EFFECTS OF CALCIUM, NITROGEN, AND PHOSPHORUS FERTILIZATION ON FOLIAR NUTRIENT DYNAMICS OF THREE NORTHERN HARDWOOD TREE SPECIES

by

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ABSTRACT

K.E. Gonzales. Effects of Calcium, Nitrogen, and Phosphorus Fertilization on Foliar Nutrient Dynamics of Three Northern Hardwood Tree Species, 116 pages, 11 tables, 14 figures, 2017.

Uncertainty remains about the relationship between plant or soil nutrient status and nutrient resorption, and this relationship may be influenced by human impacts on nutrient cycling and shifts in limitation in natural ecosystems. This research examines how experimentally increasing nutrient availability affects foliar chemistry and physical characteristics. In each of five treatments (+N, +P, +NP, +Ca, control) across eight forest stands in the White Mountains of New Hampshire, nutrient concentrations of green and senesced leaves of three dominant tree species were analyzed and resorption efficiency was calculated. Calcium silicate addition affected leaf physical characteristics, but resulted in few effects on foliar chemistry. Adding N increased green and senesced leaf concentrations and resorption efficiency of N, but had little effect on P dynamics. Adding P affected foliar P, but not N, dynamics. These results raise new questions about the influence on resorption of age class, successional stage, and degree of limitation.

Key Words: resorption efficiency, resorption proficiency, *Fagus grandifolia*, *Acer rubrum*, *Acer saccharum*.

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Chapter 1: Literature Review

Resorption

With available resources finite and competition fierce, plants have evolved mechanisms for maximizing the acquisition, conservation, and use efficiency of necessary capital. Resorption is one of the most important conservation and nutrient use efficiency mechanisms available to plants (Chapin 1980; del Arco et al. 1991; Killingbeck 1996; Eckstein et al. 1999; Oleksyn et al. 2003; Richardson et al. 2005), and preventing resorption has adverse effects on plant fitness (May and Killingbeck 1992). The construction and maintenance of leaves is a high resource investment; for deciduous trees, abscission of an intact leaf every autumn would be quite wasteful. During the process of leaf senescence, foliar components are disassembled and some nutrients are retranslocated to other plant tissues (Thomas and Stoddart 1980). This flux increases the plant's internal pool of available nutrients and allows plants to be less dependent on soil nutrient availability (Ryan and Bormann 1982; Chapin et al. 1990; Aerts 1995; Killingbeck 1996; Aerts and Chapin 2000; Lu et al. 2013). Resorption also directly affects ecosystem nutrient cycling rates, since nutrients remaining in the senescing leaf regulate the chemistry of the litter available for decomposition (Crews et al. 1995; Aerts 1997; Aerts and Chapin 2000; Kitayama et al. 2004; Kozovits et al. 2007; Wardle et al. 2009).

Resorption is often expressed in terms of efficiency (Killingbeck and Costigan 1988; Aerts 1996) and proficiency (Killingbeck 1996). Resorption efficiency is the proportion of nutrients in the green leaves that is resorbed. Estimates of global resorption means range from 50-62% for nitrogen (N) and 52-65% for phosphorus (P) (Aerts 1996; Vergutz et al. 2012). Resorption proficiency is the concentration of nutrient that remains in senesced leaves, and seems more reflective of both environmental and evolutionary constraints on nutrient conservation, since it appears more responsive to changes in nutrient supply and more indicative of the degree to which selection has acted to conserve nutrients (Chapin and Moilanen 1991; Killingbeck 1996; Aerts et al. 2003; Killingbeck 2004; Rejmánková 2005)

Resorption varies both inter- and intraspecifically (Chapin and Kedrowski 1983; Gray 1983; Killingbeck 1984; Kost and Boerner 1985; Birk and Vitousek 1986; Boerner 1986; Killingbeck and Costigan 1988). It is unclear how much influence is exerted by other factors, such as temperature, precipitation, water availability, and soil and leaf nutrient status (Del Arco et al. 1991; Nordell and Karlsson 1995; Côté et al. 2002). Studying resorption allows us to see the strategies employed by plants to cope with nutrient limitation, as well as the evolutionary and physiological constraints on maximizing nutrient use efficiency.

Ways of Quantifying Nutrients

Foliar nutrients are reported using metrics such as content per leaf mass, per leaf area, per leaf, and per leaf calcium content, with proponents for and critics of each. Nutrient content on the basis of leaf area and mass are probably the most widely used approaches, and leaf area is considered by many to produce the best representation of foliar nutrient relationships (Woodwell 1974; Chapin and Van Cleve 1996), but both are based on the assumption that these two quantities do not significantly change during senescence. The leaves sampled for nutrient analysis for resorption often belong to different cohorts, which can introduce considerable variation in mass and area. In addition, significant loss of both mass (Chapin and Kedrowski 1983; Vergutz et al. 2012) and area (van Heerwaarden et al. 2003a) due to the degradation of leaf compounds and export of solutes through leaching and resorption (Vernescu et al. 2005) can occur prior to leaf abscission, which can underestimate resorption estimates. To reconcile this issue, some have introduced correction factors for mass (Vergutz et al. 2012) or area (Rentería et al. 2005) for inclusion in calculations of resorption efficiency, while others recommend the basis of content per leaf (Lin and Sternberg 2007) or have used calcium-based estimates (Soudzilovskaia et al. 2007), based on the assumption that calcium isn't resorbed (Lambers et al. 1998). Nutrient concentration and content can also be used jointly to detect a dilution effect if leaf mass and/or area differ among treatments. However, patterns and trends tend to remain consistent within studies when compared across the various ways of quantifying nutrients, though actual values differ (van Heerwaarden et al. 2003b; Rentería et al. 2005; Soudzilovskaia et al. 2007).

Nitrogen and Phosphorus

As the macronutrients generally considered most limiting to plant growth, N and P, and their acquisition and use, have been the focus of many studies (Chapin 1980; Vitousek and Howarth 1991; Elser et al. 2007; Lebauer and Treseder 2008; Vitousek et al. 2010; Harpole et al. 2011). Both N and P are primary components of nucleic acids and ATP. Nitrogen is integral to amino acids and synthesized proteins and enzymes, such as the photosynthesis-catalyzing enzyme RuBisCo. Phosphorus is a crucial component of the rRNA required to synthesize proteins for metabolism, and can also be found in phospholipids and cellular metabolites (Elser et al. 1996; Sinclair and Vadez 2002). In leaves, both nutrients are related to foliar physical and physiological characteristics, such as specific leaf area, leaf lifespan, photosynthesis, and dark respiration, and limitation by either nutrient can restrict carbon acquisition and growth (Field and Mooney 1986; Reich et al. 1997; Wright et al. 2004; Domingues et al. 2010).

Phosphorus is added to ecosystems via mineral weathering (Vitousek et al. 2010) and deposition of dust (Chadwick et al. 1999). As soils age, P inputs decline (Walker and Syers 1976); N inputs, which come primarily from the atmosphere, tend to remain unchanged, but can be affected by anthropogenic N inputs (Holloway and Dahlgren 2002). Unlike N, P has no gaseous phase, and is much less mobile in the soil, resulting in a relatively patchy distribution spatially and temporally (Brady 1990; Aerts and Chapin 2000). Once taken up the plant, inorganic N is stored in proteins or amino acids (Chapin et al. 1990), but is primarily transported as organic N (Bloom et al. 1985). Phosphorus is stored and transported within the plant in its organic form (Bloom et al. 1985). Up to two-thirds of plant P may be stored as inorganic P in vacuoles (Sinclair and Vadez 2002).

(Co)Limitation

With both N and P playing a vital role in so many molecules important to plants, it is no surprise that their cycles are coupled across all organizational scales, from the growth and metabolism of individual plants to ecosystem-level cycling (Elser et al. 1996; Ågren 2004; McGroddy et al. 2004). Natural limitation by N and/or P may be driven by soil type and geologic history, with temperate ecosystems frequently regarded as N-limited, and the tropics P-limited (Vitousek 1984; Hedin et al. 2003; Reich and Oleksyn 2004; Lambers et al. 2008), though exceptions exist due to, for example, landscape heterogeneity (Townsend et al. 2007). It is likely, especially in species-rich ecosystems, that intra- and interspecific variation maintains a state of co-limitation by multiple resources (Arrigo 2005; Niinemets and Kull 2005; Danger et al. 2008; Harpole et al. 2011), and that limitation varies spatially and temporally across the landscape (Eviner et al. 2000). At the individual level, economic theory suggests that plants will dynamically allocate resources so as to remain simultaneously co-limited by multiple resources (Bloom et al. 1985); evidence of co-limitation in natural ecosystems has been observed (Scheu 1990; Verhoeven and Schmitz 1991; Prescott et al. 1992; Niinemets and Kull 2005; Elser et al. 2007; Harpole et al. 2011), but can sometimes be difficult to determine conclusively if indices are used to infer co-limitation.

Human activities, such as fossil fuel combustion, agricultural practices, and mining, have the potential to alter natural nutrient cycles, and, therefore, limitation and ecological stoichiometry (Vitousek et al. 1997; Galloway 2004; Vitousek et al. 2010; Peñuelas et al. 2012). Anthropogenic inputs of N, in particular, are estimated to be at least 30% greater than natural inputs (Galloway et al. 2008; Vitousek et al. 2013). Atmospherically deposited N from anthropogenic sources can affect primary production, species composition, and herbivore abundance (Ritchie 2000; Stevens et al. 2004; Bobbink et al. 2010; Li et al. 2016). There is also a concern that anthropogenic global change may influence nutrient cycling and resorption dynamics (van Heerwaarden et al. 2003b; Lu and Han 2010), especially if soil nutrient availability is affected by, for example, enhanced N mineralization rates (Rustad et al. 2001). Effects of such inputs on the cycling of other nutrients, especially over the long term, are not well understood. For example, the effects of N inputs on internal plant P cycling are inconsistent; N additions have been shown to affect plant P, but it is not clear whether this is due to changes in decomposition rates (Treseder and Vitousek 2001), phosphatase activity (Olander and Vitousek 2000; Marklein and Houlton 2012), or mycorrhizal activity (Treseder 2004). If N additions are expected to enhance primary production, and therefore, uptake of both N and P, it has been hypothesized that enhanced N availability would increase P limitation (Vitousek et al. 2010), and, therefore, plant P conservation (Perring et al. 2008; Vitousek et al. 2010), but results remain conflicting.

Foliar Nutrients and N:P Ratios

Nutrient manipulation experiments are one way to identify nutrient limitation and effects of elevated nutrient inputs. While increased biomass is a direct way to detect limitation, dry mass ratios (Vitousek 1984), foliar N:P ratios (Koerselman and Meuleman 1996; Güsewell 2004; Drenovsky and Richards 2004; Reich and Oleksyn 2004; Richardson et al. 2004), and N:P resorption ratios (Reed et al. 2012) have all been proposed as indirect, yet easily quantifiable, indicators of limitation. Foliar nutrient concentrations generally reflect soil nutrient availability (Shaver and Melillo 1984; Vitousek and Farrington 1997; Vitousek 1998; Aerts and Chapin 2000; Hobbie and Gough 2002; Han et al. 2005; Townsend et al. 2007), and resorption of N and P tend to be correlated (Güsewell 2005; Lu et al. 2013). Foliar N:P ratios increase with decreasing latitude (Hedin 2004; Reich and Oleskyn 2004), while N:P resorption ratios are higher at high latitudes (Reed et al. 2012), reflecting the putative naturally occurring shift from N to P limitation (Hedin 2004). Additions of N and P have been shown to increase plant and soil concentrations of the respective nutrient (Olander and Vitousek 2000; Galloway et al. 2008; Lu and Han 2010; Jones and Power 2012). Increased N:P and shifts in limitation from N to P resulting from anthropogenic additions of N have been observed (Aerts et al. 1992; Menge and Field 2007; Elser et al. 2009; Peñuelas et al. 2012), in fertilization studies (Vitousek and Farrington 1997; Lu et al. 2013), and in soil chronosequences (Richardson et al. 2004; Laliberté et al. 2012; Hayes et al. 2014). However, variation in response to limitation may also be strongly driven by interspecific differences among plants, as evidenced by varying responses to fertilization by different species within the same site (Davidson et al. 2004; Townsend et al. 2007). Nitrogen and P concentrations may also vary with climate (Reich and Oleskyn 2004; but see Townsend et al. 2007; Domingues et al. 2010), life history, and history of disturbance or stress (Wright et al. 2001; Bowman et al. 2003; Reich et al. 2003; Wright et al. 2004; Townsend et al. 2007). Ratios of N:P may even change seasonally due to temporal shifts in green leaf N or P concentrations (Yuan et al. 2005; Townsend et al. 2007). Ratios of N:P may not be globally applicable, and the use of N:P ratios as an indicator of co-limitation may need to be taken on a species- or ecosystem-specific basis (Drenovsky and Richards 2004; Craine et al. 2008). Despite these flaws, N:P ratios remain one of the most widely used and readily available indicators of limitation.

