRF total *	This study (estimated)	Kyushu (Nagasawa, 1970)	Peninsular Range (Gromet and Silver, 1983)	Sierra Nevada (Sawka and Chappell, 1988)	Peña Negra (Bea et al., 1994)	Madagascar (Hagerberg et al., 2003)
Ba	4.0	191	4200					
La	93	70	98800			1131	903	1200
Ce	173	148	184000	1490	509	1404	2158	
Pr	17		17800				291	300
Nd	49	51*	52000	1360	302	458	1300	1200
Sm	6.9	98*	7300	543	53	66	409	100
Eu	0.11	0.4*	120	3.3	15	10	17	10
Gd	5.3		5600			60	448	100
Tb	0.69	1.5*	730			6.6	73	10
Dy	3.71		3900	615	32		456	40
Ho	0.70		740			8.7	94	7
Er	1.96		2080	256	17		254	20
Tm	0.28		300				37	
Yb	1.72	5.0*	1820	188	14	21	222	10
Lu	0.22	0.6*	240	25		4.0	30	
Pb	1.72	16	1800				1.4	
La/Lu		112	416			283	31	
La/Yb		14	54			53	4	120
Ce/Yb		27	101	8	37	66	10	
La/Eu		156	845			116	54	120
Sm/Eu		22	62	165	3.5	6.7	25	10

* values are estimated from those reported by Eby (1992), scaled to La determined by XRF analysis on our sample.

Table 3.5 Pb isotope ratios for acid-leachable and exchangeable leaches of granite. Also shown are other endmember Pb sources in the greenhouse experiment.

	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$
acid leachable in granite	54.890	16.111	28.090	1.743
exchangeable in granite (*abiotic*)*	39.206	15.602	19.106	1.225
tapwater	38.816	15.737	19.291	1.226
hydroponic solution	38.085	15.512	18.381	1.183

* mean of 4 samples

apatite REE concentrations (Table 3.4; Hughes et al., 1991; Chu et al., 2009), these values are quite high, indicating that other weatherable accessory phases likely contributed to the nitric leach. These may include monazite (REE phosphate), allanite (REE-rich epidote), and titanite (calcium-titanium orthosilicate); (Gromet and Silver, 1983; Harlavan and Erel, 2002; Harlavan et al., 2009). Titanite and metamict allanite have been reported in Conway granite (Smith et al., 1957; Eby et al., 1992), along with trace amounts of REE-rich fluorocarbonates (Caruso and Simmons, 1985). Of these, titanite, allanite, and monazite are relatively insoluble in nitric acid (Crawford, 2009), though allanite may weather faster than apatite in soil (Harlavan and Erel, 2002). Titanite and monazite tend to have low ratios of LREE/HREE relative to apatite (Gromet and Silver, 1983; Bea et al., 1994), making them a poor fit for the observed high LREE/HREE ratios.

3.3.1.2 Pb isotope ratios in the unweathered granite leachate

Pb isotopes were highly radiogenic in the nitric acid granite leachate relative to all other samples examined (Table 3.5). Pb isotope data preclude large contributions of minerals with high Th/U ratios, such as monazite or allanite (Hurley and Fairbairn, 1956; Smith et al., 1957; Harlavan and Erel, 2002), to the nitric acid leach (Appendix 3.2). Rather, they suggest a major contribution from minerals with Th/U ratios similar to that of whole Conway granite (5.9 in our XRF analysis; other reported values range from 3.3-5.6; Billings and Wilson, 1965; Caruso and Simmons, 1985; Eby et al., 1992).

3.3.2 Greenhouse experiment results

3.3.2.1 Contribution of apatite to granite leachate and root Pb

Relative to the apatite leach, radiogenic Pb isotope ratios were much lower in the hydroponic solution and tapwater samples analyzed; values fell within the range of "common" whole-soil and environmental Pb (Table 3.5; Graney et al., 1995; Prohaska et al., 2000; Scheuhammer et al., 2003; Saint-Laurent et al., 2010).

Mixing calculations based on $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ indicated a detectable contribution of apatite-derived Pb to granite leachates, ranging from 1-14% by sample and averaging 7.4% (Fig. 3.2). The apatite contribution in biotic treatments (those with either mycorrhizal or non-mycorrhizal trees, 7.8%) were marginally significantly greater than that in the abiotic treatments (those without trees, 5.6%; one-tailed *t*-test $p = 0.03$). However, among the biotic treatments, there were no significant differences between granite leachates from pots with mycorrhizal and non-mycorrhizal seedlings, or between the two mycorrhizal innocula used. Pb isotope ratios or apatite-derived Pb estimates were not correlated when pairing roots and leachates from the same pot.

In contrast to the granite leachates, our Pb isotope mixing calculation indicated no clear contribution of apatite Pb to birch roots harvested from granite treatments (Fig. 3.2), and no clear difference in Pb isotope ratios or Pb concentrations between roots grown with and without granite (Appendix 3.3). There were not significant differences by mycorrhizal type.

Estimated relative contributions of hydroponic solution vs. tapwater to apatite Pb did not differ significantly among treatments in either roots or the granite leachates.

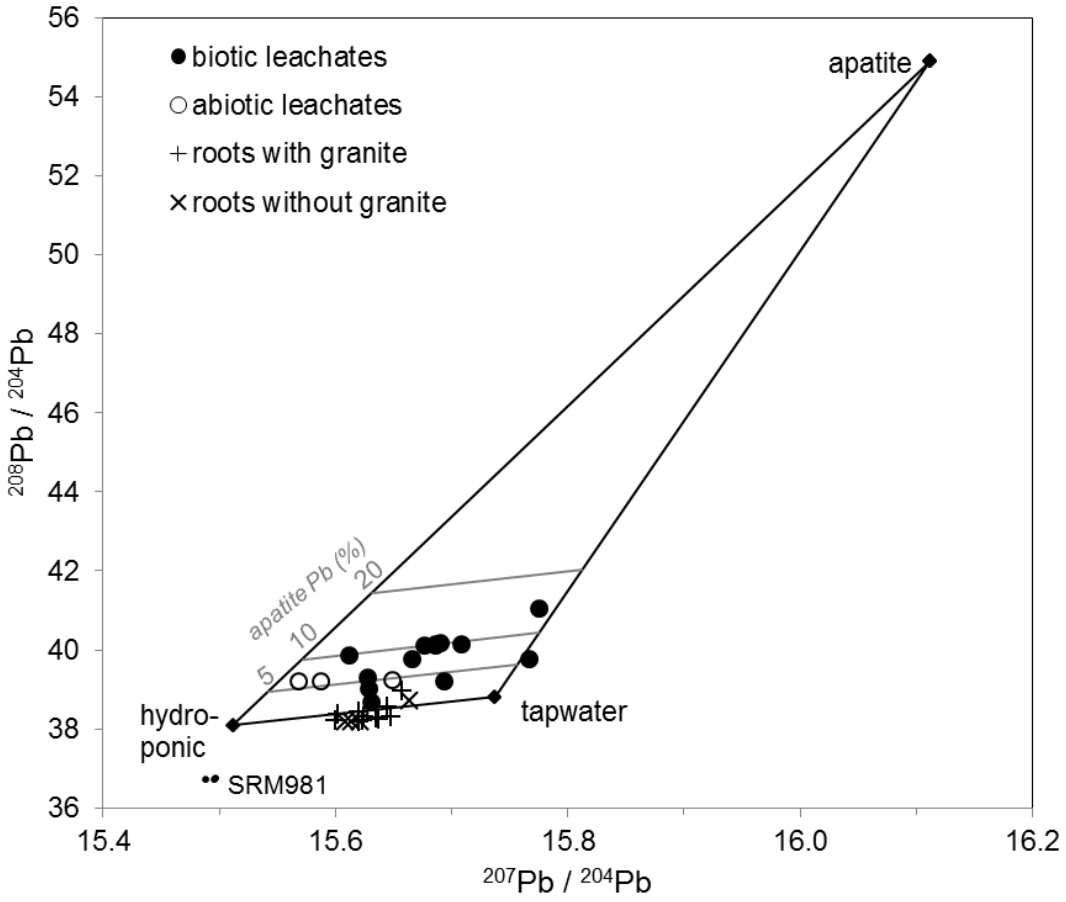


Figure 3.2 Three-part Pb isotope mixing space for roots and leachates. Multiple analyses of SRM981, a common Pb standard, indicate typical analytical precision.

3.3.2.2 REEs and Ba in granite leachates

Concentrations of different REEs were highly correlated with each other in both leachates and roots; correlation coefficients (*R*) were > 0.99 between La and all REEs analyzed except for Eu.

Exchangeable REEs from the granite deployed in experimental pots ranged from 33 pg Lu g^{-1} granite to 12.4 ng Ce g^{-1} granite. In granite leachates from the abiotic treatment, ratios among the LREEs were very similar to those from the nitric leach. However, acid leachable Eu, the HREEs, and Pb were somewhat more abundant relative to La than in the exchangeable extraction. Most notably, exchangeable Ba greatly exceeded exchangeable La, while the opposite was true of the acid leachable fraction (Fig. 3.3; Appendix 3.3). This pattern likely indicates a proportionally greater contribution of feldspar minerals to the abiotic exchangeable leach than to the acid leach.

Figure 3.3 Acid leachable and exchangeable elemental ratios in Conway granite. All elements are plotted as ratios to La.

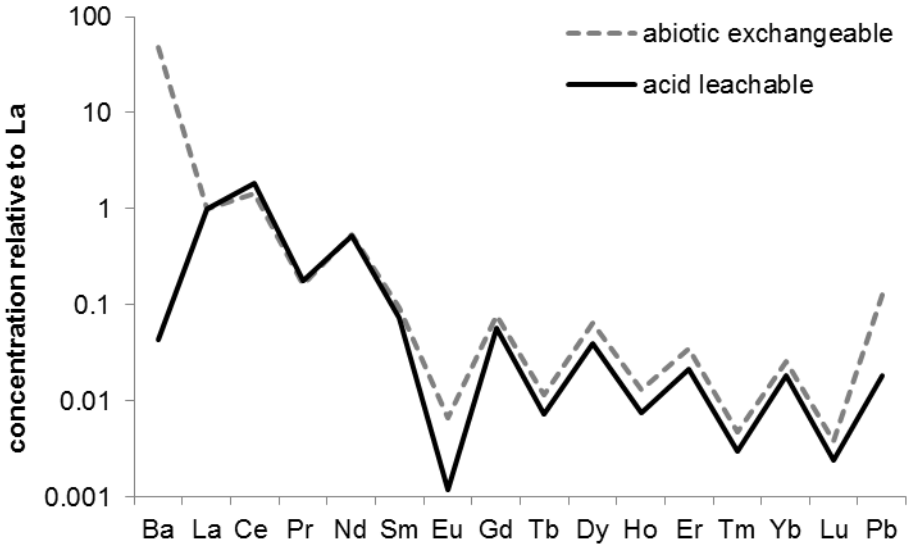
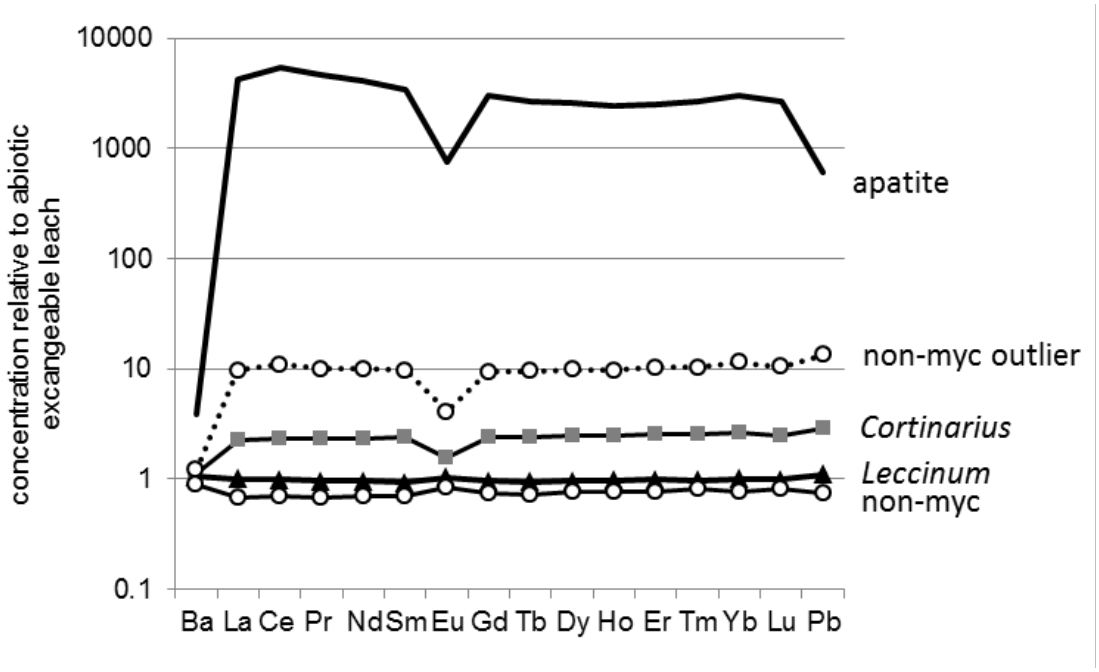


Figure 3.4 Exchangeable concentrations of Ba, REEs, and Pb in granite. Concentrations are normalized to those in the abiotic treatment. One outlier is plotted separately from the mean of the other non-mycorrhizal replicates. "Apatite" indicates acid-leachable concentrations in fresh granite, normalized in the same way for comparison.



Exchangeable REEs showed some coherent patterns by treatment, though there were no significant differences in individual REE concentrations for biotic vs. abiotic, mycorrhizal vs. non-mycorrhizal, or *Cortinarius* vs. *Leccinum* treatments. *Cortinarius* and *Leccinum* treatments did differ in La/Ce and La/Lu ratios; *Cortinarius* ratios were more similar to those of apatite than *Leccinum* were. Mean REE concentrations for *Cortinarius* samples were greater than the means of the other treatments (Fig. 3.4, Appendix 3.3). Surprisingly, the non-mycorrhizal treatment encompassed both extremes; with both the lowest median REE concentrations among all treatments, as well as the highest individual sample concentrations (plotted separately in Fig. 3.4).

3.3.2.3 REEs and Ba in roots

REE concentrations were elevated (2-tailed *t*-test, $p < 0.001$, except for Eu) in roots grown with granite relative to those without granite (Fig. 3.5). Also significant were higher La/Ba, La/Eu, La/Lu, and La/Pb ratios. High La/Ba is a feature of the acid-leachable fraction, while high ratios of La to other REEs were a feature of both the exchangeable fraction in the abiotic treatment and the acid-leachable fraction. There were no significant differences in REE concentrations or ratios between mycorrhizal and non-mycorrhizal treatments, but this appears to be due to differences between the mycorrhizal treatments. *Cortinarius*-innoculated roots had significantly lower La/Ce and greater La/Lu ratios than roots inoculated with *Leccinum*; these deviations could indicate greater contributions from acid-leachable accessory minerals. As with the granite leachates, there was one very high REE outlier (Fig. 3.5, Appendix 3.3).

3.3.2.4 Ca/Sr and Ca/Ba ratios in roots

Ca concentrations in roots averaged 3500 ppm, while Ca/Sr and Ca/Ba ratios averaged 77 and 184, respectively. Concentrations of Ca, Sr, or Ba and Ca/Ba did not differ among treatments. Ca/Sr ratios were marginally significantly greater in mycorrhizal roots grown with granite (78.4) than in non-mycorrhizal roots grown with granite (72.2; one-tailed *t*-test $p = 0.03$).

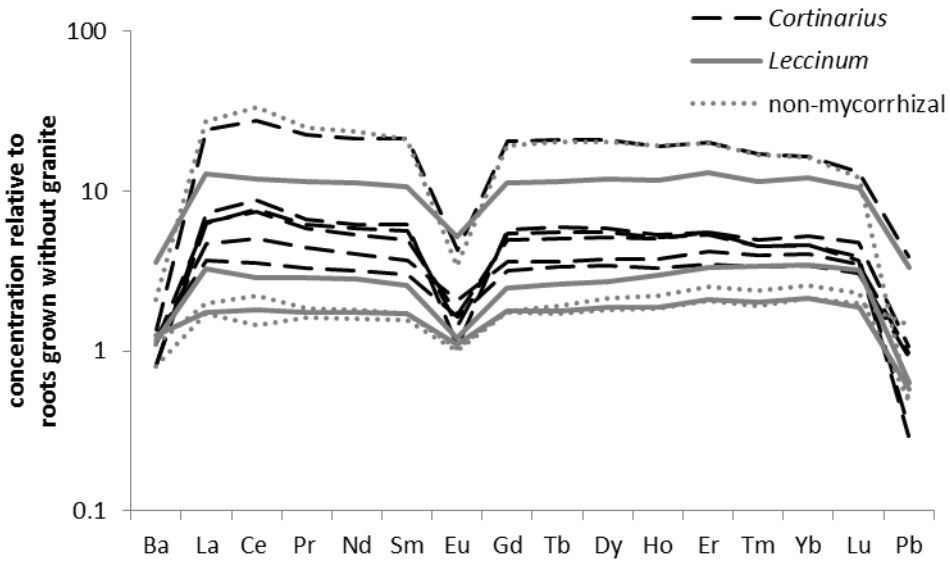
3.3.2.5 Birch production and C and N stable isotope ratios in birch seedlings

Total dry biomass of the birch seedlings that survived to the end of the experiment averaged 67 mg, and root biomass averaged 49% of this total (Appendices 3.4, 3.5). Whole-plant C/N ratios averaged 26. There were no significant differences by treatment.

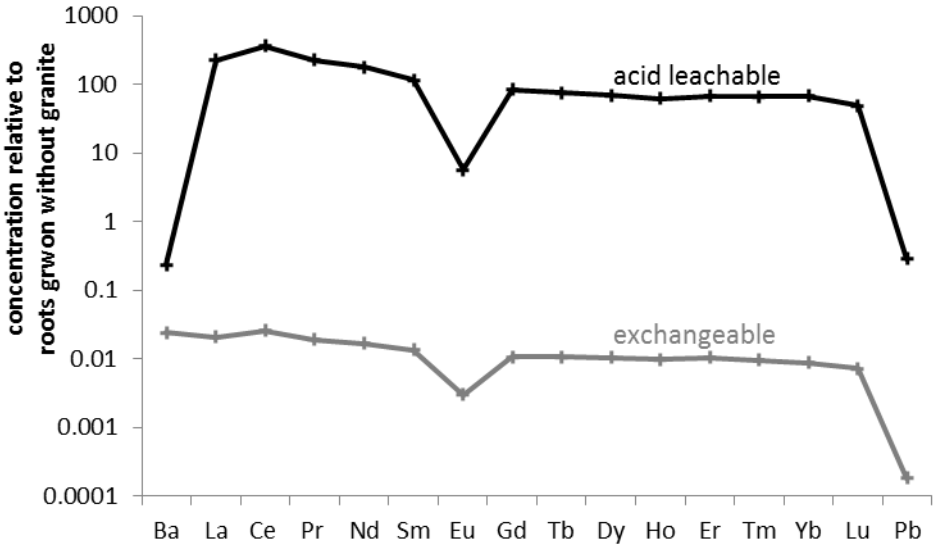
Figure 3.5 REE concentrations in roots

a. Concentrations of REEs in roots, normalized to roots grown without granite.
b. Acid-leachable concentrations in fresh granite and exchangeable concentrations in the abiotic treatment, normalized in the same way but on an expanded scale, for reference.

a)



b)



There were also no significant differences in root or whole-plant C and N stable isotope ratios (Appendices 3.4, 3.5, 3.6). All samples were depleted in ^{15}N relative to the hydroponic solution, which was the only significant source of N in the experimental pots, by an average of 2‰. Roots were almost uniformly enriched in ^{15}N and ^{13}C relative to the whole-plant value, by an average of 0.30‰ for N and 0.25‰ for C. Plant biomass, root mass fraction, and N concentrations did not correlate with C and N stable isotopes in roots or whole plants.