Resorption and Nutrient Status

Resorption, whether proficiency or efficiency, is likely a function of soil fertility, but appears to also be influenced by a variety of other factors, including the length of senescent period (del Arco et al. 1991; Killingbeck et al. 1990), disturbance history (Killingbeck 1988), leaf life span (but see Escudero and Mediavilla 2003) and water availability (del Arco et al. 1991; Pugnaire and Chapin 1992; Minoletti and Boerner 1994). Though the relationship seems to be neither unequivocal nor singular, there is a strong desire to make the intuitive connection between plant or soil nutrient status and resorption (Chapin 1980; Vitousek 1984; Field and Mooney 1986; Aerts and Chapin 2000). However, results remain conflicting (Aerts 1996; Killingbeck 1996).

Effects of Fertilization on Foliar Nutrient Status

In general, foliar nutrients reflect soil nutrient availability and fluctuations in fertility, whether natural or induced by fertilization. Plants in infertile sites generally have low foliar nutrient concentrations (Aerts and Chapin 2000), and fertilization with a nutrient generally results in an increase in foliar concentrations of that nutrient (Vitousek 1998). With fertilization, plant-available N and P concentrations increase with addition of the respective nutrient, but may also increase with addition of the other (Lu et al. 2013). Enrichment with N and P can increase soil microbial activity, thus leading to elevated rates of soil organic matter turnover (Scheu 1990; Prescott et al. 1992; Aber et al. 1995; Lovell and Hatch 1998; Grierson et al. 1999). Low soil P can limit N fixation and mineralization rates, so P additions can ameliorate low N availability (Niklaus et al. 1998; Niinemets and Kull 2005). Similarly, low soil N can limit turnover of organic P (Niinemets and Kull 2005), the amount of which can exceed plant-available P (Binkley and Vitousek 1989); N additions can increase P mineralization (Finzi 2009).

For plants, adding N may enhance P uptake via allocation towards P-acquiring phosphatase enzymes. Protein synthesis declines when P is limiting, and P additions have been shown to positively affect N uptake (Lajtha and Klein 1988; Reich and Schoettle 1988; Reich et al. 1994; Raaimakers et al. 1995; Niinemets and Kull 2005). In these cases, adding N or P leads to increased foliar concentrations of both or the other nutrient (Güsewell et al. 2003; Niinemets and Kull 2005; Jones and Power 2012; Lu et al. 2013). Others have seen the opposite effect, where adding one nutrient results in no change (Vitousek 1998) or reductions in concentrations of the other (Niinemets and Kull 2005; Menge and Field 2007). If the added element leads to enhanced growth, the reduced concentration of the other element could be a dilution effect. Alternatively, adding one nutrient may induce limitation by the other, especially if plant growth is stimulated and plant demand for both nutrients increases (Phoenix et al. 2003); this could also result in lower foliar concentrations of that limiting element.

Effects of Fertilization on Resorption Efficiency

Resorption varies both inter- (Chapin and Kedrowski 1983; del Arco et al. 1991; Hagen-Thorn et al. 2006) and intraspecifically (Birk and Vitousek 1986; Nordell and Karlsson 1995), so it is logical to associate higher nutrient use efficiency on nutrient-poor sites (Birk and Vitousek 1986; Aerts 1995). Many studies support this, with evidence that increased N or P availability reduces resorption of the respective nutrient (Shaver and Melillo 1984; Chapin and Moilanen 1991; Pugnaire and Chapin 1993; Enoki and Kawaguchi 1999; Côté et al. 2002; van Heerwaarden et al. 2003b; Li et al. 2010; Lu and Han 2010; Lu et al. 2013). In these cases, uptake of readily available nutrients from the soil may be more economically viable than spending resources on resorption, and nutrient additions reduce plant dependence on internal nutrient recycling. However, some have found resorption of N or P to increase with additions of the respective nutrient (Nambiar and Fife 1987), and a number of studies have found no association between soil or plant nutrient status and resorption efficiency of a nutrient (Birk and Vitousek 1986; Chapin and Moilanen 1991; del Arco et al. 1991; Walbridge 1991; Helmisaari 1992; Aerts and de Caluwe 1994; Aerts 1996; Vásquez de Aldana and Berendse 1997; Yuan et al. 2005; Cárdenas and Campo 2007; Soudzilovskaia et al. 2007). Responses may also depend on whether the site was initially limited by N or P (Vitousek 1998).

Few studies have looked at the response of N resorption to increased P availability. The response of P resorption to increased N availability varies (van Heerwaarden et al. 2003b). In some cases, N inputs increase P conservation due to increased plant demand (Menge and Field 2007; Perring et al. 2008). Others have found an acceleration in P cycling following N additions, with more P in litter and less resorption (Lu et al. 2013). This could be due to higher soil P availability subsequent to N additions, or could be attributable to the need to maintain a relatively rigid stoichiometric balance (Broadley et al. 2004; Yu et al. 2010; Lu et al. 2013). Resorption of N and P are usually correlated (Güsewell 2005), so it could be that reducing N resorption also reduces P resorption. Acceleration of P cycling following N additions could be a mechanism by which P limitation is delayed.

Resorption Proficiency

The lack of a consistent pattern in resorption efficiency and site fertility might be attributed to a couple of factors. It is possible that efficiency is not as evolutionarily important as proficiency, or that resorption efficiency is too subject to the vagaries of variation in nutrient concentrations between years, individuals, species, or sites (May and Killingbeck 1992; Killingbeck 1996; Eckstein et al. 1999). It has been suggested that resorption proficiency is a more objective and directly comparable measure of resorption. Resorption is an energetically expensive process (Buchanan-Wollaston 1997) and is constrained by increasingly recalcitrant foliar compounds (Chapin and Kedrowski 1983), indicating a point of maximum resorption (Cárdenas and Campo 2007). Killingbeck (1996) introduced the concepts of potential/realized and complete/incomplete resorption to describe the degree to which nutrients are resorbed. Senesced leaf nutrient concentrations, similar to green leaf concentrations, also appear more reflective of changes in soil fertility. Individuals with higher green leaf nutrient concentrations tend to have higher litter nutrient concentrations (Vitousek 1998; Hagen-Thorn et al. 2006; Soudzilovskaia et al. 2007; Lu et al. 2013), with plants in nutrient-poor sites often more proficient at resorption (Yuan et al. 2005).

Due to the interconnectedness of biogeochemical cycles, interactive effects between N and P availability, uptake, and use efficiency may be complex. Responses may depend on the ecosystem's initial limitation status (Vitousek 1998; Perring et al. 2008). Plants have different adaptations for maximizing and efficiently using and conserving nitrogen and phosphorus, and the differences in mobility, availability, use, and uptake of nitrogen and phosphorus may make it useful to consider limitation by N and P as having distinct manifestations and effects on an ecosystem, rather than as one phenomenon (Kost and Boerner 1985; Chapin et al. 1990; Lambers et al. 2008; Ostertag 2010).

Role of Calcium in the Tree

While forested ecosystems are traditionally thought of as being limited by N and/or P, other elements, such as calcium (Ca), can critically affect forest health. Calcium is a divalent cation that is taken up by plants when dissolved in the soil solution or adsorbed to soil exchange sites; when taken up into the apoplast of the root, ions are delivered to the xylem via both symplastic and apoplastic pathways (USGS 1999; White and Broadley 2003; Smith and Shortle 2013). Once deposited, Ca cannot be remobilized into the phloem, and the concentration of Ca in the xylem sap decreases with increasing distance from the point of uptake, such that the signs of a true Ca deficiency are often first apparent in the youngest and most distal branches (White and Broadley 2003; Hirschi 2004; Smith and Shortle 2013). After being translocated to its destination, Ca is thought to be generally immobile, with minimal retranslocation or resorption (Hirschi 2004; Littke and Zabowski 2007; Perakis et al. 2013).

Interestingly, Ca transport is tightly linked to transpiration, yet water flow is directed by Ca ions such that an interdependent relationship exists between water and Ca^{2+} in plants (Gilliham et al. 2011; Smith and Shortle 2013). Calcium's relationship with stomatal opening and closure may influence the efficiency of carbon (C) capture (Smith and Shortle 2013). The opening and closing of guard cells is induced in part by Ca signaling, and the efflux of potassium (K) mediated by Ca-activated pumps (MacRobbie 1998). Cell elongation and division, internal cell pH, and carbohydrate translocation are also affected by plant Ca content, but two of the most important roles of Ca are in physical structure and cell chemical signaling (Hirschi 2004).

Calcium is one of a plant's most versatile messengers, responding to more cues and stimuli than any other known component of plant signaling systems (Sanders et al. 1999). Internal cell Ca concentrations are tightly regulated, with active transport out of the cytoplasm energized by ATP hydrolysis or H+ pumps (Hirschi 2004; White and Broadley 2003). Developmental and environmental cues, such as red or blue light, heat or cold shock, mechanical stimulation, or drought, induce a rise in cytosolic Ca^{2+} concentrations through Ca-permeable channels in the cell membrane (White and Broadley 2003; Hirschi 2004). Calcium also assists in cell structural integrity and stabilization by connecting polysaccharides in the cell wall and proteins and lipids on the surface of the cell membrane (Smith and Shortle 2013; Hirschi 2004). Enzymes involved in cell wall synthesis are activated by Ca, which also impacts on the structure and composition of the cell wall (McLaughlin and Wimmer 1999).

Calcium Limitation and Effects on Ecosystem Processes

Calcium influences a broad range of ecosystem processes, both directly and indirectly. Calcium additions have resulted in increased leaf area (Juice et al. 2006; Marlow and Peart 2014), and there is a relationship between low Ca concentrations and increased crown dieback in sugar maple (Long et al. 1997) or decreased crown density in red spruce (Shortle and Smith 1988). Many have found Ca additions to be associated with enhanced growth and survivorship, and healthier crown condition, particularly in sugar maple (Long et al. 1997; Juice et al. 2006; Huggett et al. 2007; Moore et al. 2012; Marlow and Peart 2014). Similarly, simulated acid rain experiments have found high Ca levels to buffer or protect against the deleterious effects of acid rain (Ashenden and Bell 1989; Liu et al. 2011).

Litter Ca levels have a positive effect on rates of organic matter decomposition (Reich et al. 2005; Hobbie et al. 2006); in the soil, enhanced Ca availability promotes C storage in certain conditions (Paul et al. 2003). Low levels of Ca availability impede nutrient uptake by reducing fine root function and mycorrhizal infection (St. Clair and Lynch 2005; Juice et al. 2006). In plants, Ca deficiency has been linked to increased susceptibility to disease and fungal pathogens, such as dogwood anthracnose in flowering dogwood (McLaughlin and Wimmer 1999), and to winter frost injury and reduced antioxidant activity in red spruce (Hawley et al. 2006; Halman et al. 2008). Calcium-deficient plants may experience reduced photosynthetic function (Liu et al. 1997) and be more susceptible to drought, due to impaired stomatal functioning (Ruiz et al. 1993; Ridolfi et al. 1994). At the ecosystem level, watershed scale amendment of wollastonite ($CaSiO_3$) resulted in increased evapotranspiration, possibly mediated by enhanced hydraulic efficiency or changes in stomatal dynamics (Green et al. 2013; Smith and Shortle 2013). The biogeochemical cycles of other elements are linked to Ca availability; P availability, for example, may decline in association with Ca depletion (Fiorentino et al. 2003). Calcium availability affects community composition, and depletion seems to have induced declines in species sensitive to Ca availability, such as sugar maple, with a concomitant increase in species more tolerant of low Ca levels, such as beech (Van Breemen et al. 1997; Kobe et al. 2002; Duchesne and Ouimet 2009; Minocha et al. 2010). Further up the trophic web, Ca depletion and associated soil acidification can affect the abundance and shell integrity of snails (Hamburg et al. 2003).

Soil Ca availability increases following fertilization with Ca, whether as gypsum (Littke and Zabowski 2007), lime (Long et al. 1997), or wollastonite (Juice et al. 2006). Accordingly, foliar Ca also generally increases (Long et al. 1997), though effects may not appear within the first year after treatment (Littke and Zabowski 2007) and may peak and subsequently decline after several years (Juice et al. 2006).

Reasons for Calcium Deficiency

Calcium is a natural component of the soil chemical balance, obtained mainly through weathering of soil parent material; small amounts of Ca are also deposited from the atmosphere (USGS 1999). Over the last half-century, Ca depletion in forest ecosystems has increasingly been attributed to anthropogenic acidic deposition, primarily the result of emissions of sulfur dioxide, nitrogen oxides, and ammonia stemming from agricultural activities and fossil fuel combustion (Driscoll et al. 2003). Sulfuric and nitric acids and ammonium reduce the pH of precipitation; when deposited, hydrogen ions are exchanged into the soil solution and displace the base cations (Ca, magnesium (Mg), K) adsorbed to soil exchange sites (USGS 1999; Driscoll et al. 2003). These nutrient cations are released into soil water and leached, coupled with acidic anions (Aber et al. 1998; USGS 1999). With continued acid inputs and insufficient base cation supply, soil acid neutralizing capacity declines; as cations are depleted faster than they can be replenished by weathering, the loss of this buffer increases ecosystem sensitivity to acidification and reduced pH permits the mobilization of toxic aluminum (Al) and manganese (Mn) ions (Johnson et al. 1994; Driscoll et al. 2001; Driscoll et al. 2003). The effects of acidic deposition cascade throughout the ecosystem: microbial diversity and activity, and therefore nutrient recycling processes, decline (Anderson 1998; Rousk et al. 2010); trees experience enhanced leaf damage and may be predisposed to other stresses (Nihlgård 1985; Liu et al. 2011); and, owing to differences in nutrient requirements and acid tolerance, species composition may shift (Kobe et al. 2002; Moore and Ouimet 2006; Duchesne and Ouimet 2009). Nitrate deposition has remained relatively constant over the last 20-30 years, and is of particular concern, since Ca may be more susceptible to depletion than other cations at high soil N levels (Perakis et al. 2006). Meanwhile, the concentrations of cations in precipitation have also declined (Hedin et al. 1994).

Management practices may also be partially responsible for Ca depletion. Once deposited in tree tissues, Ca is relatively immobile, with minimal resorption from senescing leaves or retranslocation from sapwood as it matures into heartwood (Schaberg et al. 2001; Perakis et al. 2013). In northern hardwood forests, much of the annual Ca requirement is supplied through internal cycling and decomposition of organic matter, and leaching losses can be high (8-15 kg Ca ha^{-1} y^{-1}), even in uncut forests (Mann et al. 1988; Hornbeck et al. 1990; Blum et al. 2008). Subsequent to harvesting, Ca is more mobile, with increased hydrologic losses for several years post-harvest (Mann et al. 1988; Johnson et al. 1988). Therefore, biomass removal, particularly whole-tree and multiple rotation timber harvesting, can accelerate the onset of Ca deficiency by removing large amounts of the forest Ca capital in biomass and soils (Mann et al. 1988; Johnson et al. 1989; Perakis et al. 2013). The Ca added to the system by mineral weathering and atmospheric deposition is insufficient to meet forest demands on the relatively short timescale of forest regeneration (Huntington et al. 2000; Yanai et al. 2005; Perakis et al. 2006). Calcium is the nutrient predicted to become depleted in soils soonest as a result of vegetation uptake, repeated harvest, and hydrologic export, with a potential reduction of 20-60% of total Ca after 120 years of forest growth (Johnson et al. 1988; Federer et al. 1989; Hornbeck et al. 1990). Declines in Ca to less than that required by a merchantable forest stand in the southeastern US could occur in only 80 years (Huntington et al. 2000). This amount of time may be reduced by certain management practices, such as increased harvest intensity, shortened rotation times, and removing slash (Yanai et al. 1999).

Aging stands and changing species compositions may be yet another reason for the observed reductions in Ca supply. As stands age and accumulate biomass, trees may take up Ca in quantities exceeding that which is supplied by weathering and deposition, especially since the magnitude of recycling within the tree is lower for Ca than for other nutrients (USGS 1999). Hamburg et al. (2003) found that Ca availability in northern hardwoods forest stands depended upon stand age, with several indicators suggesting higher labile Ca in the younger stands. Over a fifteen-year sampling period in the same region, older stands exhibited the most base cation depletion, while young stands tended to gain cations in the forest floor, possibly due to the ability of younger stands or early successional species to access nontraditional Ca pools (Yanai et al. 1999; Hamburg et al. 2003). Therefore, it is possible that Ca declines are a natural aspect of forest succession.

Implications of Calcium Depletion

Species Effects

Ca depletion has been observed in ecosystems around the world, including the eastern United States (Johnson et al. 1988; Johnson and Todd 1990; Johnson et al. 1994), Europe (Malmer 1976; Ulrich et al. 1980), and China (Liu et al. 2011). Calcium depletion and the concomitant decrease in pH have been linked to declines of several forest keystone species, especially in the northeastern United States. Acidic deposition has been identified as a probable factor in soil Ca reductions in this region, and implicated in reduced cold tolerance and antioxidant activity and increased risk for winter injury and crown deterioration in red spruce (Lawrence et al. 1997; DeHayes et al. 1999; Hawley et al. 2006; Halman et al. 2008). The decline in sugar maple that has been reported across much of its range has also been attributed, at least in part, to Ca depletion.

Sugar maple is one of the most valuable northern hardwoods species. Economically, it is prized for its wood and sap-based products, especially syrup. Ecologically, sugar maple has value as an abundant, dominant canopy tree. The decline complex, manifesting as reduced growth, poor regeneration, deterioration of crown condition, and ultimately, mortality, has also been linked to drought and repeated insect defoliation as inciting factors (Mader and Thompson 1969; Kolb and McCormick 1993; Long et al. 1997; Houston 1999). Over the last several decades, nutrient stress has been suggested as a major predisposing factor (Bernier and Brazeau 1988; Kolb and McCormick 1993; Drohan et al. 2002; Horsley et al. 2002). Sugar maple can be found in soils with pH 3.7-8.1, but most commonly occurs where pH is 5.5-7.3 (Godman 1957). Sugar maple is less tolerant of low nutrient soils than some of its co-occurring northern hardwoods species, such as red maple and American beech (Abrams 1998; Duchesne and Ouimet 2009).

Though low levels of Mg, K, and P and high levels of Al and Mn have been correlated with sugar maple decline in the Northeast, Ca deficiency in the foliage and the soil has consistently and repeatedly been observed in afflicted stands (Bernier and Brazeau 1988; Kolb and McCormick 1993; Horsley et al. 2000; Drohan et al. 2002; Kogelmann and Sharpe 2006; Schaberg et al. 2006; Long et al. 2009; Bal et al. 2015). A relationship between low or decreasing soil Ca and poor sugar maple regeneration, establishment, growth, and survival, and that Ca addition ameliorated these conditions is well established (Moore et al. 2000; Kobe et al. 2002; Juice et al. 2006; Huggett et al. 2007; Long et al. 2011; Sullivan et al. 2013). It is especially interesting that the perception of the inciting factors of drought and defoliation stress and initiation of tree responses such as closure of stomata or activation of gene transcription for defense is related to internal signaling by labile Ca, meaning that Ca depletion may adversely affect a tree's ability to dynamically adjust to these stimuli (Schaberg et al. 2001).

Effects on Aluminum, Manganese, Nitrogen, and pH

In soils where base cation supply is insufficient to neutralize acidic inputs or where leaching losses and vegetation uptake have depleted native Ca supplies, the decline in pH permits the mobilization of Al and Mn. As soil acidifies, Mn will mobilize at a higher pH (<5.5) than Al (<4.0), so deleterious effects of Mn may be expected first (Watmough et al. 2007). High levels of Mn have been implicated in forest decline, though it is not unequivocally clear if Mn leads to impaired nutrient uptake by directly harming roots or by simply blocking cation uptake without producing root injury (Horsley et al. 2000; Watmough et al. 2007; Kitao et al. 2011). Increased Mn has been associated with decreased Ca uptake and reduced foliar nutrients (Kavvadias and Miller 1999; Schier and McQuattie 2000). Adverse effects of Mn upon sugar maple seedlings have been reported, but direct toxicity (i.e., mortality) occurred only at solutions above 40 mg/L, a level that is at least twenty times what may occur in a natural environment (McQuattie and Schier 2000).

Like Mn, Al mobilizes in the soil solution at low pH, and high soil Al concentrations have often been linked to Ca deficiency. Toxic effects may be due in part to inhibition of nutrient uptake resulting from direct injury to root cells, but also result from competition for root uptake and cation exchange sites (Ryan et al. 1993; Long et al. 1997; Bal et al. 2015). Aluminum can displace nutrient cations from exchange sites and decrease availability of Ca in the forest floor (Cronan and Grigal 1995; Lawrence et al. 1995). Aluminum ions may be taken up by roots at the expense of Ca, especially at an Al:Ca ratio greater than one (Rengel and Elliot 1992; Cronan 1991). Species vary in their tolerance to Al, and sugar maple appears particularly sensitive, exhibiting reduced Ca content in Al solutions of 100 µM and diminished biomass with 600 µM Al solutions (Thornton et al. 1986; Kelly et al. 1990). Low Ca:Al (<110) ratios in the soil solution have been linked with sugar maple decline in field studies (Cronan and Grigal 1995; Long et al. 1997; Drohan et al. 2002; Bailey et al. 2004). Increases in Ca:Al ratios concurrent with reduced foliar Al and increased foliar Ca have followed Ca additions (Long et al. 1997; Juice et al. 2006; Minick et al. 2011).

Plant-available N in the soil has been shown to increase via increased mineralization (Marrs et al. 1988) or decrease via increased immobilization (Lohm et al. 1984; Shah et al. 1990) or decreased net mineralization (Groffman et al. 2006; Minick et al. 2011) following Ca additions, and may be attributable to pH changes (Long et al. 1997; Perakis et al. 2006). There may be no corresponding change in foliar N concentrations (Long et al. 1997; Littke and Zabowski 2007), though others have found lower leaf N, yet higher total chlorophyll content (Juice et al. 2006), in sites fertilized with Ca. Perakis et al. (2006) noted a negative correlation between foliar N and Ca concentrations.

Conclusion

As human activities continue to affect element cycling in natural ecosystems, interspecific variation in nutrient requirements may lead to shifts in species composition and dominance. Fertilization studies can help us predict potential changes in nutrient cycling, within both individual trees and entire ecosystems. In a way, Ca, control, and N and/or P plots represent the past, present, and future states, respectively, of northern hardwood forests, and allow us to assess actual and potential changes over time.

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Chapter 2: Effects of nitrogen and phosphorus fertilization on foliar nutrient concentrations and resorption in three species of northern hardwood trees

Abstract

Anthropogenic disruption of natural biogeochemical processes is of growing concern and long-term effects of such alterations on foliar nutrient status and resorption remain largely unclear, especially since there is still little known about how nutrients interact to influence and limit leaf nutrient concentrations and resorption dynamics. In the White Mountains of central New Hampshire, plots in eight natural forest stands of two age classes have been fertilized annually since 2011 in a nitrogen (N) and phosphorus (P) addition full factorial design. I analyzed green leaves collected in August, 2015, and senesced leaves collected in October, 2015, from red maple (*Acer rubrum*), sugar maple (*A. saccharum*), and American beech (*Fagus grandifolia*) for N and P concentrations to compare foliar nutrition and resorption proficiency and efficiency within and among species, treatments, and age classes. Foliar N:P ratios increased with N and decreased with P additions. Green and senesced leaf concentrations of N and P increased with additions of the respective nutrient, but were not affected by additions of the other. Resorption of N and P were highly correlated across all treatments and sites. Phosphorus resorption efficiency declined with additions of P, alone or with N. There was no effect of N or P treatment alone on N resorption efficiency. Higher foliar N:P ratios were associated with higher resorption efficiency of both N and P. Higher litter N and P concentrations with N additions suggest that higher nutrient availability lessens tree dependence on internal nutrient recycling and accelerates ecosystem turnover of N and P.

Introduction

Since the beginning of the industrial age in the late 1700s, atmospheric nitrogen (N) deposition has increased significantly due largely to fossil fuel combustion and agricultural use of ammonia-based fertilizers, while mining and agricultural activities have altered phosphorus (P) cycling (Galloway et al. 2008; Vitousek et al. 2010; Peñuelas et al. 2012). Increases in ecosystem nutrient capital can lead to increased plant biomass and nutrient concentrations, but can also affect other ecosystem characteristics such as species composition, herbivory, and mycorrhizal abundance (Ritchie 2000; Stevens et al. 2004; Treseder 2004; Bobbink et al. 2010; Li et al. 2016).