Differences among replicates may relate to differential use of ammonium vs. nitrate as N sources (Hobbie et al., 2008); these were not measured individually in hydroponic solution. Because hydroponic solution was applied to the perlite surface with a spray bottle, some foliar uptake may have occurred. N was clearly supplied in excess; on average only 9% of the hydroponic N added to each pot was found in the harvested birch biomass. Given the slow growth of the seedlings, it is likely that P supply was also sufficient, assuming a whole-plant N/P ratio of 13 (Ingestad, 1979), 90% unutilized N, and a hydroponic N/P ratio of 33 (Table 3.2). Pots could drain freely, but most water was probably lost to evaporation from the perlite surface, leading to an accumulation of nutrient salts in the perlite. On the other hand, accessing hydroponic resources may have required significant "exploration" effort, as diffusion was likely limited in the coarse perlite substrate. Root systems were often distributed extensively in the pots (Appendix 3.1.4), and root/shoot ratios were high compared to other birch seedlings in culture studies (Ingestad and Lund, 1979), suggesting nutrient or water limitation (Rosenstock, 2009). The lack of a mycorrhizal signal in either $\delta^{15}N$ or $\delta^{13}C$ is somewhat surprising in

light of results from other field and culture studies (Hobbie, 2006; Hobbie et al., 2008), and may indicate a limited importance of fungal biomass as a sink of C and N in the plant-fungus system.

3.3.2.6 Correlations among tracer systems

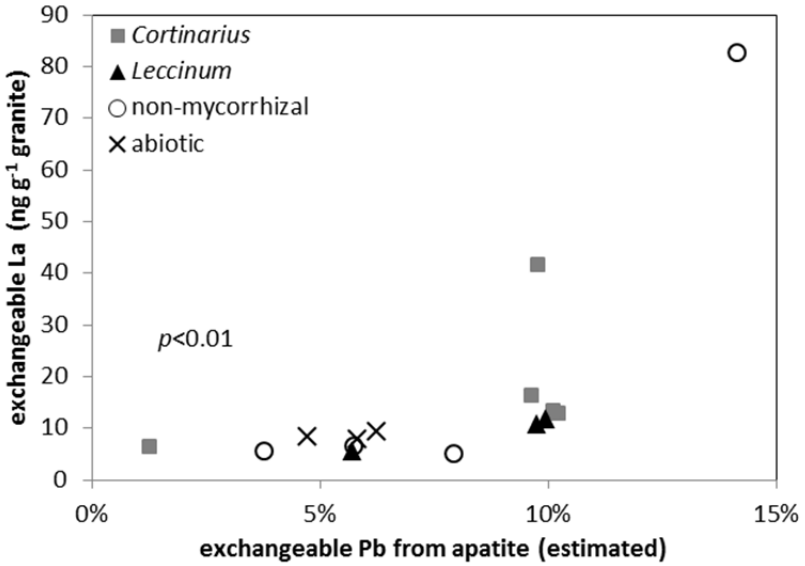
For both leachates and roots, there were significant linear correlations between Pb and La, as well as with La/Eu (Figs. 3.6, 3.7). We interpret this to indicate a common source of radiogenic Pb and the LREEs, consistent with a source similar to that extracted by the nitric acid leach of fresh granite. Access to or use of this source apparently varied widely among birch seedlings, with surprisingly little of this variation attributable to mycorrhizal treatment. In roots and leachates, the highest value for both radiogenic Pb isotopes and REE concentrations occurred in the non-mycorrhizal treatment.

In roots, there were no significant correlations between Ca/Ba or Ca/Sr and radiogenic Pb or with REE ratios. Ca/Ba did correlate ($p = 0.01$) with La concentrations, but negatively, which is the opposite trend expected if high Ca/Ba ratios indicate apatite weathering.

Pairing leachates and roots from the same pot, there were no significant correlations for REE concentrations or ratios, though the comparison was possible for only 11 samples. There were also no significant correlations of C and N stable isotopes with radiogenic Pb, REE concentrations, or REE ratios; either among all birch roots or for just the birch roots grown with granite.

Figure 3.6 Correlations between Pb isotopes and REEs in exchangeable leachates.

a)



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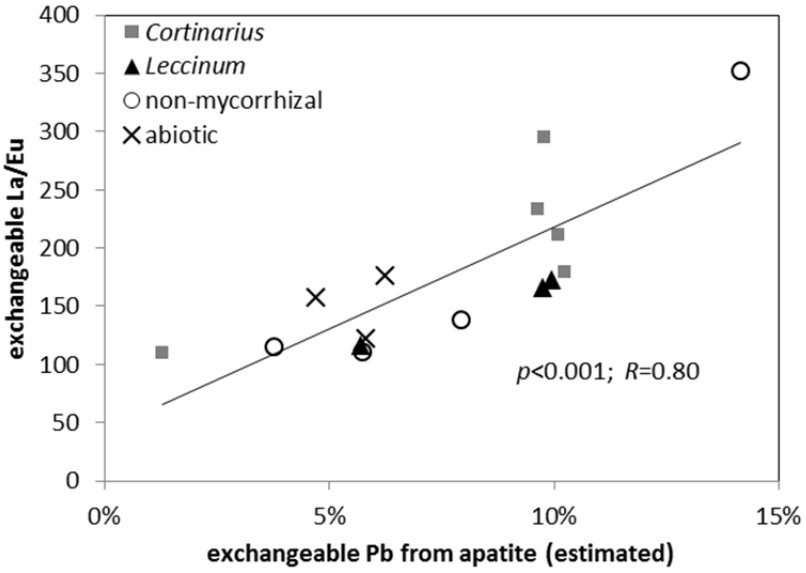
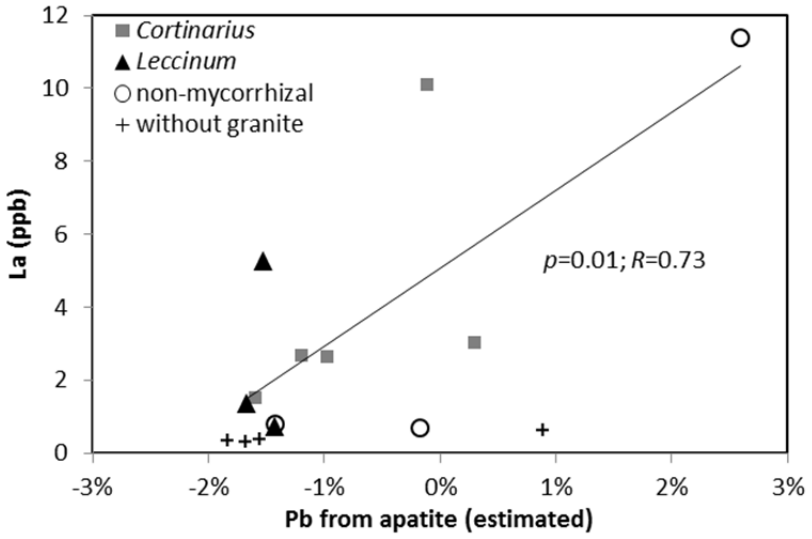
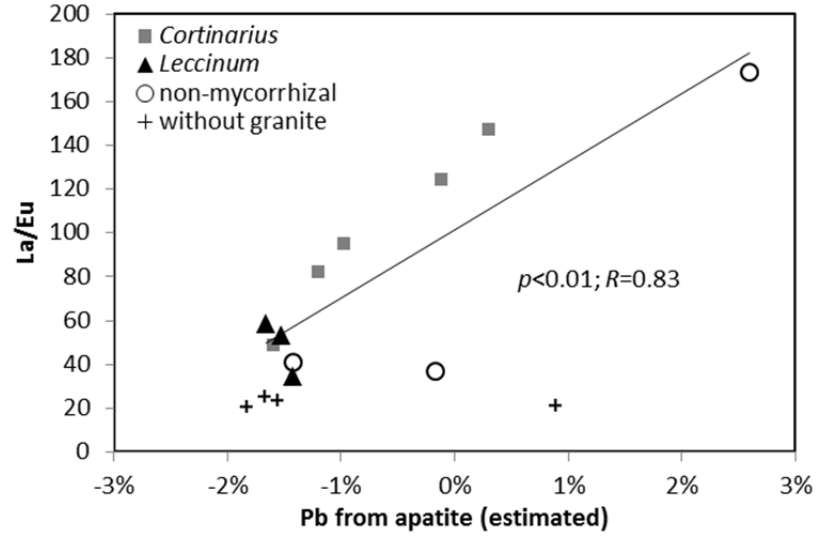


Figure 3.7 Correlations between Pb isotopes and REEs in roots.
Regressions are for roots grown with granite only. Note that correlations hold despite sometimes negative isotopically estimated apatite contributions to root Pb (Figure 3.2).

a)



b)



3.3.2.7 Summary and interpretation of greenhouse experiment findings

In the greenhouse experiment, we found clear differences between crushed granite samples incubated for four months in pots with birch seedlings and control samples without seedlings. Leachates of granite incubated with birch seedlings showed increased ratios of radiogenic Pb isotopes as well as greater REE concentrations. These excesses were qualitatively similar to, though of much smaller magnitude than, those of a 1 N nitric acid leachate of unweathered granite, which appears to have primarily dissolved apatite, but possibly also other U- and REE-rich accessory phases. These patterns indicate a biological (though not necessarily mycorrhizal; e.g. Drever, 1994) mechanism accelerating apatite weathering.

Ca/Sr and Ca/Ba ratios also weakly supported the conclusion of apatite weathering supplying Ca to roots; mycorrhizal roots had higher Ca/Sr ratios than non-mycorrhizal roots, but Ca/Ba concentrations unexpectedly correlated negatively with La concentrations, suggesting that these tracer systems are both more complicated and less sensitive than REEs and Pb isotopes in our experimental system.

REE concentrations in roots were greatly elevated in treatments with granite relative to those without granite, and among roots grown with granite, radiogenic Pb isotopes correlated with REEs, demonstrating uptake of apatite weathering products. However, a Pb isotope mixing model indicated little contribution of apatite to total Pb in roots, perhaps due to high background Pb concentrations and potentially additional uncharacterized Pb sources. These findings are also consistent with Pb being relatively immobile relative to REEs in this experimental system.

In both roots and leachates, the non-mycorrhizal treatment had high variance, with low median presence of weathering tracers relative to mycorrhizal treatments, but also the highest values among individual replicate. This suggests either that organic acid exudation by non-mycorrhizal roots also induced weathering of apatite in granite. No roots were observed growing into the bags, though some were in close contact with the mesh, which may explain some of the variation among replicates. Variation in the success of mycorrhizal inoculation may play a role; only a minority of root tips were clearly colonized when observed under 30X magnification. This may relate to the suitability of our selected taxa for low-biomass seedlings under decidedly unnatural conditions. It is also possible that the lack of fungal inoculum did not prevent some of the "non-mycorrhizal" birches from coming into contact with mycorrhizal fungal spores, which may be due to planting the pots in a lab space where EM sporocarps are routinely dried and ground.

Together, these data indicate that Pb isotopes and REE concentrations and ratios have the potential, particularly when examined together, to trace the biologically driven weathering of apatite.

3.3.3 Results of field-collected sample analyses

3.3.3.1 Pb isotopes in soils

Exchangeable Pb showed increasingly radiogenic isotope ratios with depth at both sites where they were analyzed in soil. These profiles differed somewhat among the two sites analyzed; for each depth analyzed, exchangeable Pb was somewhat more radiogenic than at HB (Fig. 3.8). In the C horizon, $^{207}Pb/^{204}Pb$ was much greater at HB than at C6, indicating a greater proportional contribution of U-rich mineral phases to exchangeable

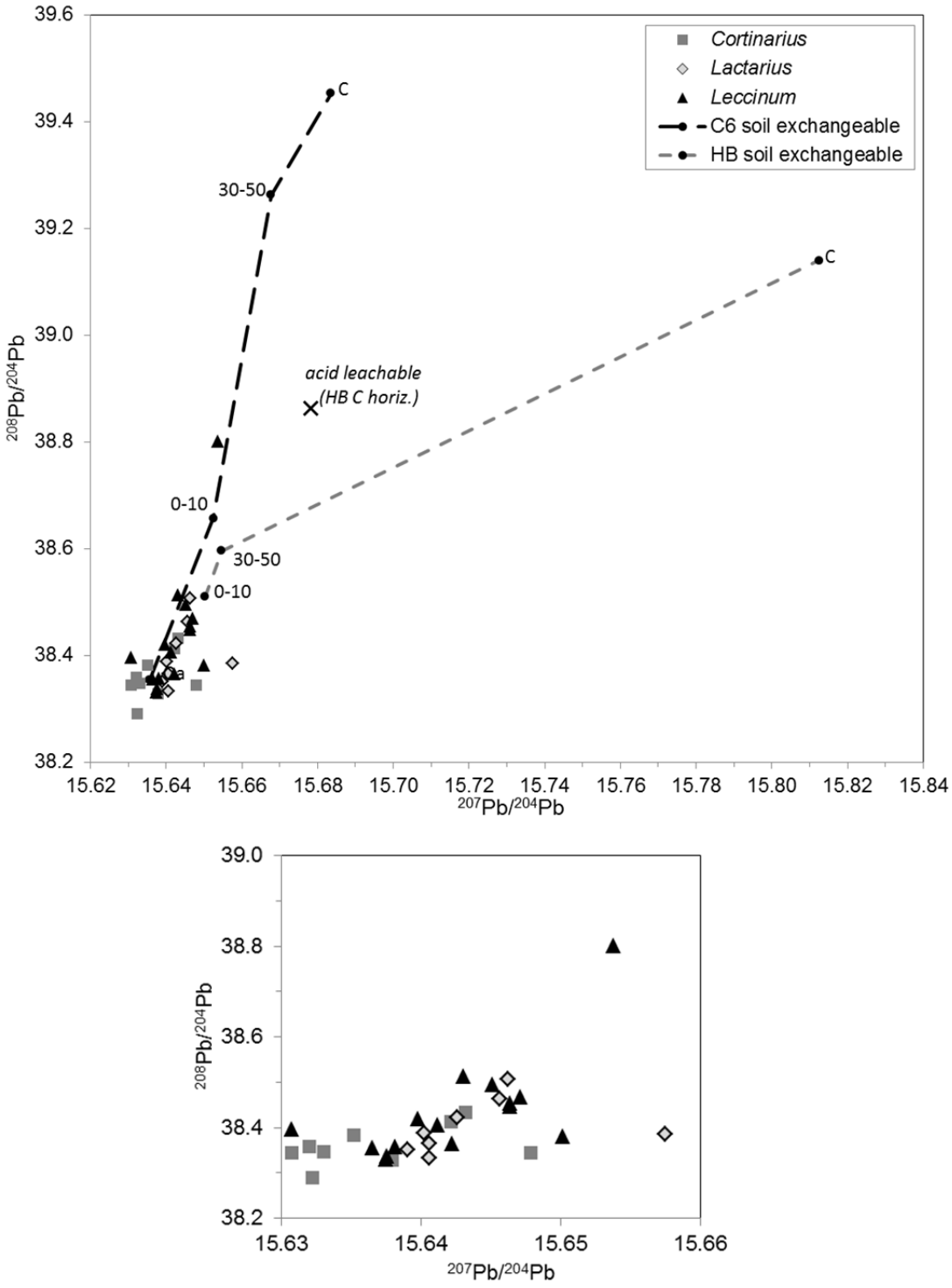
Pb at this depth (e.g. apatite or titanite; Harlavan and Erel, 2002). While the soils at these sites are likely derived primarily from local bedrock (Conway granite at Bartlett and Rangeley schist at HB), other lithologies likely contribute substantially as well (Fig. 2.1 2; Bailey and Hornbeck, 1992). Environmental Pb deposited from the atmosphere during the 20th century is generally abundant in shallow soils in the region (Johnson et al., 1981; Kaste et al., 2003; Yanai et al., 2004). This, in combination with the depletion of easily weathered minerals in the O and E horizons, likely accounts for some of the depth trend in exchangeable Pb isotopes.

Surprisingly, the acid leachable Pb in the C horizon at HB, which we intended to use as an apatite endmember, was less radiogenic than the exchangeable Pb in the same sample (Fig. 3.8). This is the opposite trend of what we expected based on our analyses of Conway granite (Table 3.5). Similar acid extractions of Hubbard Brook C horizons dissolved apatite while leaving silicate minerals intact (Nezat et al., 2007). Pb concentrations estimated from mass spectrometer collector voltage indicate that this unexpected pattern is not the result of mislabeling samples. It is possible that soil parent material was not homogenous with depth; only one C horizon sample was collected at HB due to very rocky soils, and this sample was from a fairly steep slope. Additional Pb isotope analyses of acid extractions of bedrock and deep soils will be necessary to properly interpret these unexpected values.

3.3.3.2 Sporocarp Pb and REE values

REE concentrations averaged 20 ppb La, 24 ppb Ce, and 11 ppb Nd; other REE concentrations were too close to the blank values to measure reliably. These

Figure 3.8 Pb isotope ratios in sporocarps and soils.
Soil profiles were collected at Bartlett (site C6) and Hubbard Brook Experimental Forests, and are plotted as dashed lines; the horizon or depth increment of each sample is indicated. Sporocarps were collected at all six research sites (Table 3.3). The detail panel shows sporocarps only.



concentrations are similar to those reported by Borovička et al. (2011) across a range of ectomycorrhizal sporocarps sampled in Europe. Barium and Pb averaged 600 and 690 ppb, respectively. Pb isotope ratios in sporocarps were generally similar to exchangeable Pb values from shallow soil horizons (Fig. 3.8, Appendix 3.7).