As two of the primary macronutrients essential to plant growth, the productivity of ecosystems is often considered to be limited by N, P, or both. Plants allocate assets so as to remain simultaneously co-limited by multiple resources (Bloom et al. 1985; Arrigo 2005; Niinemets and Kull 2005; Ågren et al. 2012), but anthropogenic nutrient inputs have the potential to shift limitation (Aerts et al. 1992; Menge and Field 2007; Elser et al. 2009; Peñuelas et al. 2012). It is unclear how interspecific differences in nutrient use and conservation will manifest in response to such shifts, and theories surrounding how changes in limitation status will affect the cycling of other nutrients have been in dispute. Nitrogen and P are tightly coupled ecologically (Shaver and Melillo 1984; Elser et al. 2007); additions of one nutrient can increase soil availability (Scheu 1990; Prescott et al. 1992; Aber et al. 1995; Lovell and Hatch 1998; Niklaus et al. 1998; Grierson et al. 1999; Treseder and Vitousek 2001; Finzi 2009; Marklein and Houlton 2011; Lu et al. 2013) and use efficiency (Lajtha and Klein 1988; Reich and Schoettle 1988; Reich et al. 1994; Raaimakers et al. 1995) of the other nutrient. In addition, if N and P additions increase foliar N and P, respectively, concentrations of the other nutrient would be expected to increase to maintain stoichiometric balance (Phoenix et al. 2003; Yu et al. 2010; Marklein and Houlton 2011). It has been further hypothesized that N additions may elicit more conservative plant use of P due to enhanced plant growth (Perring et al. 2008; Vitousek et al. 2010), while increased N may accelerate ecosystem P cycling (Marklein and Houlton 2012).

Changing nutrient cycles can affect tree physiological processes and phenotypic variation in leaf traits. For example, foliar photosynthetic rate increases with foliar N and P and with specific leaf area (SLA) (Reich et al. 2009), and fertilization may increase these leaf traits (Knops and Reinhart 2000). Higher leaf dry matter content (LDMC; the ratio of dry weight to fresh weight) is correlated with tougher leaves, high nutrient use efficiency, and slower growth (Garnier et al. 1999; Wilson et al. 1999; Reich et al. 1999; Wright et al. 2001; Gross et al. 2007), and may also be sensitive to fertilization.

Foliar nutrient concentrations, N:P ratios, and N:P resorption ratios have been proposed as indicators of terrestrial ecosystem nutrient limitation (Koerselman and Meuleman 1996; Tessier and Raynal 2003; Güsewell 2004; Reed et al. 2012). Nitrogen and P concentrations in foliage often reflect soil nutrient availability (Shaver and Melillo 1984; Vitousek and Farrington 1997; Vitousek 1998; Aerts and Chapin 2000; Hobbie and Gough 2002; Han et al. 2005; Townsend et al. 2007; Ordonez et al. 2009). Resorption is an important nutrient conservation mechanism (Killingbeck 1996); globally, an average of about 50% of foliar N and P is resorbed (Aerts 1996; Vergutz et al. 2012), providing approximately one-third of annual vegetation N and P demands for plant growth in northern hardwood forests (Ryan and Bormann 1982). By resorbing nutrients out of leaves prior to senescence, trees can be less dependent on variations in soil resource availability (Aerts and Chapin 2000; Yuan and Chen 2015). Thus, it is intuitively logical to presume that resorption will be higher in nutrient-poor sites (Vitousek 1982). Yet nutrient manipulation experiments and observational studies along nutrient gradients provide conflicting results vis-à-vis the relationship between soil or leaf nutrient status and resorption; soil or plant nutrient concentrations can be positively, negatively, or not significantly associated with the proportion of that nutrient resorbed (Table 1). Reviews of larger datasets have noted only weak associations between nutrient availability and resorption (Aerts 1996; Killingbeck 1996). Patterns in the link between resorption dynamics of one element and soil or plant nutrient status of another are also inconsistent, suggesting complex interactions (Yuan and Chen 2015).

Resorption is generally expressed in two ways. Proficiency is the concentration to which a nutrient has been reduced in the senesced leaves (Killingbeck 1996), while efficiency is the proportion resorbed of green leaf nutrient concentrations (Aerts 1996). Efficiency is reported as a percentage and is calculated as:

$$\frac{N_{gr} - N_{se}}{N_{se}}$$

where N_{gr} and N_{se} are the nutrient concentrations in the green and senesced leaves, respectively. Though resorption efficiency may be a better indicator of the relative degree to which nutrients are conserved, it has been proposed that natural selection acts upon absolute nutrient values (Killingbeck 1996; Yuan and Chen 2015), and that this could be one reason for the disparate results found among studies (Eckstein et al. 1999); that is, resorption proficiency may be a better measure of the evolutionary constraints of nutrient conservation, and may be more responsive to changes in nutrient availability (Killingbeck 1996; Rejmánková 2005).

In the White Mountains of central New Hampshire, eight northern hardwood stands have been fertilized annually since 2011 in a complete N and P factorial design as part of a study on multiple element limitation (Fisk et al. 2014). To reduce artifacts associated with high-level nutrient additions, only modest amounts of nutrients are being applied repeatedly over long time scales in this study. My objectives were to quantify the foliar nutrient concentrations and resorption proficiency and efficiency of three dominant tree species in these forests. I hypothesized that leaves in the fertilized plots would be larger and heavier than those in the control plots due to increased nutrient availability. I expected that our nutrient inputs would shift limitation, as evidenced by foliar N:P ratios and N:P resorption ratios. In particular, I hypothesized that green leaf and litter concentrations of N and P would increase with additions of the respective nutrient, and that resorption efficiency would decline. I also hypothesized that adding N or P would result in higher green leaf concentrations and resorption proficiency and efficiency of P or N, respectively, indicative that additions of one nutrient leads to allocation of resources towards acquisition of the other nutrient; the highest concentrations of both nutrients was expected in the NP treatment. Finally, I hypothesized that addition of both N and P would result in synergistic effects of both increased concentrations and resorption of both nutrients.

Methods

Site Description

The eight sampled stands are located across three sites in the White Mountains of New Hampshire. Four stands (two mid-aged and two mature) are located in Bartlett Experimental Forest (BEF), with two additional stands of each age class at Hubbard Brook Experimental Forest (HB) and at

Jeffers Brook (JB). The three sites are within 65 km of each other. The eight stands are characteristic of the northern hardwoods forest type (Table 1). A mixture of red (*Acer rubrum* L.) and sugar (*A. saccharum* Marsh.) maples, white (*Betula papyrifera* Marsh.) and yellow (*B. alleghaniensis* Britt.) birches, pin cherry (*Prunus pennsylvanica* L.f.), and beech (*Fagus grandifolia* Ehrh.) in the mid-aged stands transitions to a canopy dominated by beech, sugar maple, and yellow birch in the mature stands. The forests in this region are in the aftermath phase of beech bark disease (Giencke et al. 2014), and beech saplings and sprouts are numerous in the understory. The understory primarily consists of the same species that comprise the overstory, with the addition of striped maple (*A. pennsylvanicum* L.), especially in the younger stands. Some conifers, namely eastern hemlock (*Tsuga canadensis* L.) and balsam fir (*Abies balsamea* L.), and shrubs, such as hobblebush (*Viburnum lantanoides* Michx.) and Canadian yew (*Taxus canadensis* Marsh.), are also interspersed throughout.

All the soils are well-drained Spodosols formed in glacial drift, but the local bedrock differs among sites. The bedrock at both BEF and JB is igneous rock, though the Ammonoosuc volcanic material at JB is more nutrient rich than the Conway/Mt. Osceola granite found at BEF. At HB, the bedrock is metamorphic Rangeley Formation Schist. These differences result in contrasting soil nutrient availability and pH across the sites. The climate is humid continental, with an average 140 cm of annual precipitation in Hubbard Brook and 127 cm at Bartlett (Adams et al. 2003). Annual temperatures at HB range from a mean January low of -9°C to a mean July high of 18°C (Adams et al. 2003). Nitrogen deposition in this region exceeded 8 kg N ha^{-1} y^{-1} for much of the 1980s and 90s, but declined sharply in the early 21st century to about 2-4 kg N ha^{-1} y^{-1} (NADP 2014).

All stands in BEF and the mature stands at HB and JB contain four 50x50 m plots that have been treated annually since 2011 with either N (30 kg N ha^{-1} y^{-1} as NH_4NO_3), P (10 kg P ha^{-1} y^{-1} as NaH_2PO_4), both N and P together (same rates), or neither N nor P (control). In the mid-aged stands at HB and JB, the four plots are 30x30 m. All sampling was conducted in central sub-plots to avoid edge effects.

Field Methods

Beech leaves were collected in all stands, but the species of maple sampled depended on the stand. I sampled red maple in all but one (JB) of the mid-aged stands and sugar maple in all the mature stands. Sugar maple leaves were collected in the mid-aged stand at Jeffers Brook because red maple was absent. In each stand, green leaves were collected from three individuals of each species in each of the four plots August 9-15, 2015, and kept frozen until sample processing. Each sampled tree was dominant or intermediate in the canopy with DBH > 10cm. Leaves were collected from the mid-canopy of each tree with a shotgun.

Senesced leaves of the study species were collected from the ground in each stand during the period of peak fall color on October 3-4 and 10-11, 2015. I analyzed the litter from the second sampling interval except for three plots where collections from the first interval were used because of irregular samples on the later date. I composited leaf litter by species within each plot. For both green and senesced leaves, only leaves with petioles attached and free of visible damage from disease or insects were used for subsequent analysis. I photographed the green leaves and leaf litter and determined leaf surface area with ImageJ software so that foliar nutrient concentrations could be expressed on an area basis. Leaves were weighed before and after drying to constant mass at 60°C, and then ground to pass through a 40 mm mesh screen in a Wiley mill. Green LDMC was calculated as the ratio of dry mass to fresh mass, and SLA as the ratio of one-sided leaf surface area to dry mass.

To provide pre-treatment nutrient concentrations, both green and senesced leaves were collected 2008-2010 prior to the initial fertilizer application (2011). Methods may be found in See et al. (2015).

Chemical Analysis

I determined N concentrations with a FlashEA 1112 analyzer (Thermo Scientific). Aspartic acid and apples leaves (NIST 1515) were used as standards. Phosphorus concentrations were ascertained by an Optima 5300 DV inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin-Elmer) after samples were ashed and hot plate-digested with 6N nitric acid. Each round of ashing included one blank, one replicate, and two apple leaf standards (Appendix 1). During ICP analysis, I ran a blank after every ten samples, and an in-house standard as a quality control (QC) after every five. Samples were not analyzed unless the lab QC returned +/-5% recovery.

Statistical Analysis

Because litter and green leaf nutrient concentrations could not be paired by tree, green leaf data from individual trees were averaged by species within each plot, resulting in plot-level averages for all variables; the plot was considered the experimental unit in all analyses. The experimental design was a randomized complete block design with a factorial of N treatment, P treatment, and species. Stand, nested within age, was a blocking factor. The dependent variables evaluated were physical leaf characteristics (leaf mass, leaf area, SLA, LDMC), green leaf N and P concentrations, and resorption proficiency and efficiency of N and P.

Treatment effects were assessed using a nested analysis of variance (ANOVA), with age class, N treatment, P treatment, and species as predictor variables (Table 2) in the following model:

$$Y_{ijklmn} = \mu + \alpha_i + \beta(\alpha)_{j(i)} + \gamma_k + \delta_l + \zeta_m + (\gamma\delta)_{kl} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\gamma\zeta)_{km} + (\delta\zeta)_{lm} + (\alpha\gamma\delta)_{ikl} + (\gamma\delta\zeta)_{klm} + \varepsilon_{(ijklm)n} \quad [1]$$

where Y is the dependent variable, μ is the overall mean, α is age, β is stand, γ is N treatment, δ is P treatment, ζ is species, and ε is the error term. These predictor variables were treated as fixed effects.

For N and P concentrations and mass-based calculations of resorption, I also performed analysis of covariance (ANCOVA), using plot-level pre-treatment concentrations and resorption values for each species as a covariate. The experimental design and model statement were the same as above, except for the new inclusion of a continuous variable (i.e., pre-treatment concentration or resorption). For significant ($p < 0.05$) effects, means were compared by performing Tukey's post-hoc tests, though p values up to 0.2 were given additional consideration, especially for tests of interactions. All statistical tests were performed using SAS 9.4 (SAS Institute, Cary, NC).

Results

Leaf Physical Characteristics

Green leaves were larger (treatment interaction, $F_{1,42} = 9.89$, $p = 0.003$) and heavier (treatment interaction, $F_{1,42} = 12.55$, $p = 0.001$) with N or P added separately than in the NP or control plots, and leaf area and mass were highly correlated ($r_{62} = 0.59$, $p < 0.001$) (Figure 1). The two maple species showed more variation in leaf mass and area among treatments than did beech. Nevertheless, the ratio of area to mass remained consistent among treatments (treatment interaction, $F_{1,42} = 0.16$, $p = 0.69$), with average SLA values for all sampled species ranging between 13.8±1 $cm^2\ g^{-1}$ in the N treatment and 16.1±1 $cm^2\ g^{-1}$ in the P treatment. The main effect of P treatment on SLA was marginally significant ($F_{1,42} = 3.83$, $p = 0.06$): SLA was 14% higher when P was added, with SLA highest in the P and NP plots.