We found a number of significant differences in Pb isotope ratios and REE concentrations and ratios across the 32 samples analyzed (Table 3.6). *Leccinum* samples had greater $^{208}Pb/^{204}Pb$ values than other taxa examined, while *Cortinarius* samples had lower $^{207}Pb/^{204}Pb$ ratios. Lanthanum concentrations, La/Ce ratios, and Ba/La ratios were greater at Hubbard Brook than at Bartlett, and La concentrations, La/Ce, and La/Pb were greater while Ba/La was lower in mature stands than in aggrading stands. Site differences in REE ratios likely relate to the soil parent material, though differences across site age indicate potentially greater rates of apatite weathering in mature stands; this is the opposite of the trend we hypothesized. Altogether, there are not clear differences among taxa or across sites; additional soil profile data and analyses of mineral separates from soils would be required to discern and interpret such differences if they exist, or whether they simply reflect differences in the mean depth of mycorrhizae, differing contributions of lithogenic and atmospheric Pb sources, and different soil mixing history across the sites.

3.3.3.3 Sporocarp stable C and N isotope ratios

Sporocarp $\delta^{15}N$ did no differ significantly across sites, stand ages, or fungal taxa. However, $\delta^{13}C$ values were significantly greater in mature sites than in aggrading sites, with no significant differences by taxon or site. The lack of differences among taxa is

Table 3.6 Multiple regression models for sporocarp trace element and isotope data. Only significant terms are shown.

			SITE	AGE	TAXON			Interactions
	model p	model adj R^2	HB v. Bartlett	aggrading v. mature	*Cortinarius*	*Lactarius*	*Leccinum*	
$\delta^{15}N$	0.16	0.34	*no sig terms*					
$\delta^{13}C$	0.05	0.15		-				++ *agg*HB*
$^{208}Pb/^{204}Pb$	0.03	0.19	-				+	
$^{207}Pb/^{204}Pb$	0.05	0.12			-			
$^{206}Pb/^{204}Pb$	0.10	0.09	*no sig terms*					
$^{206}Pb/^{207}Pb$	0.09	0.09	*no sig terms*					
log(Ba)	0.06	0.08		(-)				
log(La)	0.33	0.10	+	-				+ *HB*Lecc* + *agg*Lecc*
log(Ce)	0.00	0.90	*no sig terms*					
log(Pb)	0.25	0.10	*no sig terms*					
log(Ba/La)	0.32	0.05	+	+				- *HB*Lecc*
log(La/Ce)	0.15	0.16	+	-				- HB*Cort; + agg*Lecc
log(La/Pb)	0.31	0.06		-				+ HB*Lecc; - agg*Lact

+ or - symbols indicate the direction of the effect listed
(+) indicates $p < 0.10$
+ indicates $p < 0.05$
++ indicates $p < 0.01$

somewhat surprising; these taxa were chosen to represent a range of ectomycorrhizal functional types. N isotope ratios are known to reflect differences in N sources and partitioning among fungal organs and between plant and fungal symbionts; long-distance hydrophobic functional types tend to be enriched in ^{15}N relative to short-distance and hydrophilic types (Hobbie and Agerer, 2010). There were no significant correlations between C and N stable isotope ratios and REE concentrations or Pb isotope ratios.

3.4 Conclusions

We examined the potential for REE abundances and ratios, as well as Pb isotope ratios to serve as tracers of apatite weathering in a simplified experimental system as well as in a real-world forest ecosystem. In the greenhouse experiment, we found a coherent signature of the accelerated weathering of apatite (plus perhaps other accessory phases rich in REEs, U, and Th) in crushed granites incubated in pots with birch trees relative to those without. This signature comprised elevated exchangeable REE concentrations, elevated ratios of LREEs to HREEs, Eu, and Ba, as well as elevated ratios of ^{208}Pb, ^{207}Pb, and ^{206}Pb to ^{204}Pb. In roots, we saw similar trends in REE concentrations and ratios between birches incubated with and without granite, and an order of magnitude variation in these tracers among birches grown with granite. While Pb isotopes did not clearly reflect an apatite contribution, there were positive correlations between REE abundance and apatite Pb, perhaps reflecting an imperfect characterization of Pb endmembers as well as the relative immobility of Pb in this system. Together, these data indicate that Pb isotopes and REE concentrations and ratios have the potential, particularly when examined together, to trace the biologically driven weathering of apatite.

Sporocarps of three ectomycorrhizal taxa harvested from six forest stands did not show clear signatures of apatite weathering. However, our ability to assess LREE/HREE ratios was limited by low overall REE concentrations. The preponderance of atmospherically-derived Pb in shallow soils where the bulk of fungal biomass occurs may also make it difficult to discern any such patterns if they exist. Because of the promise of these tracers and the importance of soil weathering rates to the sustainable management of forests, additional research into these patterns may be warranted.

CHAPTER 4

ORGANIC NITROGEN UPTAKE BY MYCORRHIZAL ROOTS IN A TEMPERATE FOREST DEMONSTRATED BY A NOVEL EXPERIMENTAL APPROACH

Abstract

The uptake of nitrogen in organic form by plants and mycorrhizal fungi is well known as an important component of the nitrogen cycle in tundra and boreal forest ecosystems, but the process is little studied in temperate forests. One criticism of previous experiments documenting organic nitrogen uptake is that concentrations of isotopically labeled amino acids added to soils may be higher than concentrations normally available to roots and mycorrhizal hyphae *in situ*. To address this issue, we developed an experimental approach in which ^{13}C- and ^{15}N-labeled whole organic matter (cyanobacteria) was added to root-ingrowth cores, allowing soil proteolytic enzymes to then release a mixture of labeled organic nitrogen molecules at a natural rate over a period of several weeks. We employed this method in eight forest stands on Inceptisols in southern New Hampshire, spanning a gradient of soil pH, nitrogen mineralization rate, mycorrhizal type, and root production. Intact uptake of organic nitrogen was subsequently detected in roots and accounted for 2-16% of label nitrogen uptake across the four forest types. Sites did not differ in levels of organic nitrogen uptake.

Confirmation of previous experimental results showing organic N uptake in similar systems suggests a need to better understand the ecosystem-scale controls on this process.

4.1 Introduction

Primary production in many ecosystems is limited mainly or in part by nitrogen (N) availability, even under high N deposition (LeBauer and Treseder, 2008; Finzi, 2009; Vadeboncoeur, 2010; Harpole et al., 2011). The vast majority of nitrogen in most soils occurs in organic form, largely as insoluble organically-bound protein and heterocyclic nitrogen, but also as soluble free protein- and non-protein amino acids, free and bound amino sugars, and inorganic nitrogen (Schulten and Schnitzer, 1997; Warren, 2013). Despite early agronomic research on the role of organic molecules in plant nutrition, for most of the 20th Century it was thought that only soil microbes had the enzymatic capabilities and cellular transport functions necessary to depolymerize and assimilate organic nitrogen, while plants relied on mineralized nitrogen released by microbial metabolism (Craine, 2009; Paungfoo-Lonhienne et al., 2012). Over the past three decades, a range of studies conducted in culture and under field conditions have demonstrated some degree of plant access to organic nitrogen in the soil, which has been described as a "short-circuit" (Chapin et al., 1993, 2003) of the microbial mineralization "bottleneck" in the nitrogen cycle as it was previously understood (Fig. 4.1).

Such uptake could potentially occur via a variety of pathways, and experimental approaches differ in their ability to distinguish among them. First, most plants form symbiotic associations with mycorrhizal fungi capable of utilizing dissolved amino acids and competing for them with other soil microbes. Second, many such fungi produce proteolytic extracellular enzymes (Chalot and Brun, 1998; Lindahl et al., 2005), which

may increase the rate of peptide and amino acid release to soil solution. Third, direct organic nitrogen uptake has also been demonstrated in some non-mycorrhizal plants including graminoids (Chapin et al., 1993; Näsholm et al., 1998; Raab et al., 1999) and the model organism *Arabidopsis thaliana* (but see Roberts and Jones, 2012). Plant roots may also have active amino acid transport mechanisms and some may exude protease enzymes directly (Paungfoo-Lonhienne et al., 2008). Finally, roots and mycorrhizal fungi may also supply labile carbon to rhizosphere microbes and indirectly up-regulate proteolytic enzyme activity (Hobbie and Hobbie, 2008; Averill and Finzi, 2011b).

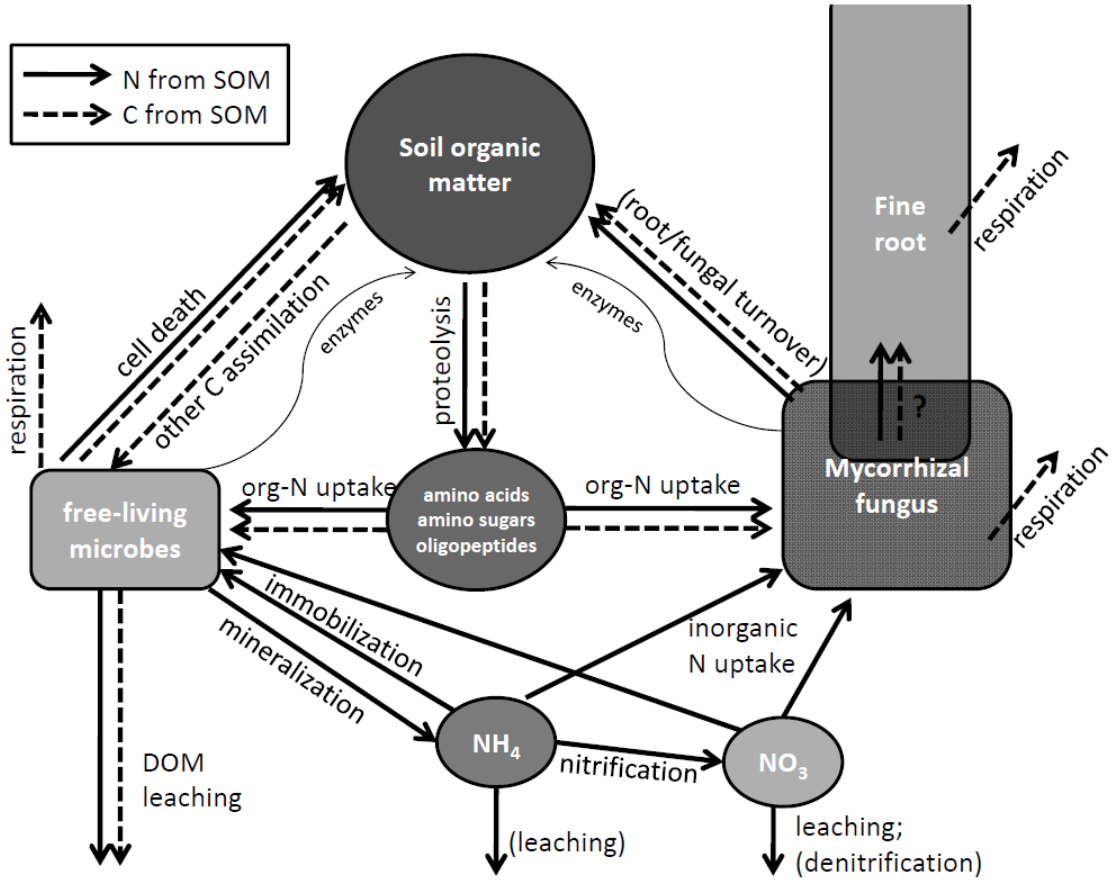


Figure 4.1 Pathways of C and N flux from soil organic matter.
Here we examine the relative importance of organic N and inorganic N uptake by mycorrhizal roots.

4.1.1 Ecosystem consequences of organic nitrogen uptake

Understanding the organic uptake short-circuit (Chapin et al., 1993, 2003) of the microbial mineralization bottleneck is important for a variety of reasons. Use of organic nitrogen by mycorrhizal and non-mycorrhizal plants appears important to ecosystem productivity where soil temperature, moisture, litter quality and secondary chemistry limit the rate of microbial nitrogen mineralization, such as in the Arctic and Antarctic (Chapin et al., 1993; Hill et al., 2011a; Inselsbacher and Näsholm, 2012), boreal forests (Näsholm et al., 1998; Persson et al., 2003; Mayor et al., 2012), alpine tundra (Lipson et al., 1999), and heathlands (Stribley and Read, 1980; Paungfoo-Lonhienne et al., 2008). However, the role and magnitude of organic nitrogen uptake is considerably less certain in other ecosystems, including in temperate forests (Neff et al., 2003; *but see* Finzi and Berthrong, 2005; Gallet-Budynek et al., 2009). Improving process models of such ecosystems, particularly under global change scenarios, will require a thorough understanding of such uptake processes and the variables that control their rates.

Differential access to organic nitrogen via mycorrhizal fungi may play a role in determining plant community composition, allowing some functional groups such as ericaceous shrubs to reinforce their own dominance via feedbacks between low-quality litter, slow nitrogen mineralization, and mycorrhizal associations that can access organic forms of nitrogen (Clark et al., 2005; Craine, 2009; Wurzburger and Hendrick, 2009). Resource partitioning by plant species for organic and inorganic nitrogen forms has been used to explain community composition in both the Arctic and Antarctic (McKane et al., 2002; Hill et al., 2011a). Recent modeling work suggests that the high competitiveness of mycorrhizal fungi for organic nitrogen and the consequent nitrogen limitation of the

microbial community may reduce overall decomposition rates and indirectly increase soil carbon storage (Orwin et al., 2011).

4.1.2 Methodological approaches assessing organic nitrogen uptake

Demonstration that roots and mycorrhizal fungi can use organic nitrogen is often conducted in culture, where different nitrogen sources are provided individually to isolated fungi (Abuzinadah and Read, 1986a), non-mycorrhizal plants (Stribley and Read, 1980; Paungfoo-Lonhienne et al., 2008; Hill et al., 2011b; Vinall et al., 2012), or mycorrhizal plants (Stribley and Read, 1980; Abuzinadah and Read, 1986b). Such methods can clearly demonstrate the existence of enzymatic and uptake functions in specific organisms but provide little insight as to the importance of such nitrogen fluxes in real-world ecosystems.

Field methods for measuring the uptake of organic nitrogen have changed little since Chapin et al. (1993) investigated organic nitrogen uptake in arctic sedges (Näsholm et al., 2009). Typically, an organic substrate labeled with ^{13}C and ^{15}N (most commonly glycine, but sometimes other amino acids or oligopeptides) is added to the soil, and roots are harvested for isotopic analysis after a period of hours. The presence of labeled nitrogen alone does not allow researchers to distinguish between uptake in organic form vs. uptake of ammonium after microbial mineralization of the substrate. However, the presence of labeled carbon in the plant is most plausibly explained by intact uptake.

A major assumption made by studies employing labeled amino acid additions is the "tracer" assumption that the amount added does not substantially change the available concentration of the substrate, and therefore does not affect uptake kinetics (Kirkham and Bartholomew, 1954; Blackburn and Knowles, 1992). However, substantial doubt has

been cast on methods commonly used to measure available amino acid concentrations in soils. Hobbie and Hobbie (2012) argued that the very large populations of bacteria in soils should maintain available free amino acid concentrations in the nanomolar range, based on aquatic culture data, while measured concentrations in soil are typically orders of magnitude greater, in the millimolar range. They suggest that most of what is measured as available in soil extracts may in fact be physically or chemically protected from absorption. Such measurement artifacts may be especially large in forest soils dominated by ectomycorrhizal species, due to high extraradical mycelium biomass in the soil (Jones et al., 2005a). If labeled amino acid addition experiments violate the tracer assumption by substantially increasing free amino acid concentrations in soil solutions, then plants may be relatively more competitive with microbes for amino acids than under natural conditions (Jones et al., 2005b; Hobbie and Hobbie, 2012). High organic nitrogen substrate concentrations may also limit the relevance of many culture studies (Näsholm et al., 2009).

Another assumption sometimes made in interpreting the results of labeled amino acid additions (e.g. Gallet-Budynek et al., 2009) is that the added substrates (most commonly glycine because of its low cost) are representative of other forms of organic nitrogen (e.g., all free amino acids measured in soil extracts) in their availability to plant roots or mycorrhizal fungi relative to free-living microbes. However, among amino acids, glycine may be both a poor carbon source for microbes due to its low C:N ratio, and more available to plants due to its high diffusion rate in soil solution (Lipson et al., 1999; Lipson and Näsholm, 2001). On the other hand, other forms of organic nitrogen such as oligopeptides and amino sugars may also be quantitatively important nitrogen

sources to plants or mycorrhizal fungi (Hill et al., 2011b; Whiteside et al., 2012), so amino acid studies may miss important organic nitrogen fluxes.

An alternative approach to assessing the importance of the organic nitrogen uptake pathway in ecosystems is to infer the fluxes from measurements of nitrogen isotopes at natural abundance ($\delta^{15}N$) in the field (Hobbie et al., 2008; Averill and Finzi, 2011a; Mayor et al., 2012). This approach avoids treatment artifacts and can potentially be applied over wide areas or in places where isotope tracers are impractical. Natural abundance isotopes also integrate over the growing season, unlike tracers which are typically added and measured over short time intervals. However, the method requires clear differences in $\delta^{15}N$ among inorganic and organic nitrogen forms and is accordingly quite sensitive to natural variation in isotope ratios and to sample collection and processing procedures. Comparisons across research sites or species may be particularly sensitive to differences in soil $\delta^{15}N$ profiles and rooting depth distribution (Hobbie and Högberg, 2012). The relationship of the $\delta^{15}N$ of free amino acids to that of bulk soil appears to vary across ecosystems and individual amino acids (Ostle et al., 1999; Bol et al., 2008; Yano et al., 2010), and so should ideally be characterized at each study site. Recycling of nitrogen within plants may also complicate the interpretation of $\delta^{15}N$ data for partitioning sources (Lipson and Näsholm, 2001), as might isotopic fractionation of nitrogen on transfer from mycorrhizal fungi to plant roots (Hobbie and Högberg, 2012). Radiocarbon abundance in protein extracted from ectomycorrhizal fungal tissue has also been used to infer uptake of soil organic nitrogen, as it contains carbon older than the current-year photosynthate used for structural compounds (Hobbie et al., 2012a). However, applications of this method may be somewhat limited, as not all EM taxa

produce aboveground fruiting structures, and the bomb-spike ^{14}C signal is dissipating over time.

4.1.3 A revised field method assessing uptake of organic nitrogen

The methods used thus far all have merit but each is insufficient to unequivocally demonstrate and quantify fluxes of organic versus inorganic nitrogen to plants in ecosystems. Additional experimental approaches that avoid the potentially problematic assumptions discussed above, even while potentially making others, are required to either confirm their results or highlight them for further scrutiny. Here, we use a new approach involving ^{13}C- and ^{15}N-labeled cyanobacterial cells to quantify uptake of isotopically labeled organic nitrogen by mycorrhizal tree roots in four common forest types in New Hampshire. We hypothesized that sites with lower nitrogen mineralization rates that were dominated by ectomycorrhizal tree species would rely more on organic nitrogen than sites with greater mineralization rates and arbuscular mycorrhizal trees.