Green foliage had 33% less surface area ($F_{1,42} = 17.29$, $p = 0.006$) and 27% less mass ($p = 0.04$) per leaf in the mature stands than in the younger stands. Despite the differences in area and mass, SLA was consistent across age classes ($F_{1,42} = 0.01$, $p = 0.83$), ranging from 14.6±1 $cm^2\ g^{-1}$ in the older stands to 14.9±1 $cm^2\ g^{-1}$ in the mid-aged stands.

The three species did not differ significantly in average surface area per green leaf ($F_{2,42} = 1.21$, $p = 0.31$), but red and sugar maple leaves were heavier than beech leaves ($F_{2,42} = 23.32$, $p < 0.001$), with correspondingly lower SLA in leaves of both maple species ($F_{2,42} = 21.67$, $p < 0.001$) (Figure 1).

Leaf dry matter content (LDMC) tended to be lower in plots with N additions (main effect, $F_{1,42} = 3.41$, $p = 0.07$), especially when added together with P (treatment interaction, $F_{1,42} = 2.01$, $p = 0.16$). The main effect of species was significant, with beech having the highest LDMC and red maple the lowest ($F_{2,42} = 3.19$, $p = 0.05$) (Figure 2). The interaction between species and treatment was not significant ($F_{2,42} = 0.72$, $p = 0.49$).

Foliar Nutrients and Resorption

I calculated nutrients as mass-based concentrations ($mg\ g^{-1}$), area-based concentrations ($mg\ cm^{-2}$), and content per leaf ($mg\ leaf^{-1}$), recognizing that there are flaws associated with each metric (van Heerwaarden et al. 2003a). I focus here on mass-based concentrations because the mass loss was less than the area loss in comparisons of green and senesced leaves, and because using mass- based concentrations permitted the inclusion of pre-treatment data as a covariate in statistical analyses. Least squares means for mass- and area-based concentrations and for leaf nutrient content can be found in the appendix. I also calculated resorption efficiency using the mass loss correction factor introduced by van Heerwaarden et al. (2003a), but as was found by Rentería et al. (2007), the patterns and trends were the same as without it. I begin with comparisons between pre- and post-treatment, followed by full ANCOVA model results using pre-treatment data as a covariate.

Comparisons between pre- and post-treatment. The average foliar N:P in the control plots was 5% higher in 2015 than when pre-treatment data were collected between 2008 and 2010 (Figure 3). Four years of addition of N alone led to a 27% increase in the N:P ratio, while N:P ratios in the NP and P plots were lower by 6 and 26%, respectively. The pattern of an increase in the N:P ratio in the control and N plots and a decrease in the P and NP plots was consistent across the two age classes, but the N:P ratio increased more with N addition in the mature than the young stands. The three species exhibited the same patterns across treatments. The N:P resorption ratio, defined as the ratio of N resorption efficiency to P resorption efficiency, in the control plots was 8% lower post-treatment compared to pre-treatment (Figure 4). There was a striking increase in the N:P resorption ratio of 41% in the P treatment plots.

Pre-treatment foliar P was not correlated with post-treatment P concentrations ($r_{62} = 0.17$, $p = 0.18$), but pre-treatment foliar N was correlated with post-treatment foliar N ($r_{62} = 0.67$, $p < 0.001$) and N:P ($r_{62} = 0.34$, $p = 0.008$). Pre-treatment foliar N concentrations were also correlated with post-treatment resorption efficiencies of N ($r_{62} = 0.47$, $p < 0.001$) and P ($r_{62} = 0.44$, $p < 0.001$). In the pre-treatment data, green leaf N concentration was not correlated with resorption efficiency of N ($r_{62} = 0.05$, $p = 0.75$) or P ($r_{62} = -0.02$, $p = 0.92$). Pre-treatment green leaf P concentrations were associated with lower P resorption efficiency ($r_{62} = -0.31$, $p = 0.04$), but higher N resorption efficiency ($r_{62} = 0.31$, $p = 0.03$).

Foliar N and N resorption. Based on the full ANCOVA, across all three species, N concentrations in the fresh foliage increased 11% with N additions (main effect, $F_{1,39} = 43.2$, $p < 0.001$) and decreased 3% with P additions (main effect, $F_{1,39} = 4.03$, $p = 0.05$) (Figure 5). Green leaf N concentrations were lowest in the P and control plots for all three species. Green leaf N was significantly higher than the control in the N plots for all three species, but the species varied in their response when P was added in combination with N (species x N x P interaction, $F_{1,39} = 5.41$, $p = 0.009$): both sugar maple and beech had foliar N concentrations in the NP plots similar to those in the N plots, but in red maple, N concentrations in the NP plots were 16% lower than those in the N plots.

Average litter N concentrations (resorption proficiency) ranged from 10.3±0.5 mg g^{-1} in the control plots to 12.2±0.5 mg g^{-1} in the NP plots, but the interaction between N and P treatments did not have a significant effect on litter N concentrations ($F_{1,28} = 0.32$, $p = 0.58$). All species were less proficient at N resorption in plots amended with N (main effect, $F_{1,39} = 5.43$, $p = 0.03$) (Figure 6). However, there was not a significant correlation between green leaf N concentrations and N resorption proficiency ($r_{62} = -0.06$, $p = 0.64$). Red maple was 20-23% less proficient at N resorption than beech or sugar maple ($F_{2,28} = 3.17$, $p = 0.06$). The range in litter N concentrations among species was widest in the P plots.

Nitrogen resorption efficiency did not vary significantly by age, and surprisingly, there wasn't a significant main or interaction effect of treatment alone on N resorption efficiency ($F_{1,26} < 0.99$, $p > 0.33$). Nevertheless, there was a strong positive correlation between green leaf N concentrations and N resorption efficiency ($r_{62} = 0.60$, $p < 0.001$). The three species responded somewhat differently to treatment (species x N x P interaction, $F_{2,26} = 2.38$, $p = 0.11$) (Figure 7). Of the three species, beech was the most efficient at N resorption in all four treatments, and its N resorption efficiency was highest in the NP and control plots at 63%. Sugar maple N resorption efficiency was highest in the P plots at 56%, and decreased below the control when N was added, alone or with P. Red maple achieved its highest N resorption efficiency when N and P were added separately, and it decreased below the control to 35% when N and P were added together.

Foliar P and P resorption. Adding P led to higher green leaf P concentrations for all three species (main effect, $F_{1,39} = 56.62$, $p < 0.001$) (Figure 5), and the response was strongest in magnitude in sugar maple, which had green leaf P concentrations 33 and 56% higher than the control in the NP and P plots, respectively (species x treatment interaction, $F_{2,39} = 2.23$, $p = 0.12$). Adding N alone resulted in green leaf P concentrations that were not significantly different from the control. Higher green leaf P concentrations were correlated with reduced P resorption proficiency ($r_{62} = 0.52$, $p < 0.001$) and efficiency ($r_{62} = -0.26$, $p = 0.04$).

Trees were more proficient at P resorption in the control plots than in any of the fertilized plots (treatment interaction, $F_{1,26} = 9.91$, $p = 0.004$) (Figure 6). Litter P concentrations were about 2-3 times higher in the NP and P plots than in the controls. Litter P concentrations were 23% higher than the control when N was added alone. Red maple was less proficient in P resorption than beech or sugar maple, especially with P treatment (species x P interaction, $F_{2,26} = 4.19$, $p = 0.03$), and had the widest range in litter P concentrations.

The two forest stand age classes followed similar patterns in P resorption efficiency by treatment with the exception of the P plots, in which P resorption efficiency in the mature stands dropped more than 50% relative to the mid-aged stands (age x treatment interaction, $F_{1,24} = 6.79$, $p = 0.02$). Trees in the control plots were most efficient at P resorption (treatment interaction, $F_{1,26} = 3.88$, $p = 0.06$) with an average efficiency of 66%, but the three species responded differently to treatment (Figure 7). Resorption efficiency of sugar maple did not vary significantly by treatment, but beech P resorption efficiency declined in the P plots, and red maple exhibited reduced efficiency in both the P and NP plots (species x treatment interaction, $F_{2,24} = 4.77$, $p = 0.02$). Red maple was less efficient than beech and sugar maple in all treatments ($F_{2,24} = 25.72$, $p < 0.001$). Resorption efficiency of N and P were highly correlated ($r_{62} = 0.80$, $p = 0.001$).

Comparing the two nutrients, resorption proficiency of N and P were highly correlated ($r_{62} = 0.58$, $p < 0.001$), as were P proficiency and N efficiency ($r_{62} = -0.76$, $p < 0.001$). Moreover, green leaf N was correlated with increased P resorption efficiency ($r_{62} = 0.55$, $p < 0.001$) and proficiency ($r_{62} = 0.51$, $p < 0.001$), while litter N concentrations were negatively correlated with P resorption efficiency ($r_{62} = -0.62$, $p < 0.001$). Finally, it was striking that, overall, the variability and the magnitude of responses were much greater for P than N. For example, P concentrations in senesced foliage were more highly variable (CV = 63%) than N concentrations (CV = 18%), and the response to nutrient additions in foliar concentrations was much higher for P than for N; in the P plots, green and senesced leaf P concentrations were 31 and 161% higher than those in the control, whereas for N concentrations in the N plots, the respective values were 13 and 15%.

N:P ratios. For all stands together, the foliar N:P ratio in the control plots averaged 20.0±0.5, and the green leaf N:P ratio was similar in the mid (19.0±0.8) and mature (18.1±0.9) stands. Not surprisingly, foliar N:P went up 13% when N was added (main effect, $F_{1,39} = 18.71$, $p < 0.001$), and declined 32% when P was added (main effect, $F_{1,39} = 92.14$, $p < 0.001$; Figure 5). Adding P, alone or with N, caused a decline in foliar N:P below the control. All three species displayed similar patterns in fresh leaf N:P ratios (Figure 6). Among species, beech had the highest and red maple the lowest N:P in each treatment ($F_{2,39} = 3.30$, $p = 0.05$). Both N ($r_{62} = 0.47$, $p < 0.001$) and P ($r_{62} = 0.51$, $p < 0.001$) resorption efficiency were higher with higher leaf N:P ratios. The patterns by species and treatment in leaf litter N:P ratios were similar to those described in the green leaves, but litter N:P ratios spanned a wider range of values than did green leaf ratios.

The resorption efficiency ratio of N:P was highest in the P plots and lowest in the control plots (treatment interaction, $F_{1,23} = 4.60$, $p = 0.04$) (Figure 7). Mid-aged and mature stands had very similar N:P resorption ratios in all but the P treatment (age x treatment interaction, $F_{1,23} = 6.26$, $p = 0.02$). With a resorption ratio range of 0.78-0.81 (i.e., proportionally higher P than N resorption), species did not differ in the control plots. The biggest range among species was in the P plots (species x treatment interaction, $F_{2,23} = 4.29$, $p = 0.03$). The pre- and post-treatment ratios of N:P resorption efficiency were correlated ($r_{62} = 0.31$, $p = 0.03$), but a paired t-test showed that pretreatment ratios were lower ($p = 0.07$).

Discussion

Previous work in these stands prior to treatment indicated that increased N availability was associated with increased P conservation, and data collected in the youngest stands in these sites suggested that N and P availability influenced the foliar dynamics of P and N, respectively. However, I found little evidence for multiple element control over foliar nutrient concentrations or resorption in these stands post-treatment. In particular, green and senesced leaf N and P were higher with additions of the respective nutrient, but there was little effect of addition of a nutrient on resorption efficiency of that nutrient. These results of the present study are in line with many previous resorption studies, but also indicate that the links between soil or plant nutrient status and resorption are more complex than previously thought. For example, the degree and duration of nutrient limitation may play important roles in determining the response of foliar nutrient dynamics to changes in availability. Stand age also may be important to consider, as sugar maple generally behaved more similarly to beech (shade-tolerant co-dominants) than to red maple (congeners), possibly reflecting successional effects on resorption.

Though there was scant evidence supporting hypotheses of co-limitation by N and P, there were several indications of P limitation in the northern hardwood forest in the present study sites. First, across the larger study of 13 forest stands, relative growth rates of trees increased significantly in the P plots but not in the N plots (Goswami et al., submitted). Second, N:P ratios in the control plots and in the pre-treatment period were mostly in the P-limited range (Koerselman and Meuleman 1996). Third, the data presented here indicate that as green leaf N:P ratios increased, P resorption proficiency also increased with no correlation with N proficiency. Fourth, P resorption efficiency was higher than N resorption efficiency (Kozovits et al. 2007); trees in the control plots had a mean N resorption efficiency near the global mean of 50% reported by Aerts (1996), and a mean P resorption efficiency more similar to the global mean of 65% reported by Vergutz et al. (2012).