Our approach involves adding a double-labeled (^{13}C and ^{15}N) whole-cell substrate to root ingrowth cores and incubating for six weeks. The added substrate is then subject to proteolysis and mineralization processes at natural rates, while roots are growing into the cores. This approach avoids the need for potentially problematic measurements of the bioavailable concentrations of dissolved organic nitrogen compounds, and requires only that the tracer addition be small relative to the total organic matter pool that is available for production of plant-available organic nitrogen forms. Additionally, by employing root ingrowth cores, we ensure that the roots analyzed are a single cohort of actively growing fine roots, improving the potential to compare across sites and soil depths, and avoiding the difficult task of separating live from recently dead roots. This method

makes a variety of assumptions as well (chiefly regarding the suitability of ingrowth cores as a proxy for the intact soil environment), but is valuable in that it serves as a complement to existing tracer and natural abundance isotope methods in establishing the magnitude of the organic nitrogen uptake pathway across ecosystems.

4.2 Methods

4.2.1 Site description

We selected forest stands representing a gradient of species composition, soil C:N ratios, pH, and nitrogen mineralization rates in Strafford County, New Hampshire (Table 4.1; Appendix 4.1). We selected two stands in each of four targeted species assemblages: (1) "maple" (*Acer saccharum* L. with some *Fraxinus americana* L.); (2) "oak-beech" (*Quercus rubra* L. and *Fagus grandifolia* Ehrh.); (3) "pine" (*Pinus strobus* L.); and (4) "spruce" (*Picea rubens* Sarg. with some *Tsuga canadensis* L.). All study sites were mature second-growth forest stands which had been cleared for grazing by the mid-19th century and abandoned by about 1930. Soils were predominantly Inceptisols (observed at all sites) and occasionally Spodosols (intermixed at sites BJ, PS, and KF).

Ingrowth cores were established in three replicate blocks in each stand. Block locations were selected for high local dominance of target species (ideally > 80% of basal area within 5 m of the cores) and a lack of obstructions or evidence of recent disturbance in the top 20 cm of the soil profile. Tree and ground-layer species composition are described in Appendix 2.

Table 4.1 Characterization of study sites and ingrowth core soil.
Sites are ordered geographically from south to north (see Appendx 4.1).

site	name	forest type	lat. (DD)	lon. (DD)	elev. (m)	aspect	slope	soil %C	soil C:N	est. BD (g cm^{-3})	soil $\delta^{15}N$ (‰)
DP	Davis Park	maple	43.10	-70.98	30	flat	< 5%	10.7	15.5	0.41	1.8
CW	College Woods	pine	43.13	-70.95	20	S	5-10%	10.8	24.7	0.40	1.8
KF	Kingman Farm	oak-beech	43.18	-70.93	40	W	5-10%	6.2	22.3	0.58	3.6
PS	Parker Mtn - upper	spruce	43.29	-71.16	400	E	< 5%	19.3	32.8	0.26	0.8
PO	Parker Mtn - lower	oak-beech	43.29	-71.16	390	E	30-40%	9.7	25.8	0.43	4.3
BJ	Blue Job Mtn	spruce	43.33	-71.12	370	W	15-50%	18.0	27.3	0.27	0.3
JP	Jones Property - lower	pine	43.47	-71.01	170	E	5-15%	8.0	21.4	0.49	4.1
JM	Jones Property - upper	maple	43.48	-71.01	180	NE	0-30%	10.5	18.2	0.41	3.7

Abbreviations: DD = decimal degrees; BD = bulk density

4.2.2 Field methods

4.2.2.1 Ingrowth core establishment

In each of three replicate blocks per study stand, two cores (control and labeled) 5.7 cm in diameter were taken to a depth of 10–12 cm after removal of the litter layer. Core locations within a block were separated by 30–50 cm. Each core was marked with three aluminum rods around its perimeter. Soil removed from the ingrowth cores was gently sieved to 4.75 mm, picked for fine roots and litter, and mixed in approximately a 1:2 ratio with soil that had been previously collected from several ~10 cm cores at each site, air-dried, and sieved to 2 mm. This was done to provide sufficient volume without an excessive amount of field sieving, which does not easily yield large volumes of root-free soil in highly organic soils. Cores were covered with leaf litter to reduce drying and prevent erosion of the fill soil.

4.2.2.2 Substrate addition

Four weeks after establishment, all sites were visited for soil sampling and label addition. A plug of soil 2 cm in diameter and 2 cm in depth was removed from each core, composited by block, and filled with reserved sieved soil.

After soil sampling, labeled nitrogen treatments were applied to each ON-labeled core using a 5-hole template and a 22 gauge, 35 mm syringe needle. A total of 1 ml of a suspension of ^{13}C and ^{15}N universally-labeled cyanobacteria (*Agmenellum quadruplicatum* strain PR-6; Cambridge Isotope Labs, Andover, MA) was injected to each ON core, containing approximately 2.5 mg N (98% ^{15}N), and 11.5 mg C (98% ^{13}C)

per core. Analysis of carbon and nitrogen in water extracts from subsamples indicated little contribution of soluble inorganic nitrogen in this material.

4.2.2.3 Core harvesting

Cores were harvested to a depth of 10 cm approximately 6 weeks after substrate addition with a sharpened PVC pipe 40.5 mm in inside diameter. Separate corers were used for each isotope treatment. Any roots protruding into the cored volume were pinched off and included in the sample. Samples were stored at 4 ºC for up to 48 hours until processing.

4.2.3 Laboratory methods

Roots were gently cleaned of soil in 1 mM $CaCl_2$ and first- through third-order roots of the target species separated from other roots. Root species was determined by gross morphology, branching pattern, color, and the presence of ectomycorrhizal fungi. Root samples were freeze-dried and weighed. Dried roots were again closely examined and rinsed as necessary before further being subsampled with scissors (1-4 mg), for analysis on a Costech 4010 Elemental Analyzer coupled to a Delta Plus XP isotope ratio mass spectrometer (IRMS). For a subset (n = 8) where sufficient sample was available, multiple fine root subsamples from the same core were analyzed. Labeled samples were run separately from control samples. To reduce isotopic carryover between labeled root samples, root analyses alternated with ~5 mg samples of tuna muscle (a natural abundance lab reference, 15% N).

For a subset of ON root samples with sufficient mass (n = 12), we extracted structural protein to more precisely measure its ^{13}C enrichment. Samples were first extracted with hexane to remove non-polar compounds, then with isopropanol to remove

soluble polar compounds, and finally with 6 M HCl at 110 ºC for 24 hours to hydrolyze non-soluble protein (Hobbie et al., 2012a). Amino acids were purified from the hydrolysate on cation exchange resin (Dowex 50WX8) and analyzed on the IRMS. Three archived samples of homogenized unlabeled roots from the Bartlett Experimental Forest were extracted in the same way to determine the mean difference between bulk root $\delta^{13}C$ and root protein $\delta^{13}C$.

4.2.4 Site characterization data

Soil plugs were stored in sealed bags at 4 ºC for up to 4 days before being picked through for roots and other litter and gently mixed. Because moisture content was low and quite variable among samples, 3 ml of distilled water was added to each sample and well mixed before further processing. A 5 g subsample of each was placed in a tied polyethylene plastic bag to be incubated for 31 days in a dark cabinet at ~20 ºC, and a separate 5 g subsample was extracted for exchangeable ions in 50 ml of 1 M KCl. Three blank KCl solutions were run with each set (pre-incubation and post-incubation). Concentrations of NH_4 and NO_3 were determined colorimetrically on an Astoria autoanalyzer. Separate subsamples of soil were oven-dried at 60 ºC to determine moisture content and then milled for C and N analysis. Core soil bulk density was estimated from %C data based on the relationship published by Federer et al. (1993) for sandy-loam till soils in NH. The remaining soil was pooled by block or site (as dictated by remaining sample mass) and pH was measured in a 1:2 solution with deionized water. Soils were oven-dried at 60 ºC, sieved to 2 mm, and a subsample homogenized in a tungsten-carbide shatterbox. Samples were run for natural abundance ^{15}N and ^{13}C

isotopic ratios. This permitted inferences on qualitative differences in nitrogen cycling among sites.

4.2.5 Organic nitrogen uptake calculations

For bulk root samples and the extracted amino acids, we calculated the fraction of nitrogen label that was taken up as an intact organic molecule (i.e. with its associated labeled carbon), based on the ratio of excess ^{13}C to excess ^{15}N in each sample. Excess was calculated as the difference in atom fraction of each heavy isotope from the baseline natural abundance. Based on control samples, mean background root $\delta^{13}C$ ranged from -28.9‰ to -26.2‰ across stands (Table 4.2). In the control sample roots, there was evidence of lateral transfer within root systems from ^{15}N labeled cores to control cores; $\delta^{15}N$ of individual analyses ranged from -2.8‰ to +34.7‰, with the high end of this range well beyond natural variability; unusually high root $\delta^{15}N$ values occurred across all forest types. Because of this, we used the bulk ingrowth-core soil $\delta^{15}N$ values for each site as the background value instead, since shallow roots closely track bulk soil $\delta^{15}N$ (Högberg et al., 1996; Ouimette et al., 2012). Site averages of bulk soil $\delta^{15}N$ ranged from +0.3‰ to +4.3‰ (Table 4.1). Due to the degree of isotopic enrichment in labeled root samples (Table 4.2), the calculations that follow are insensitive to uncertainties on the order of several per mil in baseline $\delta^{15}N$, but more sensitive to those in baseline $\delta^{13}C$.

For bulk roots, we calculated protein-carbon concentration assuming that protein was the only source of measured bulk-root nitrogen, and that protein was 16.7% N and 47.7% C (Hobbie et al., 2012b). We then calculated the molar ratio of ^{15}N excess to ^{13}C excess in root protein, assuming that all labeled carbon occurred in protein. This ratio was then converted to a ratio of labeled amino acid uptake to total labeled nitrogen

uptake, based on a molar C:N ratio of 3.34 in fungal protein (Hobbie et al., 2012b), and assuming that 50% of AA-carbon is respired on uptake; this estimate falls towards the high side of the range observed in microbial cultures (Hobbie and Hobbie, 2012), and was chosen to allow for the possibility of respiration by both the fungal and plant symbionts. Equations are shown in Appendix 4.3. The fraction of total labeled nitrogen uptake that occurred in organic form was averaged by core (where there were multiple root analyses) and then by site.

With protein extractions of root samples, we were able to directly measure the $\delta^{15}N$ and $\delta^{13}C$ of root protein, simplifying the calculations and providing a check on bulk-root estimates. For protein, we again assumed that baseline $\delta^{15}N$ was equal to that of the bulk soil, but that baseline $\delta^{13}C$ was enriched by 2.1‰ (equivalent to an atom

Table 4.2 Mass and C and N isotope ratios from harvested ingrowth roots. Values are means 1 ± SD

Site (type)	**mean target root mass per core (mg)**	**control & IN* root $\delta^{13}C$ (‰)**	**ON root $\delta^{13}C$ (‰)**	**ON root $\delta^{15}N$ (‰)**
DP (maple)	30.8 ± 40.8	-27.6 ± 1.2	-18.3 ± 6.8	16100 ± 11600
CW (pine)	6.8 ± 4.5	-26.5 ± 0.8	-23.8 ± 2.2	1760 ± 1500
KF (oak-beech)	39.1 ± 21.4	-28.1 ± 0.9	-25.1 ± 3.1	2890 ± 1880
PS (spruce)	8.1 ± 5.0	-26.8 ± 1.1	-21.9 ± 3.3	5110 ± 5660
PO (oak-beech)	42.7 ± 16.1	-27.8 ± 0.9	-25.2 ± 3.5	6470 ± 6050
BJ (spruce)	2.1 ± 2.2	-26.5 ± 0.6	-21.1 ± 6.3	3790 ± 5510
JP (pine)	9.7 ± 6.0	-27.6 ± 0.9	-27.5 ± 1.5	910 ± 790
JM (maple)	18.9 ± 19.0	-28.0 ± 1.3	-24.7 ± 1.7	1660 ± 1040

*IN cores were amended with $^{15}NH_4Cl$; see Appendix 4.4.

fraction difference of 0.0000234) relative to the mean $\delta^{13}C$ of bulk roots from control cores at each site. Subsequent calculations proceeded as described above for bulk roots.

Metrics of organic N uptake were related to individual site characteristics including soil C:N, pH, exchangeable NH_4^+, N mineralization, nitrification, and total root ingrowth mass. Regressions were conducted at both the site scale (n = 8) and the core scale (n = 23; one core at site CW contained no pine roots) with site as a random effect.

4.3 Results

4.3.1 Site characteristics

Our data confirm large and statistically significant differences among study sites in soil pH, C:N, and organic matter percentage (Table 4.1, Fig. 4.2; single-factor ANOVA p values all < 0.001). Spruce stand soils had the greatest C:N ratios and lowest pH, while maple stand soils had the lowest C:N and highest pH; oak-beech and pine soils were intermediate. Spruce soils had by far the greatest concentrations of organic carbon (the top 10 cm was mostly or entirely in the Oa horizon where sampled), and maple-ash soils had relatively thick and organic-rich A horizons relative to the oak-beech and pine soils. Bulk N concentrations were similar in spruce and maple soils, and greater than in oak-beech and pine soils. Exchangeable NH_4^+ and potential N mineralization per gram of soil was greatest in the maple and spruce stands and lowest in the oak stands. However, because bulk density was estimated to be 35-50% lower in the spruce soils than at the other sites (Table 4.1), N availability was greatest in the maple sites. No soils removed from the cores had detectable nitrate prior to the lab incubation. Net mineralization (including nitrate production) per g soil varied widely among replicate cores, but significant differences were observed across stands (n = 8; ANOVA p = 0.01) and across

forest types (n = 4; ANOVA p = 0.02). In general, maple stands had about 3 times greater N mineralization than pine and spruce sites. In the lab incubation, net nitrification occurred in some replicates of incubated soils from all stand types and did not differ significantly by stand or forest type.

Target species root ingrowth mass also varied significantly across the four stand types but not across core labeling treatments (Table 4.2; 2-way ANOVA $p < 0.001$ and $p = 0.50$, respectively). Oak-beech stands had the greatest root ingrowth, followed by maple-ash, while in the spruce stands we were quite sample-limited. These differences in root production are consistent with those observed over several years across a similar species gradient at the Bartlett Experimental Forest (M.A. Vadeboncoeur and A.P. Ouimette, unpublished data).

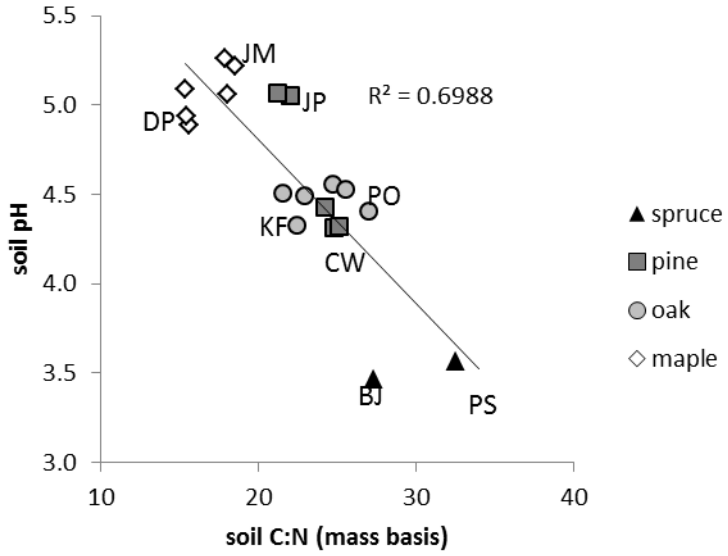
4.3.2 Uptake of organic nitrogen

Across all study sites, organic uptake of labeled nitrogen averaged 5% of total labeled nitrogen uptake; in individual cores it ranged from 0 to 50% (Fig. 4.3). There was no significant difference by site or forest type (single-factor ANOVA p = 0.38 and 0.07, respectively), although the mean value for spruce stands (16%) was notably higher than those of the other forest types, which averaged 2% to 4%.

Uptake of labeled N in organic form correlated with negatively soil pH and positively with soil C:N at the site and core levels; these trends were driven largely by the higher mean organic N uptake observed at the spruce sites (Fig. 4.4), which have high C:N and low pH relative to the other sites. Soil %C and %N also correlated positively with organic N uptake, again driven largely by spruce site differences. Regressions against metrics of N availability based on the lab incubation were not significant.

Figure 4.2 Chemical characterization of soils used to fill ingrowth cores.
a) Soil pH and C:N ratio across all study sites.
b) Soil total N concentration and exchangeable NH_4^+.
Regressions are significant to $p < 0.001$.

a.



b.

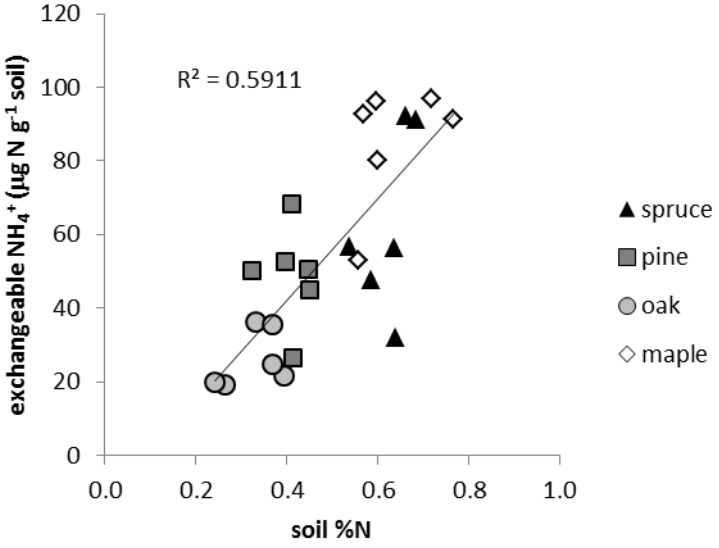


Figure 4.3 Protein ^{13}C excess and bulk ^{15}N excess in bulk root analyses.
Straight lines show ratios that correspond to a range of values for the percentage of labeled nitrogen taken up in organic form. Replicate analyses of root samples are plotted independently here, but are averaged in subsequent analyses.

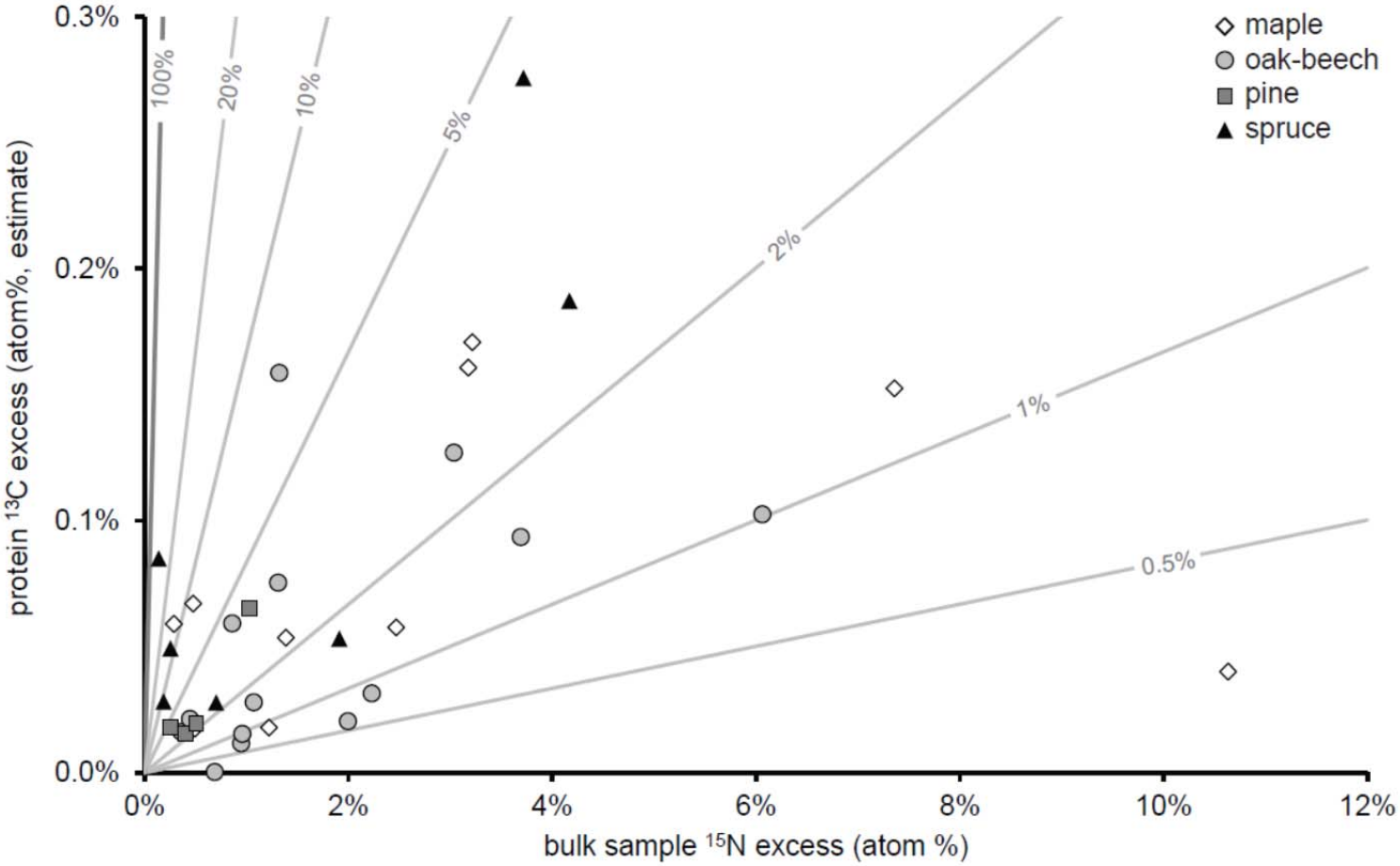


Figure 4.4 Relationships between labeled N uptake and soil chemistry. Regressions against C:N and pH are at the core level.

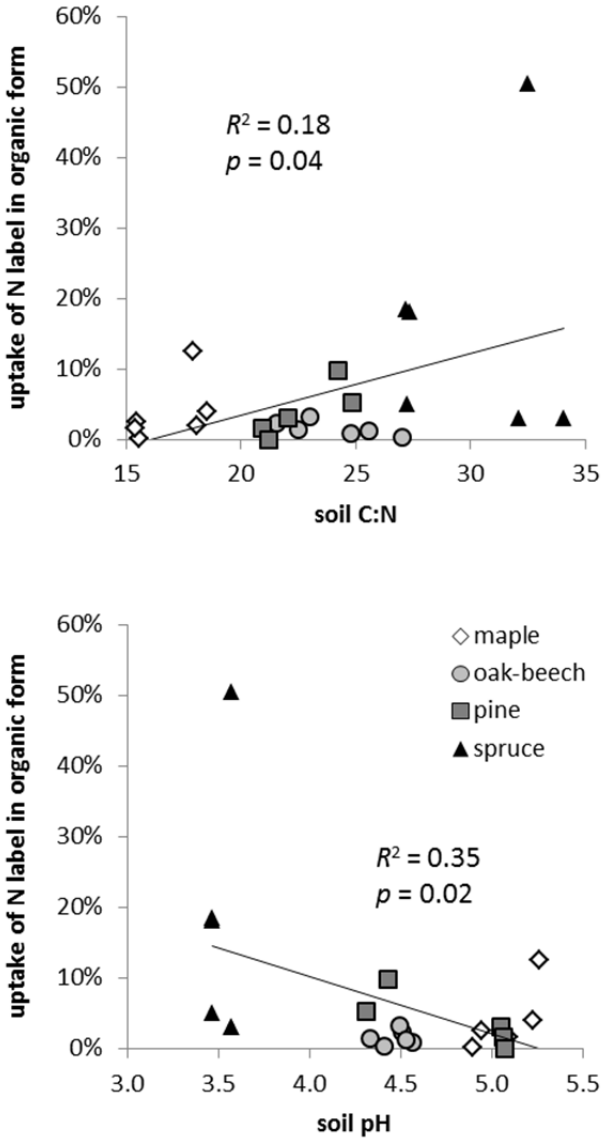
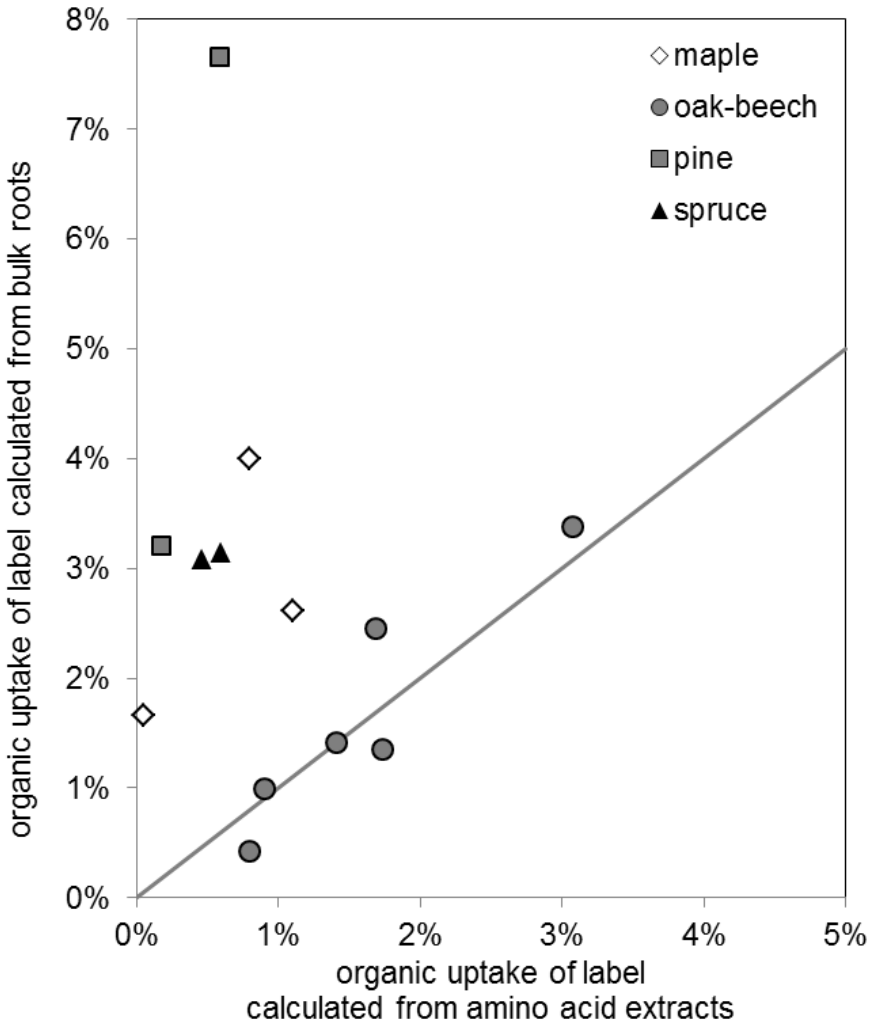


Figure 4.5 Organic N uptake estimated from amino acid and bulk root analyses. The solid line shows the expected 1:1 relationship. Oak-beech samples show good agreement between the two methods, but overall bulk analyses yielded significantly greater estimates of organic N uptake than amino acid analyses. This may be due to sample heterogeneity, surface contamination of the bulk samples, or fungal tissue that was lost in the extra processing steps for the amino acid extraction. There is no indication that protein stoichiometry assumptions used in bulk analysis (Appendix 4.3) were systematically wrong; under this condition, we would expect a linear relationship other than 1:1.



Amino acid extractions of roots yielded estimates of organic uptake of the ^{15}N label in the same general range as those estimated from bulk roots (Fig. 4.5). Overall, there was not a significant linear relationship between the two estimates across replicate cores. However, among the oak-beech samples only, there was a strong correlation (R^2 = 0.86; p = 0.01) and good agreement with the expected 1:1 relationship (Fig. 4.5). Across all samples, there was a slight bias towards high estimates of organic label uptake in the bulk analyses than in the amino acid analyses (paired *t*-test, p = 0.01); this bias only appears in the non-oak-beech samples. Some of this variation likely reflects isotopic inhomogeneity with root samples; replicate analyses of 1-4 mg subsamples of non-homogenized bulk roots had coefficients of variation ranging from 20-115% in the ratio of ^{13}C excess to ^{15}N excess. The reason for the difference between the oak-beech and the other samples is unclear. The larger oak-beech samples may have been more isotopically homogenous or may have been easier to clean of surface contamination and fungal biomass than other species.

4.4 Discussion

4.4.1 Organic nitrogen uptake across sites

Our results provide strong evidence for the process of intact uptake of organic nitrogen compounds by fine roots or their associated mycorrhizal fungi, at ecologically relevant soil concentrations. However, the mean contribution of these nitrogen forms to the total root nitrogen budget was generally relatively small and highly variable.

We observed little systematic difference among stands, despite obvious soil and species differences that might be expected to correlate with differences in organic

nitrogen uptake, though spruce sites had a generally greater fraction of labeled uptake in organic form, high C:N ratios, and low pH. Based on nitrate concentrations below detection limits in initial ingrowth-core soil at all sites (in late July), it appears that microbial and root demand for ammonium was high despite low root densities. However, the lab incubations indicate a wide range in potential for nitrification, which implies that some soil microsites in all study sites may mineralize N in excess of short-term microbial and plant demand. The lack of correlation of isotopic metrics of mineralization and organic uptake with lab-measured mineralization potential may relate to large difference in lability between the added labeled substrate and native soil organic matter at the different sites.

4.4.2 Methodological considerations

The greatest limitation associated with the method we developed is that ingrowth cores do not perfectly represent the typical soil environment. Substantial disturbance effects may be associated with sieving roots from soil, including perhaps enhanced organic matter mineralization and altered microbial communities, and the reduced root density in the ingrowth core may result in an increased the supply of inorganic N relative to demand. Additionally, in longer-term incubations, it is necessary to account for respiration of amino acid carbon. We used a value of 50% of AA-carbon respired when estimating organic uptake of labeled N; lower fractions respired would reduce our estimates of organic N uptake by as much as half (Appendix 4.3). However, this method has a variety of advantages over lower-disturbance methods that involve short-term additions of isotopically labeled single amino acids to the soil. First and most critically, labeled amino acids and other forms of organic nitrogen (including amino sugars and

oligopeptides) are released from the added organic substrate and then mineralized at approximately natural rates, rather than added as a pulse of a single amino acid that are probably large relative to truly available pools. The ingrowth core method therefore does not require assumptions about the relevance of such available concentrations and about whether a given amino acid is representative of other forms of organic nitrogen in the soil. Soluble nitrogen chemistry in soil may be more complicated than previously appreciated (Warren, 2013).

If organic N uptake varies across the growing season, based on substrate availability, mineralization rates, and competition for uptake (McKane et al., 2002), our experiment and most assays of labeled N uptake offer only a one-time snapshot of a dynamic process. Our six-week late-summer incubations occurred during a time when both enzymatic proteolysis and N mineralization would be expected to be limited by the availability of labile substrate and at least intermittently by soil moisture, but not by temperature (Brzostek and Finzi, 2011). This situation might be expected to result in strong competition by soil microbes and mycorrhizal fungi for free available organic N compounds. Longer incubation times could potentially provide results more representative of growing-season mean contributions of organic N uptake. However, if the characteristic turnover time of the added organic substrate is short, the power to resolve this process diminishes with longer incubation times. Because we did not attempt to quantitatively recover the added tracer, we cannot comment on whether a longer or shorter incubation time would be optimal.

The forms of N transferred from the mycorrhizal fungus to the plant host appear to vary; evidence supports both organic forms such as glutamate and argninie have been

suggested as well as ammonium (Chalot and Brun, 1998; Chalot et al., 2006; Smith and Read, 2008; Jin et al., 2012). To the extent that N acquired in organic form is transferred to the root in organic form or associated with a different organic skeleton than that with which it was acquired, our estimates of organic uptake based on the ratio of ^{13}C excess to ^{15}N excess are underestimates. Our root samples included some fungal tissue, but presumably the majority of extraradical fungal biomass was removed with rhizosphere soil prior to analysis; the large majority of N and protein-C measured in each analysis was from root tissue.

The method we introduce here would be improved by procedures that could reduce within-core heterogeneity of label application. Some of this variability may result from the heterogeneous distribution of tracer application within each ingrowth core and the overlapping spatial distribution of roots and mycorrhizal fungi in the core. One alternative would be to incorporate the tracer into the homogenized fill soil prior to filling the ingrowth cores, but this introduces the potential problem that the majority of the fairly labile substrate added might be mineralized before roots grew into the core. Differences in mineralization rates among study sites would add to these complications. Alternatively, the substrate could be injected when soil is at or above field capacity; the dry soils we encountered when applying the label likely contributed to the heterogeneous distribution of the isotopic label within the cores. Also, to avoid laboratory contamination with highly enriched material, we did not thoroughly homogenize root samples thoroughly prior to analysis, which likely introduced additional variability that could be reduced by careful homogenization.

4.4.3 Comparison to other organic N uptake studies

In temperate forests, N mineralization often explains much of the observed variation in primary production (Pastor et al., 1984; Carlyle and Nambiar, 2001; Newman et al., 2006), suggesting an often dominant role for DIN uptake in in meeting plant N requirements (Wu, 2011). On the other hand, in colder climates with low N mineralization rates, plants in boreal and tundra ecosystems appear to rely more heavily on organic N forms (Schimel and Chapin, 1996; Näsholm et al., 1998). Our sites were selected to span a range in N mineralization rates, from spruce sites with recalcitrant litter and thick organic horizons, to maple sites with high-quality litter and high N mineralization. We saw non-significantly greater reliance on organic N in the spruce sites, though the very low root production at these limited our ability to assess uptake precisely.

The few direct uptake experiments involving short-term isotope label uptake in comparable study systems show a similar patterns. Maple-ash forests in Connecticut took up relatively little labeled glycine relative to inorganic N (about 20% of total), while glycine represented 48-77% (by horizon) of total uptake in nearby hardwood-hemlock forests (Gallet-Budynek et al., 2009). Excised roots from these and a pine-dominated site took up DIN at 2-6 times the rate of glycine (Finzi and Berthrong, 2005). In spruce-fir-birch forests ~100 km north of our study area, uptake of glycine-N increased with elevation and exceeded DIN at high elevations, where presumably temperature limits N mineralization to a greater degree (Averill and Finzi, 2011a).

Our estimates of organic N uptake are generally lower than those provided by short-term labeled uptake studies in similar systems, though this could be attributable to

stand-scale or temporal differences in mineralization and uptake processes. It is not clear the extent to which relative uptake of labeled single amino acids and inorganic N over short incubation times can be compared to our estimates of organic and inorganic N uptake based on longer-term incubations of a complex organic matter substrate. It is possible that inherent biases in each method bracket a range of realistic uptake rates. For example, if organic uptake of the labeled substrate we added dominates early in the incubation period, but most of the label is mineralized relatively quickly, this would serve to reduce the fraction of organic uptake of the label over increasingly long incubation periods.

Direct methods comparisons in the same forest stands may be warranted, and could shed light on the relevance of the various assumptions required by each method. However, comparing short- and longer-term rates is inherently difficult, and may require multiple short-term measurements. The difference between adding a short-term substrate to intact soils containing high densities of live and dead roots, and adding a longer-term substrate to disturbed cores with low root density must also be considered. Lateral transfer of labeled N from one treatment location to another nearby (as seen in the ^{15}N enrichment of control-core roots in this study) could also complicate such a comparison; the optimal spacing to take advantage of spatial autocorrelation in soil properties but avoid isotopic transfer between paired cores is unclear.

4.5 Conclusions

We used a six-week double-labeled organic matter incubation to demonstrate a low but significant (2-16%) degree of organic nitrogen supply to mycorrhizal tree roots in the temperate forest ecosystems examined. This method allowed us to avoid potentially

problematic assumptions about available concentrations of amino acids in soil solution or the identity of quantitatively important plant- and mycorrhizal-available organic N compounds. Our estimates of organic N uptake are notably lower than those done in similar forests in the region, but it is unclear whether this is due to methodological differences. Such low values indicate that under current conditions the process of organic N uptake is not quantitatively very important to ecosystem function. The degree of organic N deamination and transamination in fungal tissue prior to fungus-root transfer is unclear, but may affect the accuracy of these estimates. In spite of methodological limitations, the method we introduce confirms that the capacity exists across a range of temperate forest ecosystem types. Future applications of this method, perhaps in concert with shorter-term approaches, could shed light on whether a change in the importance of organic N uptake might occur in situations where N limitation is increased relative to present-day, such as under intensified biomass harvesting without fertilization, accompanied by a decline in atmospheric N deposition.

CHAPTER 5

SUMMARY OF PRINCIPAL FINDINGS AND RECOMMENDATIONS

In the preceding four chapters, I have presented the results of several studies targeted at answering questions important to understanding forest nutrient cycles in a changing environment. Principal findings, which may be useful in designing further research relevant to management in northeastern forests, are summarized here.

In a meta-analysis of 35 fertilization experiments, I found that N is clearly a limiting nutrient in hardwood forests of the northeast, but fertilization experiments indicate significant responses to P and Ca fertilization as well (Chapter 1). Co-limitation among multiple resources is becoming recognized by the ecological community as somewhat common among ecosystems globally (Elser et al. 2007), and the forests of the northeast are no exception. Despite the large number of fertilization studies previously conducted in the region, additional factorial fertilization studies are needed to examine the mechanisms of nutrient limitation and the processes by which co-limitation is maintained (Craine, 2009), to screen for threshold effects beyond which these processes change, to separate pH from Ca fertilization effects, and to document potential regional differences due to soil parent material (Crowley et al., 2012). One such project,

MELNHE, (Multiple Element Limitation in Northern Hardwood Ecosystems) was initiated in 2011 by a team including Ruth Yanai, Melany Fisk, Tim Fahey, and myself.