After four years of treatment, the P and NP plots had average foliar N:P ratios lower than the control, but only the P plots reached the narrow range of co-limitation defined by Koerselman and Meuleman (1996). The mean foliar N:P ratio in the control plots was 5% higher post-treatment than pre-treatment. This may be attributable to inter-annual variation in green leaf nutrient concentrations (May and Killingbeck 1992) or to increases in foliar N over time (Yang et al. 2015), possibly stemming from sustained anthropogenic atmospheric N deposition, as has been found in both hardwood (Duquesnay et al. 2000) and conifer (Mohren et al. 1986) forest stands in Europe. It has been suggested that N:P ratios are driven more by availability of P than of N (Townsend et al. 2007) and that foliar N concentrations may be more constrained by foliar P than the inverse (Vitousek 1998). In the present study, green leaf N:P ratios were only slightly more strongly correlated with green leaf P than N concentrations. The foliar N:P ratio in the NP plots was more similar to the N:P ratio with P alone than with N alone; additionally, the average N:P ratio in the P and NP plots was lower than that in the control plots, illustrating the importance of P in the regulation of biogeochemical cycles (Ostertag 2010; Peñuelas et al. 2012).

Han et al. (2011) hypothesized that, across ecosystem and plant functional types, the nutrients most often limiting to plants and needed in the highest concentrations will be the least variable ones. I found that foliar P concentrations and resorption were more variable than for N, despite the fact that, by several measures, these forests appeared to be primarily P limited. Nitrogen was less variable in the present study than in the aforementioned study, but P variability was similar (Han et al. 2011). As in other studies (Vitousek 1998; Güsewell and Koerselman 2002; Ostertag 2010; Hayes et al. 2014), foliar P concentrations were more variable than foliar N concentrations, and the increase in P concentrations in both green and senesced leaves following P fertilization was greater than for N subsequent to N fertilization. The patchy distribution (Brady 1990) and generally low availability of P in soil may exert pressure on plants to develop efficient mechanisms for P uptake and accumulation (Chapin et al. 1990; Lambers et al. 2008; Ostertag 2010), possibly leading to an inability to downregulate P uptake when availability is high (Lambers and Shane 2007). This would agree with low P resorption at very high P availability, as was found in my study.

Since fertilization with N or P was previously shown to increase soil availability of N and P, respectively, in these sites (Fisk et al. 2014), and foliar N and P concentrations generally reflect soil N and P availability, I hypothesized that addition of a nutrient would cause an increase in both green and senesced leaf concentrations of that nutrient. As expected, and in agreement with others (Chapin and Moilanen 1991; del Arco et al. 1991; Austin and Vitousek 1998; Vitousek 1998; Harrington et al. 2001; Rentería et al. 2007; Ostertag 2010; Lu et al 2013; Yuan and Chen 2015), N or P additions resulted in an increase in foliar concentrations of these respective nutrients.

I hypothesized that nutrient resorption efficiency would decline in response to nutrient addition, and the response of P resorption to P addition supported this hypothesis. However, N resorption efficiency did not decrease following the addition of N. This is inconsistent with previous studies that reported reduced N use efficiency with increasing N supply (Kost and Boerner 1985; Aerts and de Caluwe 1994; Vásquez de Aldana and Berendse 1997). Both green leaf and litter N concentrations increased with N additions so that the proportion of N resorbed did not differ significantly by treatment or by species among treatments. Higher P resorption efficiency in the control than in the P addition plots is in agreement with the finding that nutrient use efficiency is enhanced on infertile sites (Boerner 1984; Kost and Boerner 1985; Birk and Vitousek 1986; Aerts 1995). Resorption is an energetically expensive process (Buchanan-Wollaston 1997), but is likely a function of favorable cost/benefit relationships in nutrient-poor sites where the cost of nutrient uptake from the soil exceeds the cost of retranslocating nutrients for reuse (Chapin and Kedrowski 1983). Similarly, reductions in P resorption efficiency with fertilization suggest that increased nutrient availability decreases dependence on internal nutrient conservation (Lu et al. 2013).

The strong correlations between litter N and P concentrations and between N and P resorption efficiency reinforce the idea of a tight coupling between the cycles of these two nutrients (Güsewell 2004). Based on this stoichiometric coupling of N and P, I hypothesized that adding N would also stimulate an increase in foliar P concentrations, and vice versa, as others have observed in fertilization experiments (Bowman 1994; Güsewell et al. 2003; Niinemets and Kull 2005; Lu et al. 2013), natural nutrient gradients (Jones and Power 2012), and models (Perring et al. 2008). In contrast, in the present study, foliar N concentrations were actually slightly lower than the control in the P plots. This result may be explained in part by a dilution effect caused by increased leaf mass (see Vitousek 1998) in the P plots. That is, expressed on a per leaf basis, green leaf N content was nearly 20% higher than the control in the P plots, lending support for this explanation. Phosphorus concentrations, however, were only slightly higher than the control in the N plots, whether expressed as concentration or on a per leaf basis. Increased N availability following N fertilization may induce P limitation (Perring et al. 2008), manifested by low tissue P concentrations (Menge and Field 2007). Thus, the hypothesis that green leaf N and P would increase with additions of P and N, respectively, was supported for N, but not for P.

Expecting a synergistic effect of adding both N and P on acquisition, uptake, and use efficiency of both nutrients, I hypothesized that N and P concentrations would be highest in the green leaves and lowest in the leaf litter in the combined NP plots. This was not the case for green leaves or litter. Because there were no significant differences in leaf mass or area between the NP and control plots, this result is not explained by a dilution effect. Following patterns in the green leaves, litter concentrations of N and P were higher with the respective nutrient alone than with both combined, agreeing with a recent global analysis (Yuan and Chen 2015). Furthermore, additions of N or P alone resulted in higher litter concentrations of P or N, respectively, compared to the control. While this result is in agreement with previous studies in marshes (Shaver and Melillo 1984), subarctic bogs (van Heerwaarden et al. 2003b), temperate forest (Hagen-Thorn et al. 2006), alpine tundra (Soudzilovskaia et al. 2007), and grassland (Lu et al. 2013), it is contrary to my initial hypothesis that higher availability of one nutrient would result in increased resorption proficiency, and thus, conservation, of the other nutrient (See et al. 2015). This result of accelerated ecosystem cycling of P could represent an external mechanism that delays P limitation induced by N inputs (Lu et al. 2013).

I also hypothesized that resorption efficiency of one nutrient would be correlated with green leaf concentrations of the other nutrient. This hypothesis follows from previous work in these stands (See et al. 2015), a California grassland (Menge and Field 2007), and in models (Perring et al. 2008) which demonstrated an association between increased soil N availability and enhanced P conservation, and thus suggested multiple element control over resorption. In the present study, neither N nor P resorption efficiency were significantly different from the control in the N plots (Figure 7), so it is possible that the need to maintain a stoichiometric balance between N and P resorption precluded the possibility of observing a treatment effect of N additions on P resorption efficiency (Lu et al. 2013).

It is also possible that some trees were already achieving maximum, or complete (Killingbeck 1996), P resorption, and higher efficiency was not possible. Litter N concentrations were generally higher than reported values for the sampled species, but P concentrations were similar to or lower than the ranges reported by others (Grizzard et al. 1976; Boerner 1984; Berg and McClaugherty 1989; Aerts 1996; Moorhead and McArthur 1996; Finzi et al. 2001; King et al. 2001; Côté et al. 2002). Thus, no species in any treatment appeared to achieve complete resorption of N, but sugar maple reached intermediate resorption of N in the control and P plots (Killingbeck 1996). Phosphorus resorption was intermediate or complete for beech and sugar maple in all treatments, but intermediate in the control and N plots and incomplete in the P and NP treatments for red maple.

My observations about incomplete resorption may be related to the timing of litter sampling and alterations to the phenology of senescence induced by elevated nutrient availability: one possible source of error in this and many other resorption studies is the timing and brief interval of single sampling periods. To avoid excessive leaching of nutrients from tissues, leaf litter was collected over a relatively short interval near the time of peak litterfall. Nutrient concentrations in leaf litter change during the litterfall period, typically declining early and then stabilizing during mid and late stages (C. See, in prep.). I deliberately timed the litter collection in the present study in the middle period of litterfall when nutrient concentrations tend to stabilize in these forests to minimize this source of error. However, if the phenology of litterfall was altered by nutrient addition, then the timing of litter collections may confound comparisons between treatments. In particular, it was observed at the time of leaf litter collection that trees in the fertilized plots appeared to have more leaves remaining than trees in the control plots It is possible higher nutrient availability was coupled with longer leaf retention prior to abscission, such that the relative cost of holding leaves longer to maximize photosynthesis, even if those leaves were lost due to wind or frost prior to complete resorption, was more beneficial than realizing higher resorption earlier in the season. Results could thus be confounded by single date sampling if leaves of higher nutrient status trees senesced later and have not yet completed resorption; conversely, the earlier senescing leaves of trees on control plots may have been subject to more leaching losses (Chapin and Moilanen 1991). Thus, these potential confounding effects of the sampling design limit the strength of conclusions about resorption.

A striking and unexpected result in the present study was the contrasting response of green leaf size and mass to separate N and P additions compared to both nutrients added together; green leaves were larger and heavier with N or P additions separately (Figure 1), but leaf size and mass were not significantly different from the control when both nutrients were added together. Increases in leaf area have also been observed in studies of fertilization with N alone in conifers (Binkley and Reid 1984) and hardwoods (Heilman and Fu-Guang 1994). It was surprising that the largest increases in area or mass didn't occur in the NP plots, as has been found in other N x P factorial-design studies; Feller (1995) observed leaf area increases in mangroves fertilized with P or NPK, but not N alone, while Chang (2003) found that leaf area increased in sweetgum seedlings fertilized with N or NP, but not P alone. These single nutrient responses indicate single element limitation in these studies. In the present study, the separate but convergent responses of leaf size and mass to N or P additions, as well as some of the foliar N:P ratios (Figure 5), suggest co-limitation, so the addition of nitrogen and phosphorus together might have been expected to produce a synergistic biomass response (Elser et al. 2007; Harpole et al. 2011), yet leaf mass and area in the NP plots were not significantly different from the control plots. If other plant processes besides growth, such as reproduction, were co-limited by N and P, the most efficient use of increased availability of both nutrients would be to allocate these resources to those other processes if investment will yield greater returns than would increased investment in growth (Chapin et al. 1987). For example, I did not examine growth and reproduction concurrently, so it is possible that nutrients were allocated to reproduction rather than growth in the combined NP plots. The fact that area and mass were similar in the NP and control plots could indicate balanced nutrition in the NP plots, which in our case was at an N:P addition ratio of 3:1. Furthermore, the N:P resorption efficiency ratio was closest to 1 in these plots, suggesting balanced nutrient availability relative to plant demand (Reed et al. 2012). As noted previously, several lines of evidence indicate P-limitation in the study sites, so increased biomass and SLA in the P plots may be a result of adding this limiting nutrient (Niinemets and Kull 2003). In the N plots, the observed increases in leaf area and mass could be a compensatory response to the nutrient imbalance imposed by surplus N (Chapin 1980). Finally, the finding that LDMC decreased with N fertilization is in direct contrast to at least one previous study (Feller 1995), but indicates that the larger, thinner leaves with higher N concentrations subsequent to N additions will result in green leaves that are more palatable to herbivores, which has important implications for forest health in this era of high N deposition (Li et al. 2016).

Conclusions

In these forests where P appears to be the most limiting mineral nutrient, it seems that P conservation through resorption is more closely related to soil and leaf nutrient status than is N conservation. This result may also be related to stronger selective pressure to acquire and conserve P whenever it is available. Litter P concentrations are more variable and seem to more closely track soil and leaf P concentrations than N concentrations (Vitousek 1998; Cárdenas and Campo 2007; Rentería et al. 2007; Townsend et al. 2007). The results in the present study suggest that the nutrient status-resorption debate should not be considered settled. However, the length of the sampling period and the number of sampling intervals should be considered for a better representation of complete and incomplete resorption in future studies, particularly in nutrient manipulation studies.

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Chapter 3: Effects of calcium addition on foliar nutrient concentrations and resorption in three co-dominant hardwood species in the White Mountains, NH.

Abstract

Calcium (Ca) depletion resulting from acidic precipitation, aging forest stands, and timber harvesting is purported to be associated with reductions in forest health and productivity. In 2011, six forest stands of two age classes in the White Mountains, NH, received a calcium amendment of 1150 kg Ca ha^{-1} in the form of wollastonite. Green and senesced leaves of American beech, red maple, and sugar maple were collected in August and October, 2015, respectively, and analyzed for concentrations and leaf content of ten elements to determine the effect of calcium fertilization on foliar physical and chemical characteristics. Leaf mass, area, thickness, and toughness were enhanced by the addition of calcium. Trees in the calcium-treated plots had leaves with higher calcium content, but other effects of Ca addition on foliar nutrition were scarce. There were few differences between the two age classes, suggesting that any responses to calcium addition or depletion in these forests are systemic. Foliar nutrient concentrations were generally in healthy ranges for sugar maple, even though this species is thought to be particularly adversely affected by calcium depletion.