Given that N, P, and Ca all have the potential to limit forest productivity, it is critical to proper forest management that the cycles of these nutrients are adequately understood at relevant spatial and temporal scales. With collaborators, I constructed harvest-rotation nutrient budgets under a range of harvesting scenarios and under various assumptions about ecosystem inputs and outputs, and the availability of various ecosystem stocks at management-relevant time scales. We compared these budgets to soil nutrient stocks measured in mature and aggrading northern hardwood stands across the White Mountain region of New Hampshire (Chapter 2). We found that whole-tree harvesting, even on a 100-year rotation, substantially increases the rate at which soil stocks of nutrients are depleted. Even assuming that apatite stocks of P and Ca in the B horizon are available for accelerated mycorrhizal weathering, there is the potential whole-tree harvesting could deplete these stocks over 1-5 rotations. The substantial variation among stands is due in part to till depth and in part to soil mineralogy. We recommend a cautious approach to harvesting stands on thin soils, particularly on low-apatite substrates such as Conway granite. Luckily, the long harvest rotation lengths currently common in the region leave sufficient time for more research to improve our understanding of how nutrient cycles change after harvest. To the extent that low-value forest biomass is burned locally to generate electricity, it may be prudent to investigate the economic and ecological impacts of returning the nutrients in wood ash to forest ecosystems as fertilizer.

While weathering rate estimates are important to determining sustainable biomass harvest rates as shown in Chapter 2, available methods lack the precision to make such recommendations at the site scale with confidence (Klaminder et al., 2011; Futter et al., 2012). Causes include soil heterogeneity, the inability to detect short-term changes in long-term nutrient stocks, and errors in measurements of other components of nutrient budgets. If mycorrhizal weathering is accelerated under high biotic demand in aggrading forest stands as suggested by some ecosystem budgets (Hamburg et al., 2003; Yanai et al., 2005), it is important to have a method that can validate this finding independently. Rare earth element ratios and Pb isotope ratios are distinct in apatite compared with other minerals. In a greenhouse experiment where birch seedlings were grown with and without bags of crushed granite, we found that these tracers indicated weathering of apatite (and possibly other accessory minerals rich in LREEs and radiogenic Pb) was accelerated in the presence of birch seedlings, though there were no consistent effects of different mycorrhizal treatments. This suggests that these tracers may find use in field applications. However, birch roots showed only a significant REE signal from granite and not a clear Pb isotope signal. The lack of a mycorrhizal effect may have been an artifact of poor mycorrhizal growth under greenhouse conditions, or may reflect the fact that non-mycorrhizal birch roots have significant ability to induce weathering on their own. Our application of these tracer systems showed few differences among mycorrhizal sporocarps including *Leccinum*, *Cortinarius*, and *Lactarius* species collected at six field sites. This may be due to atmospheric Pb and differences in mycorrhizal depth and substrate at the scale of individual sporocarp samples overwhelming any apatite signal. Additional replication within and across research sites, as well as sampling a wider

taxonomic diversity, would be required to determine whether these tracer systems are potentially useful in field settings.

Finally, recent declines in atmospheric N deposition (Bernal et al., 2012) at the regional scale call into question whether such inputs will continue to offset harvest removals as suggested by Chapter 2; N is already a limiting nutrient even under chronic N deposition (Chapter 1; Thomas et al., 2009; Crowley et al., 2012). Under these conditions, the uptake and use of organic N by mycorrhizal roots may represent a greater fraction of the total nutrient budget, bypassing the microbial mineralization "bottleneck" (Chapman et al., 2006). There is clear evidence of this pathway in tundra and boreal ecosystems, but the methods thus far applied in temperate forests (e.g. Gallet-Budynek et al., 2009) have been criticized for introducing potentially unrealistic concentrations of free amino acids (Hobbie and Hobbie, 2012). We used a novel approach of adding isotopically labeled whole organic matter and allowing proteolytic enzymes to release labeled amino acids at natural rates to confirm that organic N uptake does occur in a range of common northeastern forest types (Chapter 4). Estimated rates of organic uptake of the N label are relatively low (generally < 10%), but we confirmed that organic uptake occurred in all four forest types examined over a 6-week incubation in mid-late summer. Additional studies that explicitly compare the two methods and assess differences in organic N uptake seasonally, and across successional, climate, and fertility gradients, would further improve our understanding of the controls on organic N uptake in temperate forests, and allow us to better model the effects of various global change drivers on forest ecosystem N cycles.

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APPENDICES

Appendix 1.1 Table of experimental data sets included in the meta-analysis.

#	Reference	Location(s)	species (some may not be used here)	stand or cohort age	exp. design	fert. schedule	growing seasons	usable reported obs.	limiting resources
1	Bigelow and Canham 2007	Great Mountain Forest (CT)	QURU, ACRU, ACSA, FRAM, FAGR, TSCA, PIST	saplings	N X Ca factorial	continual	3	7	Ca and N
2	Côté et al. 1995	Entrelacs (QUE)	ACSA	110	N, Ca separately	pulse	5	2	not Ca or N
3	Ellis et al. 1979	Grey County (ONT)	ACSA, FRAM, PRSE	35 - 85	NPCa	pulse	5	6	N; not-N
4	Elvir et al. 2003	Bear Brook (ME)	ACSA	mature	N	continual	10	1	N; not-N
5	Fahey et al. 1998	White Mountains (NH)	PRPE. BEPA, BEAL, FAGR, ACSA, ACPE	6 - 23	NPCa	continual	5	3	one or more of N,P,K,Ca, Mg
6	Finn and Tyron 1942	Black Rock Forest (NY)	QURU	43	N and P separately	pulse	3	3	Ca and/or P; N
7	Finn and White 1966	southwest MI	LITU (plantation)	20	N and NPK	pulse	5	4	N
8	Finzi 2009	Great Mountain Forest (CT)	ACSA, FRAM, QURU, FAGR, TSCA	120	NxP full factorial	continual	2	6	primarily N; secondarily P
9	Fyles et al. 1994	Station Biologique des Laurentides (QUE)	ACSA	80	base cations only	pulse	3	1	Ca, pH or other base cations
10	Gradowski and Thomas 2008	Halliburton Forest (ONT)	ACSA	mature	PxCa full factorial	continual	2	3	not P
11	Juice et al. 2006	Hubbard Brook (NH)	ACSA, FAGR, BEAL	1	Ca	pulse	5	1	Ca and/or pH
12	Karnig 1972	Black Rock Forest (NY)	QURU	mature	N	pulse	6	1	N
13	Kobe et al. 2002	Hubbard Brook (NH)	ACSA, FAGR, BEAL	2	Ca	continual	2	3	Ca
14	Lea, Tierson, and Leaf 1979	Huntington Forest (NY)	BEAL, ACRU, ACSA, FAGR	70	NxPxCa full factorial	pulse	2	5	N; P
15	Leech and Kim 1990	Flos (ONT)	ACSA	100	NxPxCa partial factorial	continual	6	2	N or N+P
16	Mader et al. 1969	Conway (MA)	ACSA	mature	NPK only	pulse	2	1	N
17	Magill et al. 2004	Harvard Forest (MA)	QURU, QUVE, ACRU, FAGR, BELE	90	N	continual	14	2	N
18	Mercer 1974	central NB	ACSA	37	NxP factorial	pulse	2	3	N
19	Mitchell and Chandler 1939	Black Rock Forest, Arnot Forest (NY)	ACRU, ACSA, CAGL, FAGR, FRAM, LITU, NYSY, POTR, QU**, TIAM	35 - 60	N	pulse	2	38 *	N

Appendix 1.1, continued

20	Mitchell et al. 2001	Huntington Forest (NY)	FAGR, BEAL ACSA, ACRU	70	N	continual	3	2	not-N
21	Moore and Houle 2009	Lake Clair watershed (QUE)	ACSA, FAGR, BEAL	mature	N	continual	3	2	not-N
22	Moore and Ouimet 2006	Lake Clair watershed (QUE)	ACSA	mature	Ca	continual	10	7	Ca, Mg, or pH
23	Ouimet and Fortin 1992	Beauce (QUE)	ACSA	mature	P + base cations	pulse	2	1	P, K, Ca, Mg, or pH
24	Pregitzer et al. 2008	Michigan Gradient Study	primarily ACSA	90	N	continual	10	4	N
25	Safford 1973	Bartlett (NH)	BEAL, BEPA, ACSA, FAGR	60	NxPxCa partial factorial	pulse	7	8	one or more of N,P,K. Also Ca or pH effect
26	Safford 1982	Bartlett (NH) and Massabesic (ME)	BEPA	7	NxPxCa partial factorial	pulse	3	14	Bartlett: N, then P. Massabesic: N only
27	Safford and Czapowskyj 1986	Clifford Burn (ME)	POGR, POTR, BEPA, ACRU	14	NxPxCa full factorial	continual	10	28	ranked by number of significant effects: N+P+Ca, N, P+Ca+Mg, Ca+Mg
28	Safford and Filip 1974	Bartlett (NH)	PRPE, BEPA, BEAL	just cut	NPK lime	pulse	4	1	one or more of NPK. Also Ca or pH effect
29	Schmitt et al. 1981	Clifford Burn (ME)	POGR, BEPA	22	NPK lime	pulse	4	2	one or more of N, P, Ca, or pH
30	Stanturf 1983	multiple sites (NY)	ACSA, FRAM, PRSE, QURU, TIAM	100	N	pulse	10	18	N and non-N
31	Stone 1980	Hurley (WI)	ACSA, ACRU	48	NxP full factorial	pulse	10	3	N; P?
32	Tripler et al. 2002	Great Mountain Forest (CT)	BEAL, FAGR, PRSE, QURU	saplings	N	pulse	1	8	N; not-N
33	Wallace et al. 2007	Millbrook (NY)	QUPR, QURU, CA**, ACSA, ACRU, PIST	60 - 80	N	continual	8	1	not-N
34	Wilmot et al. 1996	multiple sites (VT)	ACSA	mature	Ca, Mg, and K	continual	3	2	Ca, Mg, and/or K. Larger pH effect
35	Zaccherio and Finzi 2007	Great Mountain Forest and Housatonic State Forest (CT)	ACSA, ACRU, QURU	3-6 yr seedlings	N x Ca full factorial	continual	4	24	N for red maple, Ca for sugar maple

Species are abbreviated using the first two letters of the genus and the first two letters of the species;
e.g. ACRU = *Acer rubrum*; AC** = multiple *Acer* species A complete species list is included in Table 1.2.

* Only observations including at least 5 control and 5 treatment trees were used from Mitchell and Chandler (1939).

Appendix 2.1 Weathering rate calculations for phosphorus.

Because of its fixation in secondary minerals in the soil, short-term soil weathering rates for P based on hydrologic losses probably underestimate the rate of primary P weathering in the soil, and indeed these calculations are published less often than for major rock-forming elements such as Ca, Mg, K, Al, Fe, Si, etc. (Table 2.1). To construct ecosystem budgets for P, we must therefore make indirect estimates from other available data. Below, we discuss a range of published estimates of P weathering at HBEF and other data that might be used to constrain these estimates.

Long-term weathering rate estimates

Yanai (1992) estimated weathering at **0.11 kg P ha^{-1} yr^{-1}**, which represents the long-term mean required to supply current biomass and soil organic matter over ~10,000 years of ecosystem development. This estimate likely represents a minimum long-term mean, since there must have been some degree of P loss from the ecosystem over these timescales as secondary mineral P fixation and sediment loss.

Nezat et al. (2004) also estimated long-term mean weathering at **0.11 kg P ha^{-1} yr^{-1}** based on P depletion relative to Ti in soil profiles excavated at HBEF Watershed 1 prior to fertilization. We used this value for **Scenario I at HBEF**. This value represents the weighted mean over all pre-fertilization W1 soil profiles examined based on a total digest of the soil, so it represents the *net* effect of primary mineral weathering minus secondary mineral formation and organic P accumulation storage. This method makes few assumptions other than that the reference element is immobile in the profile at pedogenic time scales, and that the deep C horizon material (which is fairly homogenous over 50+cm when examined; Schaller et al. ,2010) represents the initial composition and bulk density of the full till profile.

For **scenario I in other stands**, we used a similar profile-based approach to estimate long-term P weathering across 13 study stands, with a mean of **0.05 kg P ha^{-1} yr^{-1},** and a range of **0.02-0.08 kg P ha^{-1} yr^{-1}**. Weathering rates for base cations are reported by Schaller et al. (2010), though P is reported here for the first time. One difference between these values and those reported by Nezat et al. (2004) is that these are not based on total P but on P extractable in room-temperature 1N nitric acid, after removing organic P with a 30% hydrogen peroxide leach. This rate might be an underestimate by about ~20%, if we account for silicate-shielded P not accessible to the nitric acid leach. On the other hand, this estimate avoids counting organic P and likely most secondary P, which has already been weathered from primary minerals, as part of the total.

It could be argued, that since weathering rates in general follow an exponential decay function (Taylor and Blum, 1995) under unchanging conditions, current rates would be expected to be approximately a third of the long-term mean. We did not apply this correction, as the specifics may vary across landscapes, and also because long-term mean weathering rates may underestimate current short-term weathering rates due to increases in in acid deposition and biomass removals over the past 100-200 years.

Short-term (current) P weathering rates

Wood et al. (1984) published the first estimate of current P weathering at HBEF, **1.5-1.8 kg P ha^{-1} yr^{-1}**, though the steps of this calculation were not published. Yanai (1998) reported that this estimate was based on early Ca weathering estimates from wathershed budgets (later published by Likens and Bormann 1995), and a whole-rock or whole-soil Ca:P ratio (Johnson et al., 1968). This calculation assumes congruent weathering of soil material; i.e. that mineral all weather at the same rate and the relative

abundance of each mineral remains constant over time. We have not been able to precisely replicate this calculation based on these published data, but in any case we judge this first published value to be an overestimate due to later corrections to the Ca weathering rate on which it is based (Likens et al., 1998). This rate is clearly unsustainable over the long term; the initial unweathered total solum P stock would represent only about a 2,000 year supply at these loss rates, despite 14,000 years of soil development. Yanai (1998) notes that the Wood et al. (1984) estimate was approximately the right magnitude to balance P demand by the aggrading forest for which it was calculated, but that it likely did not accurately represent a steady-state system.

To update this calculation, one could use the same total P:Ca mass ratio (0.075) in the C horizon till reported by Johnson et al. (1968), and multiply this ratio by a more recent budget-based estimate of Ca weathering from Likens et al. (1998), (Table 2.1), which yields a much lower estimate of **0.15-0.23 kg P ha^{-1} yr^{-1}**. Using the ratio from unweathered Littleton Formation rock gives a somewhat higher **0.40-0.45 kg P ha^{-1} yr^{-1}**. Again, these estimates assume congruent weathering of mineral material.

For the short-term weathering rate estimate used in **scenario II**, we started with the most recent budget-based estimate of Ca weathering from Likens et al. (1998) (Table 2.1), and assumed that 17% of Ca weathering is from apatite (midpoint the 12-22% rate estimated by Nezat et al. 2004). This leads to an apatite weathering estimate of 0.39 kg Ca ha^{-1} yr^{-1}, which based on a 3:5 molar ratio of P:Ca in apatite, converts to a weathering release of **0.18 kg P ha^{-1} yr^{-1}**.

Appendix 3.1 Greenhouse experiment photographs.

Photo 1.

Nylon 50-μm mesh bag containing 10 g of Conway granite crushed to 250-500 μm.

Photo 2.

Mesh bags were placed mid-depth in standard polypropylene greenhouse pots 10 cm in diameter. The remainder of each pot was filled with perlite, saturated several times with deionized water, and allowed to drain freely to field capacity before planting.

Photo 3.

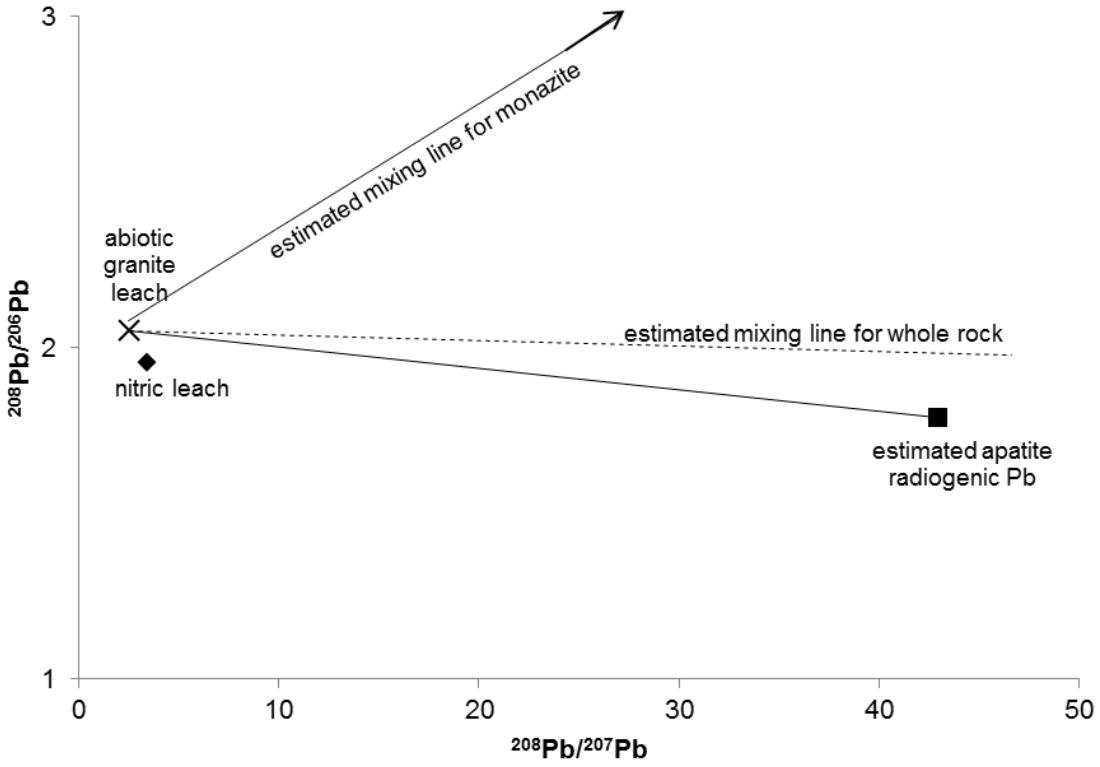
After the inoculation and planting of one yellow birch germinant per pot, they were placed under an automatic mist in the greenhouse for two weeks, then moved and allowed to grow for an additional 4 months.

Photo 4.

At harvest, stems and leaves were separated from roots with stainless steel blades. Roots, which grew throughout the pots and often immediately against the mesh bags, were carefully separated from the perlite in deionized water baths, then thoroughly rinsed before drying and weighing.

Appendix 3.2 Sources of acid leachable Pb in Conway granite.

Pb isotope comparison of the nitric acid leach with the mixing space expected for contributions from monazite, with high Th/U ratios, and apatite, which discriminates little between U and Th, assuming an age of 180 Ma (Eby et al., 1992), Kd values from Bea et al. (1994), and whole-rock Th/U of 5.9 based on the XRF analysis. Initial non-radiogenic Pb is represented by the abiotic greenhouse leach from the greenhouse experiment. This analysis should be regarded as approximate, but the nitric leach falls closest to the expected mixing line for apatite, suggesting that acid leachable Pb came primarily from phases with relatively low Th/U ratios and little was contributed by mineral phases with high Th/U ratios.



Appendix 3.3 Birch and leachate elemental concentrations and Pb isotope ratios

		Pb isotope ratios				Major elements (ppm)				
pot #	Treatment	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{206}Pb$	Mg	Ca	Fe	Zn	Sr
Birch roots										
2	BCW	38.254	15.637	18.390	0.8503	2449	4061	86.0	216.5	44.0
4	BXW	38.411	15.620	18.429	0.8476	1388	2715	87.5	61.1	39.0
13	BLW	38.314	15.648	18.449	0.8372	1637	3299	130.2	54.0	43.7
19	BXW	38.273	15.635	18.380	0.8507	2316	4466	73.2	63.5	59.5
26	BLW	38.190	15.620	18.342	0.8516	2789	3229	70.2	58.5	43.7
36	BLP	38.168	15.614	18.118	0.8619	1929	2638	30.0	61.8	36.0
42	BCW	38.306	15.625	18.397	0.8492	1609	2642	60.8	40.2	35.8
45	BCW	38.363	15.603	18.286	0.8533	2051	3726	132.2	692.6	47.3
52	BLP	38.170	15.622	18.287	0.8543	1555	4290	104.7	54.9	46.9
60	BCW	NA	NA	NA	NA	2163	3539	174.1	59.7	41.9
63	BLP	38.710	15.664	18.704	0.8374	1658	4262	99.7	165.3	56.4
64	BLP	38.170	15.609	18.199	0.8577	1509	3773	118.2	336.7	52.1
65	BCW	38.560	15.644	18.597	0.8413	1727	3057	235.3	42.2	43.3
67	BLW	38.220	15.623	18.313	0.8531	1807	3007	69.8	69.9	39.6
68	BXW	38.942	15.657	18.908	0.8281	2032	4073	132.0	110.7	56.6
76	BCW	38.195	15.600	18.167	0.8587	1832	3659	135.7	89.9	45.7
Exchangeable leachates of granite										
2	BCW	NA	NA	NA	NA					
4	BXW	39.764	15.767	19.401	0.8124					
7	XXW	39.193	15.587	19.048	0.8184					
9	BLW	39.310	15.628	19.153	0.8158					
13	BLW	39.860	15.612	19.613	0.7961					
19	BXW	39.023	15.629	18.915	0.8256					
26	BLW	NA	NA	NA	NA					
30	XXW	39.232	15.649	19.151	0.8173					
39	BXW	39.763	15.666	19.464	0.8049					
42	BCW	40.122	15.677	19.773	0.7926					
44	XXW	NA	NA	NA	NA					
45	BCW	40.156	15.708	19.775	0.7943					
56	BXW	41.042	15.776	20.414	0.7730					
60	BCW	38.661	15.631	18.641	0.8386					
65	BCW	40.184	15.690	19.812	0.7919					
67	BLW	40.131	15.687	19.748	0.7942					
68	BXW	39.209	15.694	19.274	0.8139					
69	XXW	39.195	15.568	19.119	0.8142					
76	BCW	40.105	15.687	19.735	0.7950					

Appendix 3.3, continued

	Trace element concentrations															
pot	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Pb
Birch roots (ppb)																
2	20977	1520	1685	244	872	177.2	31.3	201.3	30.7	180.3	37.4	100.9	14.4	87.2	14.0	5508
4	13759	715	693	120	439	91.2	19.3	109.8	15.7	96.0	20.8	59.7	8.2	54.6	9.0	8604
13	21428	729	852	130	481	100.5	21.0	112.2	16.4	100.5	21.4	60.8	8.6	53.8	8.5	3412
19	18735	822	1051	136	502	101.1	20.0	113.6	17.7	113.8	25.0	73.9	10.2	65.6	10.4	2885
26	19649	1358	1370	214	772	151.4	23.1	157.6	24.1	144.9	33.5	97.2	14.4	88.1	14.5	3787
36	11705	330	345	46	170	33.1	13.4	38.3	5.2	29.8	6.7	16.8	3.0	15.4	3.2	5228
42	14182	2618	3636	459	1614	328.2	27.7	344.5	50.5	295.4	57.5	154.1	19.2	115.8	17.5	1989
45	23304	10098	12930	1679	5905	1238.6	81.4	1288.1	190.6	1099.3	214.5	582.0	72.3	414.5	59.1	23191
52	15241	336	495	61	221	43.1	16.7	43.8	6.5	39.6	8.5	24.3	3.7	23.0	4.0	1750
60	19243	1929	2371	329	1116	218.2	39.6	228.9	33.2	199.8	42.0	120.9	16.9	101.5	15.7	5825
63	23177	626	547	120	458	108.8	30.5	120.3	17.6	99.0	20.6	49.4	6.4	38.3	6.6	2816
64	17976	374	513	70	256	50.4	16.4	51.4	7.5	44.4	9.5	25.9	4.1	24.7	4.4	14101
65	14104	3023	4189	495	1698	359.6	20.6	364.7	54.3	312.2	60.5	159.2	19.4	116.0	16.8	1757
67	60685*	5278	5580	845	3117	621.2	99.2	716.5	104.5	633.3	131.9	379.3	49.0	307.0	47.4	19826
68	35794	11387	15672	1858	6427	1229.5	65.6	1215.5	186.8	1084.7	216.7	574.8	72.6	412.8	55.0	2822
76	19657	2665	3536	431	1479	290.1	32.6	314.0	46.6	274.6	57.1	160.6	21.2	131.2	21.5	6359
Exchangeable leachates (ng g^{-1} granite)																
2	882	25.97	38.17	4.418	14.323	2.587	0.145	2.203	0.321	1.864	0.383	1.047	0.141	0.769	0.109	3.64
4	452	6.61	9.63	1.086	3.577	0.615	0.060	0.539	0.080	0.477	0.094	0.238	0.034	0.179	0.028	1.03
7	488	7.92	11.03	1.325	4.421	0.781	0.065	0.707	0.106	0.595	0.118	0.335	0.043	0.237	0.036	1.15
9	407	5.56	7.63	0.887	3.008	0.534	0.048	0.475	0.069	0.384	0.079	0.214	0.029	0.146	0.021	0.52
13	464	10.83	15.40	1.719	5.701	0.927	0.065	0.786	0.110	0.647	0.133	0.374	0.051	0.288	0.040	1.54
19	378	5.65	7.75	0.937	3.144	0.559	0.049	0.488	0.072	0.425	0.088	0.238	0.032	0.167	0.025	0.53
26	418	5.95	8.25	0.901	2.984	0.435	0.053	0.412	0.060	0.335	0.069	0.183	0.025	0.143	0.022	1.01
30	409	8.45	13.48	1.417	4.558	0.801	0.054	0.674	0.099	0.569	0.109	0.297	0.041	0.229	0.034	1.10
39	265	5.08	8.30	0.835	2.815	0.502	0.037	0.465	0.064	0.372	0.078	0.218	0.032	0.168	0.025	0.86
42	341	13.02	18.97	2.171	7.117	1.285	0.062	1.115	0.158	0.920	0.177	0.484	0.062	0.361	0.051	1.62
44	403	8.59	12.44	1.383	4.445	0.757	0.057	0.607	0.088	0.499	0.104	0.271	0.040	0.205	0.030	1.04
45	277	16.09	23.04	2.699	8.965	1.605	0.069	1.472	0.206	1.196	0.237	0.653	0.089	0.469	0.065	3.20
56	491	82.74	135.39	14.164	46.248	7.680	0.235	6.373	0.938	5.489	1.079	3.137	0.423	2.571	0.342	14.68
60	486	6.08	8.89	0.956	2.992	0.515	0.055	0.436	0.062	0.376	0.076	0.199	0.031	0.155	0.023	1.18
65	386	12.63	19.43	2.122	7.130	1.252	0.071	1.042	0.151	0.831	0.174	0.450	0.064	0.353	0.049	1.35
67	418	11.89	17.32	1.894	6.012	1.038	0.069	0.934	0.136	0.760	0.150	0.425	0.054	0.315	0.045	1.71
68	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
69	333	9.40	12.53	1.532	5.040	0.835	0.053	0.723	0.104	0.575	0.115	0.312	0.039	0.225	0.032	1.09
76	331	41.23	65.69	7.132	23.907	4.219	0.140	3.495	0.529	3.108	0.612	1.760	0.237	1.415	0.191	7.92

* may be erroneous; root 67 was not a Ba outlier in the major element analysis

Appendix 3.4 Birch biomass and C and N concentrations and isotope ratios

			foliage					stems					roots				
pot #	block	Trt.	mass (mg)	$\delta^{15}N$	%N	$\delta^{13}C$	%C	mass (mg)	$\delta^{15}N$	%N	$\delta^{13}C$	%C	mass (mg)	$\delta^{15}N$	%N	$\delta^{13}C$	%C
2	1	BCW	30.2	0.96	1.71	-30.31	42.0	22.6	1.78	1.35	-29.62	45.3	46.2	1.43	1.50	-29.67	45.8
4	1	BXW	6.8	0.63	1.95	-30.86	39.7	9.2	0.14	1.56	-29.93	41.2	20.8	1.33	1.86	-30.23	42.8
5	1	BLP	44.6	0.36	1.61	-29.93	41.5	35.6	0.84	1.78	-29.48	43.5	93.2	0.21	1.26	-28.68	46.4
9	1	BLW	18.6	0.10	2.24	-30.86	41.3	32.8	0.27	1.35	-29.81	41.6	81.5	0.66	1.35	-29.79	46.3
13	2	BLW	46.3	0.17	2.22	-30.94	41.9	78.5	0.66	0.67	-29.49	45.1	98.6	1.74	1.14	-29.91	45.2
19	2	BXW	9.4	0.83	2.32	-30.33	40.1	17.5	0.75	1.59	-29.39	43.3	31.2	1.15	1.32	-29.20	42.7
26	3	BLW	6.6	-1.21	2.38	-31.30	38.1	11.6	-0.75	1.92	-29.65	43.9	15.1	-0.48	1.54	-29.52	44.7
36	4	BLP	8.7	-0.63	1.94	-30.43	40.6	17.4	0.08	1.85	-29.60	44.6	21.7	0.29	1.67	-29.49	46.0
39	4	BXW	2.8	-1.76	2.02	-31.90	41.6	6.7	-0.65	1.93	-30.61	45.5	8.5	-0.78	1.54	-30.37	45.7
42	4	BCW	7.3	-1.31	1.85	-29.76	41.8	15.3	0.60	2.39	-28.46	45.7	23.3	1.00	1.74	-28.35	43.7
45	5	BCW	5.5	0.48	2.74	-31.16	39.0	9.8	0.45	1.81	-29.55	42.7	10.0	0.43	2.01	-29.29	42.0
50	5	BLW	14.7	0.21	2.45	-31.60	41.7	20.9	0.37	1.63	-30.50	41.5	27.3	1.34	1.74	-30.52	42.8
52	5	BLP	11.0	-0.73	2.18	-30.08	41.9	16.7	-1.20	1.75	-28.92	44.4	23.1	-0.51	1.55	-29.16	44.3
56	6	BXW	23.6	0.25	1.85	-30.54	41.3	44.5	1.63	1.77	-29.40	45.3	61.1	1.71	1.36	-29.24	44.8
60	6	BCW	8.2	-1.02	2.49	-30.73	42.0	13.9	0.76	1.89	-29.26	41.3	20.6	2.57	2.20	-28.81	37.8
63	6	BLP	1.7	-0.38	2.50	-30.09	41.0	3.4	-0.88	1.97	-29.42	42.8	9.0	0.11	1.89	-28.81	42.9
64	7	BLP	5.9	-0.02	2.63	-29.58	41.8	7.3	0.20	1.81	-27.97	44.3	11.6	1.30	1.59	-27.98	43.1
65	7	BCW	13.9	1.27	1.79	-30.76	41.3	22.7	1.01	1.24	-29.77	44.6	34.6	1.33	1.28	-29.46	45.1
67	7	BLW	10.7	0.89	2.17	-31.04	35.4	26.5	1.49	1.91	-28.67	44.5	30.6	1.36	1.65	-28.53	44.9
68	7	BXW	6.2	2.18	2.93	-30.97	41.6	7.2	1.44	1.88	-30.03	45.2	12.3	1.85	1.60	-30.11	45.6
76	8	BCW	1.0	-0.35	2.74	-31.89	39.1	13.5	0.45	2.04	-30.01	44.5	18.5	0.19	1.49	-30.34	45.4

Treatment codes:

B indicates birch

X indicates non-mycorrhizal, **C** indicates *Cortinarius,* and **L** indicates *Leccinum*

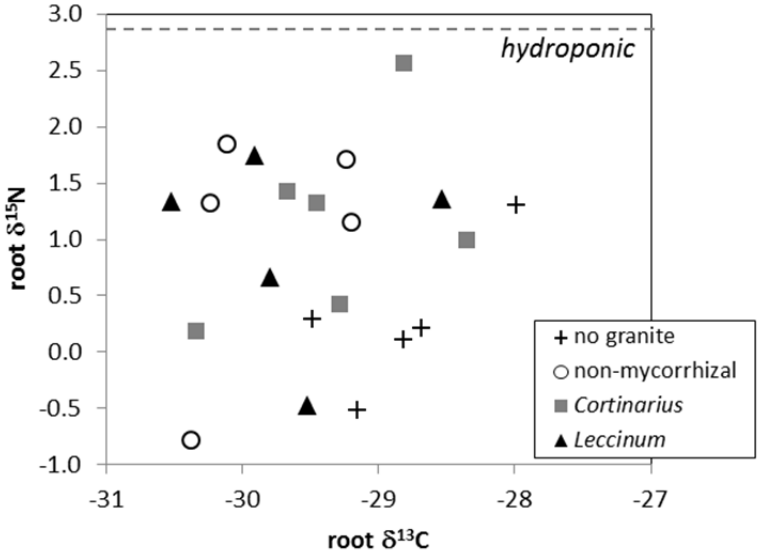
W indicates whole (unleached) granite substrate, **P** indicates no granite (perlite only)

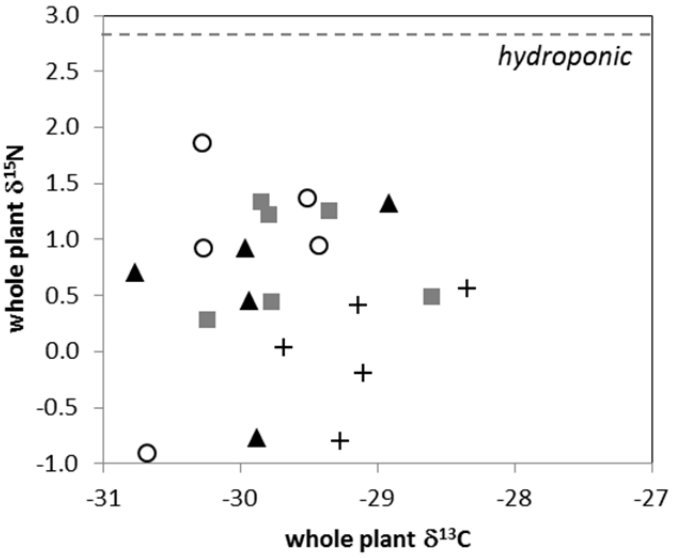
Appendix 3.5 Summary statistics for birch biomass and C and N data.

Mean biomass and stable C and N isotope ratios from the birch cultures for roots and whole plants. Δ^{15}N root and Δ^{13}C root indicate differences in δ^{15}N and δ^{13}C between roots and whole plants.

	n	whole plant biomass (g)	root/whole (mass ratio)	δ^{15}N whole (‰)	Δ^{15}N root (‰)	δ^{13}C whole (‰)	Δ^{13}C root (‰)
Cortinarius	6	0.053 ± 0.027	0.48 ± 0.05	0.84 ± 0.48	0.3 ± 0.5	-29.6 ± 0.6	0.28 ± 0.23
Leccinum	5	0.104 ± 0.076	0.48 ± 0.08	0.53 ± 0.79	0.4 ± 0.3	-29.9 ± 0.7	0.23 ± 0.14
non-mycorrhizal	5	0.054 ± 0.045	0.51 ± 0.04	0.84 ± 1.05	0.2 ± 0.2	-30.0 ± 0.5	0.20 ± 0.11
without granite	5	0.062 ± 0.064	0.51 ± 0.08	0.00 ± 0.54	0.3 ± 0.3	-29.1 ± 0.5	0.29 ± 0.14
ANOVA *p*		0.42	0.82	0.25	0.89	0.09	0.83

Appendix 3.6 Stable C and N isotope ratios in birch roots and whole seedlings.