Introduction

Acidic deposition fueled by a century of industrial development has left behind a legacy of ecosystem decline in the northern hardwood forests of the northeastern United States. The resultant soil acidification, aluminum (Al) mobilization, and loss of soil nutrient cations have degraded growing conditions for vegetation and reduced the resiliency of forest stands (Driscoll et al. 2003). In particular, these effects have been causally linked to the widespread decline of several tree species, including sugar maple, an economic and ecological keystone species in these forests (Bal et al. 2015).

In the early 1970s, reports began emerging of high acidity in precipitation at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire (Likens et al. 1972). Since then, long-term sampling efforts there have demonstrated a decline in soil available calcium (Ca) and little recovery in surface water acid neutralizing capacity, likely due to continued effects of acidic deposition and reductions in cations in precipitation (Likens et al. 1996). Decades later, above-ground net primary production at Hubbard Brook was 14% lower than predicted by a simulation model, potentially indicating the effects of an ongoing, presumably Ca-related, stress (Fahey et al. 2005).

Forest management practices and stand age are other possible drivers of Ca depletion. Biomass removal, particularly whole-tree and multiple rotation timber harvesting, can accelerate the onset of Ca deficiency by removing large amounts of the forest Ca capital residing in woody plant biomass (Mann et al. 1988; Johnson et al. 1989; Perakis et al. 2013). In addition, streamwater export of Ca from the soil can remain elevated for several years post-harvest (Mann et a. 1988; Johnson et al. 1991). The Ca added to the system by mineral weathering and atmospheric deposition may be insufficient to meet forest demands on the relatively short timescale of forest regeneration (Huntington et al. 2000; Yanai et al. 2005; Perakis et al. 2006). The observed Ca depletion in many forests may also be partly a natural aspect of compositional shifts and successional change in biogeochemical cycling (Hamburg et al. 2003).

Calcium depletion, whether a result of acidic deposition, reductions in cation deposition, biomass removal, or vegetation uptake, has also been reported in forest ecosystems in the southeastern United States (Huntington et al. 2000), Europe (Kirchner and Lydersen 1995), and Asia (Liu et al. 2011), with far-reaching effects across the individual, community, and ecosystem scales. Calcium influences a diverse array of ecosystem processes and characteristics, from mycorrhizal colonization (Juice et al. 2006) to snail abundance (Hamburg et al. 2003). Within the tree, Ca functions in cell structure, enzymatic processes, and signaling pathways (Hirschi 2004). Calcium imparts structure and strength to plant cell walls, and deficiency can result in cell wall disintegration and tissue collapse (Hirschi 2004; Littke and Zabowski 2007). Reduced leaf area has been observed in calcium-deficient plants, while Ca addition experiments have demonstrated increased leaf area three or more years post-treatment (Yeh et al. 2000; Juice et al. 2006; Marlow and Peart 2014). Greenhouse studies have noted a link between foliar lime application and leaf thickness and greenness (Harrison 1898). Others have associated crown dieback in sugar maple (Long et al. 1997) or decreased crown density in red spruce (Shortle and Smith 1988) with low Ca concentrations. The physiological response to several of the inciting factors related to sugar maple decline, including defoliation and drought, are initiated by Ca through its signaling role (Schaberg et al. 2001).

Calcium depletion is not the only possible effect of acid precipitation. When strong acid anions, such as nitrate and sulfate, are deposited in forest ecosystems, hydrogen ions displace base cations (Ca, potassium (K), magnesium (Mg), and sodium (Na)) from soil exchange sites, causing them to be coupled with acid anions and leached from the system. As pH declines, cations like Al and manganese (Mn) mobilize in the soil solution and occupy cation exchange sites. These elements, in certain forms, can have deleterious effects on plants, either through direct toxicity to the roots or by indirectly interfering with nutrient uptake (Van Diest and Schuffelen 1967; Shortle and Smith 1988; Cronan and Grigal 1995). High levels of Al and Mn in the soil solution can reduce photosynthetic activity, constrain root growth, and inhibit cation uptake in sugar maple, ultimately resulting in increased mortality (Cronan and Grigal 1995; McQuattie and Schier 2000; Kobe et al. 2002; St. Clair and Lynch 2004).

In acidic soils, phosphorus (P) is likely to be immobilized as aluminum phosphate, potentially reducing plant-available P in the soil and inducing P limitation (Pare and Bernier 1989). With continued acidification, labile P pools in the mineral soil are depleted (Bal et al. 2015). Additionally, mycorrhizal abundance decreases, which can further reduce P uptake (SanClements et al. 2010; St. Clair and Lynch 2005). Calcium depletion has also been linked to reductions in soil P availability (Fiorentino et al. 2003). Reductions in cation availability and pH, as well as the concomitant increases in Al and Mn mobility, have been particularly linked to declines in sugar maple, a species that grows best in relatively rich soils and pH conditions between 5.5-7.3 (Godman 1957; Kolb and McCormick 1993; Horsley et al. 2000; Hallett et al. 2006; Bal et al. 2015).

In 2011 in the White Mountains of central New Hampshire, Ca was added as wollastonite ($CaSiO_3$) to one treatment plot in each of six hardwood dominated forest stands, with the long-term goal of restoring soil Ca that had been depleted by acidic deposition. To determine if Ca amendment had changed foliar physical and chemical characteristics, and to distinguish between effects of species and stand age, I collected fresh and senesced leaves from three dominant northern hardwood forest tree species across two age classes and quantified foliar concentrations, content, and resorption of ten elements. Given the structural role of Ca and the mechanisms trees have for maintaining low internal concentrations of labile Ca, I hypothesized that the leaves from trees in Ca plots would be heavier, thicker, and tougher, as evidenced by leaf mass, specific leaf area, and leaf dry matter content, due to increased Ca concentrations in the foliage. I also thought trees in the Ca-treated plots would exhibit less dieback and defoliation. I hypothesized that Ca-treated trees would have lower foliar concentrations of K and Mg as a consequence of the added Ca displacing other cations in the soil. Alternatively, base cation concentrations might be higher if Ca facilitated the uptake of these nutrients. I further predicted that foliage from Ca-treated trees would have lower concentrations of Al and Mn, higher concentrations of P, and lower N:P ratios. Finally, I expected significant interactive effects of treatment and stand age, as well as between treatment and tree species, with stronger responses to treatment in the older stands and in sugar maple due to the effects of calcium depletion observed on this species in particular.

Methods

Site Characteristics

Sampling took place in six stands in Bartlett Experimental Forest (BEF), Hubbard Brook Experimental Forest (HBEF), and Jeffers Brook (JB), all located in the White Mountains of New Hampshire (Figure 1). Three stands were mature, dominated by American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), and yellow birch (*Betula alleghaniensis* Britt.), and three stands were successional, primarily composed of a mix of beech, pin cherry (*Prunus pennsylvanica* L.f.), sugar maple, red maple (*A. rubrum* L.), white birch (*B. papyrifera* Marsh.), and yellow birch (Table 1). The understory was denser in the younger stands, and included striped maple (*A. pennsylvanicum* L.), conifers such as eastern hemlock (*Tsuga canadensis* L.) and balsam fir (*Abies balsamea* L.), and various shrubs such as Canadian yew (*Taxus canadensis* Marsh.) and hobblebush (*Viburnum lantanoides* Michx.), in addition to the species comprising the overstory. The beech sprouts characteristic of the aftermath phase of beech bark disease were also present.

Soils are Spodosols developed in glacial drift. Temperatures at HBEF range from an average January low of -9°C to a mean high of 18°C in July (Adams et al. 2003). The climate is humid continental, and annual precipitation averages 127-140 cm (Adams et al. 2003).

Each of the three studied stands in BEF and the stand at HB has two 50x50 m plots: one untreated control and one Ca treatment plot. At JB, the plots are 30x30 m. Each stand has three additional plots in a nitrogen (N) and P fertilization factorial that are part of a long-term study on multiple element limitation (see Ch. 2; Fisk et al. 2014). The Ca plots received 1150 kg Ca ha^{-1} as $CaSiO_3$ in 2011. This Ca application rate was calculated to be the amount thought to be lost due to acid rain with an additional 30% margin of error.

Field Methods

I collected green leaves and leaf litter of American beech in all six stands, red maple in two of the successional stands (C1 and C6), and sugar maple in the other successional stand and the three mature stands (C8, HBCa, JM, and JO). I was unable to collect red maple from the third mid-aged stand, JM, due to this site's species composition (Table 1). Sun-exposed green leaves were collected August 9-15, 2015, with a shotgun from the mid-canopy of three individuals of each desired species; collected leaves were kept frozen until subsequent analysis. Sampled trees were dominant or intermediate in the canopy with >10 cm dbh.

I collected recently fallen leaf litter of each species from the ground of each plot following a rain-free period on the weekend of October 10-11, 2015. All sampling was conducted in central sub-plots to avoid edge effects.

Laboratory Analysis

All green and senesced leaves were dried at 60°C to constant mass. Before drying, leaves were weighed and photographed for estimation of leaf surface area with ImageJ software (Schneider et al. 2012). After drying, leaves were weighed again and then ground in a Wiley mill to pass through a 40 mm mesh screen. I calculated specific leaf area (SLA) as the ratio of leaf surface area to leaf dry mass, and leaf dry matter content (LDMC) as the ratio of dry mass to fresh mass.

For each sample, one quarter-gram subsample was ashed at 470°C and hot plate-digested with 6 N nitric acid. The subsequent solutions were analyzed for Al, Ca, K, Mg, Mn, P, strontium (Sr), and sulfur (S) with inductively coupled plasma optical emission spectrometry (Optima 5300 DV ICP-OES, Perkin-Elmer) using five-point linear calibration curves for each element. I included one replicate, one blank, and two standards (apple leaves, NIST 1515) with every round of ashing (Appendix 1). During ICP analysis, I analyzed a lab standard solution before and after every five samples for quality control. Analyses only proceeded if the QC returned +/-5% recovery; otherwise, the instrument was recalibrated. Carbon (C) and N concentrations were determined through complete combustion of 2-3 mg subsamples (FlashEA 1112 analyzer, Thermo Scientific).

Data Analysis

Since litter of each species was collected across each whole plot, I averaged individual tree values to obtain a plot-level estimate for green leaves, and considered the plot to be the experimental unit. The experimental design was a randomized complete block design, with stand as the blocking factor, nested within age (Table 2).

A univariate analysis of variance (ANOVA) was conducted for each dependent variable, including leaf physical characteristics, green and senesced leaf element concentrations and content, and resorption efficiency. Proficiency is reported as the concentration to which a nutrient has been reduced in the leaf litter. Efficiency was calculated as:

$$\frac{N_{gr} - N_{se}}{N_{se}} * 100$$

where N_{gr} and N_{se} are the nutrient concentrations (by mass or area) in the green and senesced leaves, respectively. Least squares means were compared using Tukey's honestly significant difference tests. All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, NC).

Results

Leaf Physical Characteristics

Leaves were 13% larger ($F_{1,12} = 4.36$, $p = 0.10$) and heavier ($F_{1,12} = 5.92$, $p = 0.07$) in the younger stands than mature stands. However, the ratio of area to mass (SLA) remained consistent among age classes ($F_{1,12} = 0.04$, $p = 0.86$), evidenced by the lack of variation by age in SLA, which ranged from 13.2±0.85 $cm^2 g^{-1}$ (LSMean±SE) in the younger stands to 13.5±1.08 $cm^2 g^{-1}$ in the mature stands. There was an increase in SLA in Ca plots in the mature stands, but not in the younger stands (age x treatment interaction, $F_{1,12} = 3.48$, $p = 0.09$) (Table 3). Green leaves were also 16% larger ($F_{1,12} = 5.76$, $p = 0.03$) and 22% heavier ($F_{1,12} = 4.37$, $p = 0.06$) with Ca treatment compared to the control. However, this pattern was not uniform across species; the mass and surface area of sugar maple leaves were not significantly affected by treatment (species x treatment interaction), but with Ca addition, surface area increased in beech and red maple leaves ($F_{2,12} = 5.71$, $p = 0.02$). In red maple, leaf mass also increased with Ca addition, though not significantly ($F_{2,12} = 2.08$, $p = 0.17$; Figure 2). The increase in beech surface area with Ca addition resulted in a significantly higher SLA compared to the control plots ($F_{2,12} = 8.65$, $p = 0.005$) (Figure 2). Between the Ca and control treatments, the average SLA of the two maple species only varied by 1-3 $cm^2 g^{-1}$, while beech SLA increased by 34% from the control to the Ca treatments (Figure 2). Leaf dry matter content was higher in the Ca-treated plots ($F_{1,12} = 3.30$, $p = 0.09$) (Table 3); beech had the highest LDMC among the three species ($F_{2,12} = 8.74$, $p = 0.005$) (Figure 3).