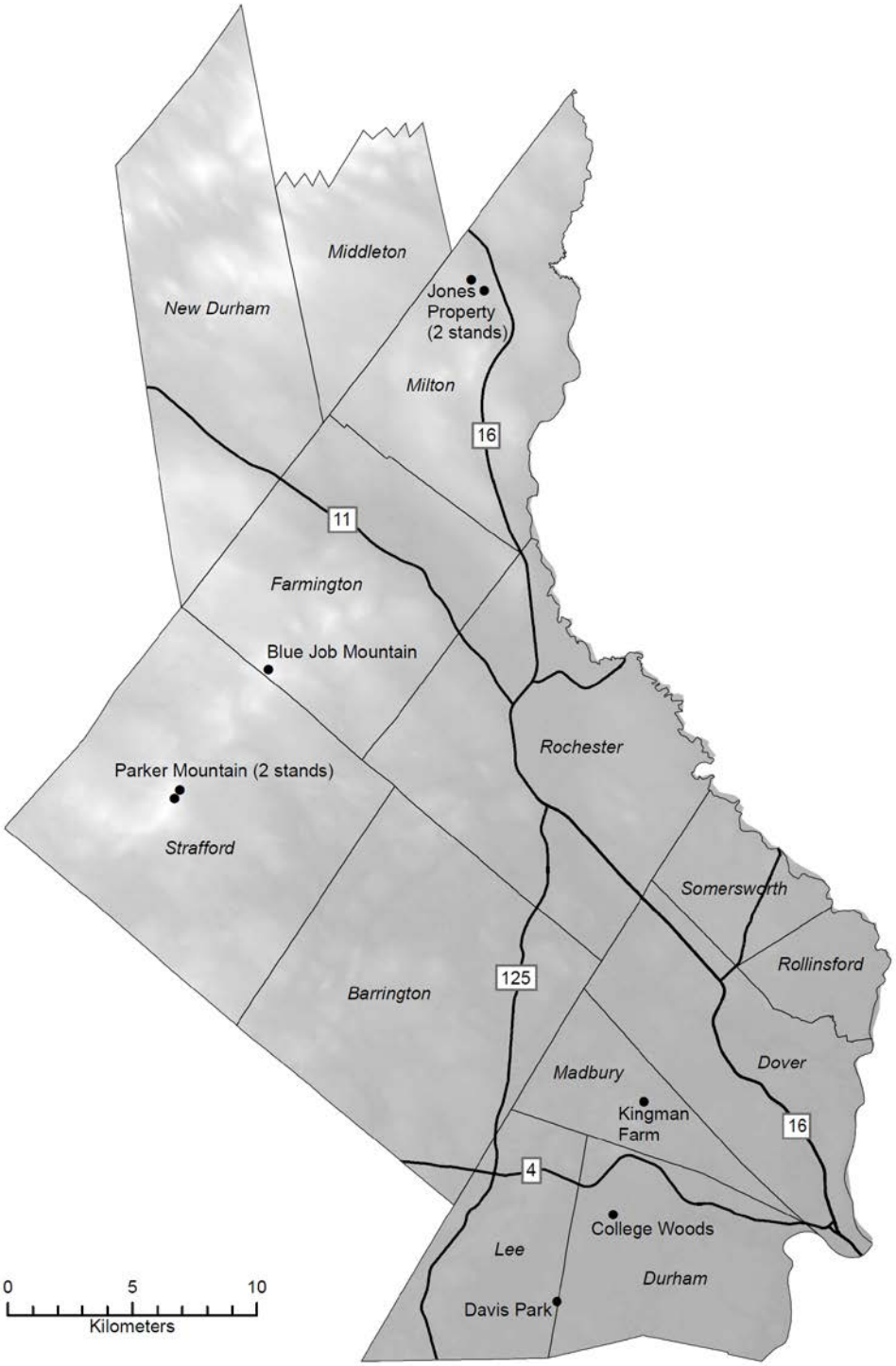


Appendix 3.7 Isotope ratios of C, N, and Pb, and trace element concentrations in sporocarps analyzed

					Pb isotope ratios				Trace elements (ppb)					
Sample	Site	Taxon	$\delta^{13}C$	$\delta^{15}N$	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{206}Pb$	Ba	La	Ce	Pr	Nd	Pb
711	C9	Cort	-25.94	10.35	38.412	15.642	18.739	0.8348	239	69.0	27.2	5.8	23.4	544
713	C8	Lecc	-25.20	7.05	38.396	15.631	18.664	0.8375	344	16.0	26.8	2.6	9.4	460
722	C6	Lecc	-25.61	10.40	38.406	15.641	18.690	0.8369	1126	4.8	8.5	0.8	2.7	372
725	C6	Cort	NA	NA	38.348	15.633	18.685	0.8366	98	36.5	2.8	2.6	7.4	222
732	C9	Lact	-23.28	7.35	38.463	15.646	18.761	0.8339	177	84.6	179.5	20.8	79.7	2369
733	HBM	Lact	-24.54	12.91	38.351	15.639	18.677	0.8373	354	3.4	4.1	0.4	1.5	262
743	C6	Lact	-26.24	10.60	38.506	15.646	18.735	0.8350	894	66.5	53.5	10.4	34.4	336
750	HBO	Lecc	-26.10	4.68	38.382	15.650	18.720	0.8360	732	4.0	7.2	1.4	4.7	420
751	C6	Cort	-26.50	13.44	38.345	15.648	18.643	0.8394	245	0.8	2.4	0.3	1.2	1393
781	C8	Lecc	-25.94	4.38	38.496	15.645	18.762	0.8339	341	2.6	3.4	0.4	1.3	29
782	C6	Lecc	-25.19	2.08	38.357	15.638	18.710	0.8358	325	1.8	3.2	0.5	1.5	43
793	C8	Lact	-25.78	3.42	38.386	15.658	18.658	0.8394	398	12.8	7.6	1.4	4.1	1428
794	HBO	Lact	-26.33	4.32	38.389	15.640	18.669	0.8378	1139	17.2	15.7	2.3	7.3	863
798	HBM	Lecc	-25.77	7.98	38.421	15.640	18.724	0.8353	267	15.4	32.2	3.3	12.3	1123
820	HBO	Lecc	-27.00	2.75	38.332	15.637	18.707	0.8359	323	4.8	7.7	0.5	1.8	735
829	C9	Lecc	-24.57	15.66	38.455	15.646	18.775	0.8336	678	37.7	120.8	8.0	27.8	399
862	HBM	Lecc	-26.12	5.38	38.448	15.646	18.742	0.8348	664	3.0	1.5	0.3	1.0	252
878	C6	Cort	-26.75	8.65	38.344	15.631	18.679	0.8368	494	47.9	93.8	10.6	36.9	2018
882	HBO	Lecc	-26.02	3.58	38.338	15.638	18.671	0.8376	2058	3.1	4.2	0.5	1.3	157
887	C9	Lecc	-26.36	8.87	38.514	15.643	18.746	0.8345	1273	1.6	6.0	0.8	3.2	150
896	C9	Lact	-24.80	2.46	38.422	15.643	18.708	0.8362	3612	6.0	7.7	0.8	2.3	232
920	C4	Lecc	-26.50	10.15	38.802	15.654	18.921	0.8273	58	0.6	1.9	0.1	0.5	893
927	HBM	Lecc	-25.04	8.87	38.357	15.637	18.644	0.8387	365	105.1	43.9	12.9	39.4	247
937	C6	Cort	-25.66	16.11	38.433	15.643	18.721	0.8356	250	6.1	10.2	1.2	3.9	164
939	C8	Lact	-25.87	9.71	38.333	15.641	18.521	0.8444	231	0.9	1.9	0.3	0.8	2753
942	C4	Cort	-25.67	7.89	38.382	15.635	18.667	0.8376	660	26.6	45.0	5.5	20.7	889
943	HBO	Cort	-26.34	10.17	38.328	15.638	18.713	0.8357	854	35.6	21.3	3.9	13.2	227
950	HBM	Cort	-25.93	9.36	38.290	15.632	18.636	0.8389	179	0.0	0.7	0.1	0.5	1243
955	C9	Lecc	-26.37	5.85	38.469	15.647	18.762	0.8340	205	7.3	9.4	1.1	3.8	509
956	C4	Cort	-25.90	4.39	38.359	15.632	18.693	0.8362	165	3.1	4.3	0.5	1.8	318
958	C4	Lact	-25.96	8.79	38.365	15.641	18.667	0.8379	320	0.9	3.2	0.4	1.6	264
962	C4	Lecc	-26.53	7.82	38.366	15.642	18.677	0.8375	250	2.2	4.3	0.6	2.1	746

Appendix 4.1 Map of study sites used in the organic N uptake study.

Strafford County, New Hampshire



Appendix 4.2 Floristic characterization of study sites.

a) Basal area (m^2 ha^{-1}) of trees and shrubs > 2 cm in diameter at breast height within 5 m of core locations at each study site. Nomenclature follows the USDA PLANTS database. Target species at each site are shown in bold.

	DP	CW	KF	PS	PO	BJ	JP	JM
Arbuscular mycorrhizal species								
Acer pensylvanicum L.							1	
Acer rubrum L.	4	1	1	3	2	< 1	< 1	
Acer saccharum Marsh.	**9**						< 1	**16**
Fraxinus americana L.	**21**							**18**
Hamamelis virginiana L.				< 1	< 1			
Ectomycorrhizal species								
Abies balsamea (L.) Mill.							< 1	
Betula alleghaniensis Britton			< 1	< 1				< 1
Betula lenta L.		1		< 1	< 1		1	
Betula papyrifera Marsh.				1				
Betula populifolia Marsh.				< 1				
Carya ovata (Mill.) K. Koch	< 1							
Fagus grandifolia Ehrh.		< 1	**7**		**10**		< 1	4
Ostrya virginiana (Mill.) K. Koch					< 1			
Picea rubens Sarg.				**24**		**37**		
Pinus strobus L.		**112**			< 1		**51**	
Quercus rubra L.			**35**	4	**28**			
Tsuga canadensis (L.) Carrière		13		2		< 1	2	1
Total	**33**	**128**	**43**	**35**	**41**	**38**	**56**	**39**

b) Estimated percentage cover by species in the ground layer at each study site.

Species	DP	CW	KF	PS	PO	BJ	JP	JM
Abies balsamea (L.) Mill.						5	5	
Acer pensylvanicum L.		1		1	1		1	1
Acer rubrum L.			1	1		1		
Acer saccharum Marsh.	2						1	3
Aralia nudicaulis L.	3				2		11	
Arisaema triphyllum (L.) Schott								1
Betula alleghaniensis Britton			1		1		1	
Carya ovata (Mill.) K. Koch	2							
Carex spp.	2				12			1
Cornus canadensis L.						5		
Dennstaedtia punctilobula (Michx.) T. Moore						10	3	4
Epigaea repens L.					10			
Epifagus virginiana (L.) W. Bartram								1
Fagus grandifolia Ehrh.			15		1			1

Appendix 2b, continued

Species	DP	CW	KF	PS	PO	BJ	JP	JM
Fraxinus americana L.							1	1
Gaultheria procumbens L.					3			
Goodyera pubescens (Willd.) R. Br.					5			
Hamamelis virginiana L.				3	3			
Lycopodium annotinum L.								1
Lycopodium dendroideum Michx.			5			5		
Maianthemum canadense Desf.	34	3	10		16	5	23	
Maianthemum racemosum (L.) Link	14				1			
Melampyrum lineare Desr.					1			
Medeola virginiana L.								1
Mitchella repens L.		1	1					
Monotropa uniflora L.			1		1			
Oclemena acuminata (Michx.) Greene							10	
Onoclea sensibilis L.								1
Ostrya virginiana (Mill.) K. Koch					1			
Picea rubens Sarg.				1	1	6		
Pinus strobus L.	1	1		1	1	1	1	1
Platanthera orbiculata (Pursh) Lindl.								1
Polypodium virginianum L.					3			
Prunus virginiana L.	1							
Pteridium aquilinum (L.) Kuhn							4	
Quercus rubra L.	1	1	1	1	1	1	1	1
Rubus spp.							1	
Streptopus amplexifolius (L.) DC.			1					10
Thelypteris noveboracensis (L.) Nieuwl.								5
Toxicodendron radicans (L.) Kuntze	2							
Trientalis borealis Raf.	1	1	1		1	5	5	
Trillium erectum Willd.								1
Tsuga canadensis (L.) Carrière		1	1	1		10	1	
Uvularia sessilifolia L.	6				1			5
Vaccinium angustifolium Aiton				3	8	22	5	
Viburnum acerifolium L.					1			
Viburnum nudum L.					1			
Viola rotundifolia Michx.								5
All mosses				20		20	23	1
All lichens				5				
Total species present	12	7	11	10	23	13	17	20

Appendix 4.3 Equations used to calculate the uptake of labeled organic N.

All isotope data were converted to atom fraction following Coplen (2011). Excess atom fraction of ^{15}N and ^{13}C in ON treatment root amino acid extracts were calculated as:

$$x^E\left(^{15}\mathrm{N}\right)_{ON} = x_{AA}\left(^{15}\mathrm{N}\right) - x_{control\ soil}\left(^{15}\mathrm{N}\right) \quad [1]$$

$$x^E\left(^{13}\mathrm{C}\right)_{ON} = x_{AA}\left(^{13}\mathrm{C}\right) - x_{control\ roots}\left(^{13}\mathrm{C}\right) \quad [2]$$

Excess atom fraction of ^{15}N was calculated in the same way from bulk root analyses, based on the assumption that non-protein N was negligible in bulk roots:

$$x^E\left(^{15}\mathrm{N}\right)_{ON} = x_{root}\left(^{15}\mathrm{N}\right) - x_{control\ soil}\left(^{15}\mathrm{N}\right) \quad [3]$$

Excess atom fraction of ^{13}C in root protein was calculated from bulk root analyses with a mixing equation:

$$x^E\left(^{13}\mathrm{C}\right)_{ON} = \frac{x_{bulk}\left(^{13}\mathrm{C}\right) - x_{baseline}\left(^{13}\mathrm{C}\right)\times\left(1-f_{AA}\right)}{f_{AA}} - x_{baseline}\left(^{13}\mathrm{C}\right) \quad [4]$$

where f_{AA}, the fraction of root C in amino acids, is estimated as

$$f_{AA} = \frac{2.86 \times [N]_{root}}{[C]_{root}} \quad [5]$$

assuming a C:N mass ratio of 2.86 in protein (Hobbie et al., 2012b)

$$x_{baseline}\left(^{13}\mathrm{C}\right) = x_{control\ root}\left(^{13}\mathrm{C}\right) + 0.0000234 \quad [6]$$

based on the ^{13}C offset between proteins and bulk analyses of archived fine roots. Finally, intact organic N uptake as a fraction of total N uptake was calculated as:

$$f_{intact} = \frac{x^E\left(^{13}\mathrm{C}\right)_{ON}}{0.5 \times 3.34 \times x^E\left(^{15}\mathrm{N}\right)_{ON}} \quad [7]$$

assuming a C:N mole ratio of 3.34 in protein (Hobbie et al., 2012b), and that half of protein-C is respired (Hobbie and Hobbie, 2012). One ON root sample (from site JP) was more depleted in ^{13}C than the control roots in two replicate analyses. This sample was assigned an organic uptake value of 0.

Appendix 4.4 Attempted assessment of total organic N uptake

In addition to the ON labeled and control cores, cores amended with isotopically labeled inorganic N were deployed in parallel. Also, cation exchange resin strips were added to each core, with the hope that characterizing N isotope ratios of NH_4^+ in ON cores and paired "IN" cores with known additions of $^{15}NH_4$ would allow us to infer fluxes of unlabeled inorganic and organic N to roots. Estimates of these values would be more generally useful than simply reporting the fate of tracer N and C, because the fates of added substrates do not necessarily reflect those of native soil organic matter. However, this approach does not appear to have provided much usable information, and is reported here only in the interest of completeness.

Field and laboratory methods

Each block of cores included one with each of the following treatments: control (no addition), "ON", labeled organic nitrogen addition, and "IN", labeled inorganic nitrogen addition. To each IN core, 15 ml of 1.0 mM $^{15}NH_4Cl$ (certified 99% ^{15}N) was injected, for a total addition of approximately 225 μg ^{15}N per core.

A strip of cation exchange resin was inserted vertically into each core at the time of label addition. Strips GE Cation Exchange Membrane CR67-HMR were cut to 1.5 cm × 5 cm and rinsed twice in 6 M HCl and stored in 0.01 M HCl until deployment. Cation exchange capacity was about 1 meq per strip. After core harvest, resin strips were gently cleaned of soil particles under a distilled water rinse and frozen until extraction. Exchangeable cations were extracted from each strip in 25 ml of 2 M KCl for 24 hours with gentle agitation. The solution was then adjusted to pH ~ 13 with 1 ml of 10 M NaOH and sealed in glass jars for 21 days; NH_3 gas diffusing out of solution was

collected on glass filters acidified with 150 µl 1M $KHSO_4$ (method adapted from Kelley et al., 1991; Sebilo et al., 2004). Filters were dried in a sealed desiccator containing calcium sulfate and a vial of 1 M H_2SO_4. Multiple blanks and natural abundance NH_4Cl reference samples were run with each batch of samples.

Calculations

Ratios of $^{15}N_{excess}$ to $^{13}C_{excess}$ in ON core roots allowed us to estimate the relative importance of organic and inorganic uptake of N in the added organic substrate (Fig. 4.3, Appendix 4.3). We intended to use the resin data as a time-integrated proxy for the N isotope ratio of DIN in each core. This, along with the N isotope ratios in roots, would allow us to estimate the unlabeled inorganic N uptake by roots, and by difference, the unlabeled organic uptake. Resin and root N isotope ratios in the labeled IN amended were intended to allow us to verify these relationships. Labeled N uptake as a fraction of total N uptake could be calculated as:

$$f_L = \frac{x^E(^{15}\mathrm{N})_{ON}}{0.98 - x_{control\,soil}(^{15}\mathrm{N})} \quad [8]$$

and partitioned into inorganic labeled and organic labeled components:

$$f_{OL} = f_L \times f_{intact} \quad [9]$$

$$f_{IL} = f_L - f_{OL} \quad [10]$$

Unlabeled inorganic uptake as a fraction of total uptake would then be:

$$f_{IU} = f_{IL} \times \frac{0.98 - x_{ON\,resin}(^{15}\mathrm{N})}{x^E(^{15}\mathrm{N})_{ON\,resin}} \quad [11]$$

Finally unlabeled organic uptake would be:

$$f_{OU} = 1 - f_{IL} - f_{OL} - f_{IU} \quad [12]$$

Note that the fraction of total N uptake occurring as unlabeled organic N is calculated by difference, and therefore subject to the accumulated error of all other measured or estimated values. If roots and the exchange resin do not "see" the same N isotope ratios in DIN, due to heterogeneous distribution of the labeled substrate or DIN preferentially moving towards or away from the center of the core (where the resin strip was located), this calculation is subject to fairly large errors.

Additional calculations that are theoretically possible with these data include gross mineralization and the mineralization of the labeled organic substrate. Using an isotope dilution approach (modified from Davidson et al., 1991; Hart et al., 1994), gross mineralization rate (m_{gross}) over incubation time *t* could be estimated as:

$$m_{gross} = \frac{225\ \mu g\ N \times 99\%}{x^E(^{15}N)_{IN\ resin} \times t} \quad [13]$$

where resin ^{15}N excess was calculated relative to bulk soil ^{15}N, (as in eqs. 1, 3).
The fraction of the labeled cyanobacteria substrate mineralized over the course of the incubation could be estimated based on the ratios of ^{15}N excess in the IN and ON core resins as follows:

$$f_{min} = \frac{\frac{x^E(^{15}N)_{ON\ resin}}{2500\ \mu g\ N}}{\frac{x^E(^{15}N)_{IN\ resin}}{225\ \mu g\ N}} \quad [14]$$

This calculation assumes that "background" N mineralization is equal in paired cores.

Results and Discussion

Unfortunately, resin N isotope ratios were highly variable among cores. Site means ranged from 0.9% to 7.2% (atom fraction) in the ON cores and 0.41% to 1.23% in the IN cores. Coefficients of variation were as high as 101%. This variation likely reflects both real differences among paired cores in gross mineralization rates, as well as

the possibly larger effect of inhomogenous distribution of the IN and ON tracers within the core, and consequent differential distribution of the ^{15}N tracer among parts of the core occupied by roots and resin.

This is problematic because small errors resulting from these inhomogenities are magnified in importance when calculating f_{OU} from the available data. Averaging within sites only marginally improved the values we calculated for unlabeled ON uptake; calculations of unlabeled ON to total core N ranged from -28% to 92% by site, and ranged widely within sites, with little meaningful information about means across sites or differences among sites. This variation also complicated attempts to characterize gross N mineralization in the IN cores using a modification of the isotope dilution technique, and to estimate the fraction of the organic N label mineralized in each core.

Further methods development is needed in order to estimate the isotopic parameters required to convert root tracer uptake data to estimates of the ratio of total organic to total inorganic root N uptake.