Metal Cations

Foliar Al concentrations and levels of accretion (i.e., accumulation in senescing leaves rather than resorption) were highest in beech ($F_{2,12} > 3.09$, $p < 0.08$), but surprisingly, there were no significant effects of treatment on foliar Al concentrations or content (Figure 5, $F_{1,12} < 1.25$, $p > 0.37$). Aluminum resorption efficiency was near zero in both treatments in the younger stands (Table 4). In the older stands, it increased from -15% in the control plots to 10% in the Ca-treated plots (age x treatment interaction, $F_{1,12} = 1.75$, $p = 0.21$). This difference may be partly attributable to treatment and age effects on leaf size and mass, because on a content basis, there were no significant effects of age or treatment on Al resorption efficiency ($F_{1,12} < 0.67$, $p > 0.43$).

Sugar maple had higher green and senesced leaf Sr concentrations than beech or red maple (Figure 5, $F_{2,12} > 2.89$, $p < 0.09$). Averaged by species, Sr resorption efficiency decreased below zero in the young stands and increased above zero in the old stands when Ca was added (Table 4; $F_{1,12} = 4.14$, $p = 0.06$). This effect of treatment and age is due to differences in green leaves ($F_{1,12} = 2.70$, $p = 0.13$) rather than litter ($F_{1,12} = 0.54$, $p = 0.48$); green leaf Sr concentrations were higher than the control with Ca addition in the mature stands, but lower than the control in the younger stands (Table 4). However, there was no effect of treatment, age, or species on Sr content in green or senesced leaves (Table 4; Figure 5).

When Ca was added, the older stands had 27% more and the young stands had 23% less Mn in the green foliage compared to the control (Table 4; $F_{1,12} = 6.10$, $p = 0.03$). Litter Mn did not differ by treatment in the mature stands; in the successional stands, litter Mn concentrations were 55% lower than the control when Ca was added (Table 4; $F_{1,12} = 2.56$, $p = 0.12$). Higher litter concentrations in the control plots also meant that these trees accreted nearly twice as much Mn as trees in the Ca-treated plots (Table 4; $F_{1,12} = 4.44$, $p = 0.06$). Accretion of Mn in the control plots was higher on a content basis, as well ($F_{1,12} = 8.56$, $p = 0.01$). Foliar Mn concentrations did not vary significantly among the three sampled species (Figure 5).

Base Cations

Green leaf Ca concentrations were higher in the Ca plots compared to the control plots, but only slightly and not significantly (Figure 6; $F_{1,12} = 1.36$, $p = 0.27$). However, there was an interactive effect of age and treatment: trees in the mature stands had elevated foliar Ca in the Ca plots, but trees in the younger stands had similar Ca concentrations in both treatments (Table 4; $F_{1,12} = 2.40$, $p = 0.15$). This interaction between treatment and age was not present on a content basis, though green leaf Ca content was higher in the Ca plots compared to the controls ($F_{1,12} = 2.37$, $p = 0.15$) and in the younger stands compared to the mature stands ($F_{1,12} = 10.84$, $p = 0.03$). While Ca concentrations in the Ca plots were only 8% higher than those in the control plots, Ca content per leaf in the Ca plots was 26% higher than in the control plots, pointing to a dilution effect in the Ca plots. Sugar maple had the highest green leaf Ca concentrations and content in both treatments (Figure 6; $F_{2,12} > 4.82$, $p < 0.03$). There was an interaction between treatment and age affecting Ca resorption efficiency as calculated from foliar Ca concentrations ($F_{1,12} = 2.95$, $p = 0.11$): the young stands accumulated nearly six times more Ca in the litter than was present in the green leaves in the Ca plots, while the old stands exhibited more Ca accumulation in the control plots (Table 4). When calculated from leaf Ca content, there was a significant interaction between species and treatment ($F_{2,12} = 2.87$, $p = 0.10$).

Calcium additions resulted in slightly lower foliar K concentrations in both green ($F_{1,12} = 2.46$, $p = 0.14$) and senesced ($F_{1,12} = 3.32$, $p = 0.09$) leaves, but did not affect resorption efficiency ($F_{1,12} = 0.97$, $p = 0.34$) (Figure 6). On a content basis, K resorption efficiency was higher in the Ca plots ($F_{1,12} = 2.83$, $p = 0.12$), especially for red maple (species x treatment interaction, $F_{2,12} = 4.15$, $p = 0.04$).

Beech had the highest green leaf Mg concentrations (main effect, $F_{2,12} = 9.75$, $p = 0.003$), and there was little effect of Ca treatment on this species (Figure 6; species x treatment interaction, $F_{2,12} = 3.50$, $p = 0.06$). Green leaf Mg concentrations were lowest in sugar maple and dropped 25% below the control when Ca was added (Figure 6). Sugar maple also had the lowest litter Mg concentrations ($F_{2,12} = 3.92$, $p = 0.05$). In red maple, green leaf Mg concentrations and content were higher in the Ca plots compared to the control plots (species x treatment interaction, $F_{2,12} > 3.50$, $p < 0.07$). Calcium-treated trees in the older stands had higher green leaf Mg concentrations ($F_{1,12} = 2.80$, $p = 0.12$) and resorbed more Mg (Table 4; $F_{1,12} =.4.41$, $p = 0.06$). On a content basis, Mg resorption efficiency was not affected by age, but was more than two times higher in the Ca plots than in the control plots ($F_{1,12} = 3.98$, $p = 0.07$).

Other Nutrients

There were higher green leaf S concentrations in the control plots ($F_{1,12} = 3.12$, $p = 0.10$), but I found no effects of treatment on litter S ($F_{1,12} < 0.62$, $p > 0.44$) or S resorption efficiency ($F_{1,12} < 0.31$, $p > 0.59$) on the basis of concentration or content (Figure 7). Sugar maple had the highest green leaf S concentrations of the three species ($F_{2,12} = 4.00$, $p = 0.05$).

Trees receiving Ca additions in the older stands had marginally significantly higher green leaf P concentrations (age x treatment interaction, $F_{1,12} = 1.68$, $p = 0.19$), but also were less proficient ($F_{1,12} = 3.93$, $p = 0.07$) and efficient ($F_{1,12} = 3.79$, $p = 0.08$) at P resorption (Table 4). Calcium-treated trees in the older stands were also less efficient at P resorption when efficiency was calculated from foliar P content ($F_{1,12} = 3.12$, $p = 0.10$). Red maple was the least efficient ($F_{2,12} = 7.13$, $p = 0.009$) and proficient ($F_{2,12} = 5.21$, $p = 0.02$) species in terms of both concentrations and content (Figure 7). On a content basis, green leaf P concentrations were 29% higher than the control in the Ca plots ($F_{1,12} = 9.70$, $p = 0.009$), and this result was particularly driven by red maple (species x treatment interaction, $F_{2,12} = 3.62$, $p = 0.06$).

Green leaf N was lower in the older stands when Ca was added (Table 4; age x treatment interaction, $F_{1,12} = 3.46$, $p = 0.09$). Among species, red maple was less efficient ($F_{2,12} = 20.69$, $p < 0.001$) and proficient ($F_{2,12} = 11.49$, $p = 0.002$) at N resorption than beech or sugar maple (Figure 7). While adding Ca helped beech and red maple to be slightly more proficient at N resorption, sugar maple litter N concentrations increased when Ca was added (Figure 7; species x treatment interaction, $F_{2,12} = 2.85$, $p = 0.10$). Foliar N content was not affected by treatment, but there was a significant interactive effect of species and treatment on N resorption efficiency on a content basis ($F_{2,12} = 6.57$, $p = 0.01$).

Though the three species had similar green leaf C concentrations in the control plots (492-495 mg g^{-1}), beech C declined to 480 mg g^{-1} when Ca was added (Figure 7; species x treatment interaction, $F_{2,12} = 4.69$, $p = 0.03$). However, there was no interactive effect of Ca treatment and species on C resorption efficiency ($F_{2,12} = 1.30$, $p = 0.31$) or proficiency ($F_{2,12} = 0.32$, $p = 0.73$) (Figure 7). Higher C concentrations in the control plots may be related to the increases in mass and surface area in the Ca treatments, since C content was significantly higher in the Ca plots ($F_{1,12} = 4.14$, $p = 0.06$), especially in red maple (species x treatment interaction, $F_{2,12} = 2.41$, $p = 0.13$). Carbon resorption efficiency based on content was also higher in red maple in the Ca plots (species x treatment interaction, $F_{2,12} = 4.08$, $p = 0.04$).

Ratios

The N:P ratio averaged 20.3 in the control plots and 18.0 in the Ca plots, with the largest decrease in the old stands (Table 5; age x treatment interaction, $F_{1,12} = 6.88$, $p = 0.02$). Among the three species, the N:P ratio ranged from 15.2 in red maple to 22.0 in beech (species main effect, $F_{2,12} = 13.87$, $p < 0.001$). The Ca:Al and Ca:Mn ratios were 17 and 9% higher, respectively, in the Ca plots, but this difference was not significant (Table 5; Ca:Mn $F_{1,12} = 0.21$, $p = 0.7$; Ca:Al $F_{1,12} = 2.03$, $p = 0.2$). Sugar maple had the highest Ca:Al ratio in both treatments.

Discussion

The majority of the observed treatment effects were associated with leaf physical characteristics rather than foliar chemistry. As hypothesized, trees in the Ca plots tended to produce leaves that were larger, heavier, and tougher, as evidenced by higher surface area, leaf mass, and LDMC in the calcium-treated plots. To my knowledge, an increase in leaf mass or toughness in response to Ca addition has not been previously reported. Others have found an increase in leaf surface area for up to eight years following Ca addition (Juice et al. 2006; Marlow and Peart 2014). My hypothesis that these differences in leaf physical characteristics would be associated with higher foliar Ca levels was supported for Ca on a content basis, but not for concentration, suggesting a dilution effect. If a Ca deficiency was ameliorated by the Ca addition, trees may have been able to allocate more resources to aboveground biomass, such as foliage, resulting in larger, heavier leaves with higher Ca content, but Ca concentrations similar to leaves in the control plots. The relationship between aboveground growth and foliar Ca following Ca treatment is variable, with reports of increased foliar Ca concentrations (Long et al. 1997) or no significant difference in foliar Ca by treatment (Littke and Zabowski 2007). Juice et al. (2006) reported peak foliar Ca three years after whole watershed wollastonite addition, with Ca concentrations five years post-treatment similar to Ca levels recorded in both control and treated trees in the present study.

Calcium concentrations in the leaves of sugar maple, specifically, were only slightly higher in Ca-treated trees ($8.5 \text{ mg g}^{-1} \pm 0.71$) compared to trees in the control plots ($8.1 \text{ mg g}^{-1} \pm 1.21$), and were similar to Ca concentrations reported in sugar maple leaves on lower slopes in Pennsylvania (Horsley et al. 2002) and to those in sugar maples following nine years of liming in the unglaciated Susquenhannock State Forest (Long et al. 1997). Mean foliar Ca concentrations were below the optimum leaf Ca concentrations for sugar maple (Côté et al. 1993), but within the putative healthy range (Kolb and McCormick 1993). In the present study, the highest foliar Ca values in sugar maple were observed at Jeffers Brook, possibly owing to this site's moist, nutrient-rich soil to which sugar maple is well-suited. The lowest foliar Ca concentrations were measured at Hubbard Brook, and were actually below the range found in pretreatment and reference watersheds (5.7-6.9 mg g^{-1}) in the same study region in the White Mountains (Juice et al. 2006). Both soil Ca (Johnson and Hamburg 2013) and streamwater export of Ca (Likens 2014) have declined in the Hubbard Brook reference watershed over the last fifteen years, which lends support to the idea that Ca declines with forest age due to biomass uptake and may be a reason for the lower foliar Ca concentrations there. At the same time, Ca concentrations at the plot level did not differ by stand age, as might be expected if a Ca deficiency were intensified in the older stands. However, compared to control plots, older trees in Ca plots accreted less Ca and had higher concentrations of Sr, an element believed to substitute for and be taken up similarly to Ca (Blum et al. 2008).

Of the three species examined, leaf surface area was higher in both red maple and beech with Ca addition, but only beech exhibited increased SLA in response to Ca additions. Specific leaf area tends to correlate positively with rates of photosynthesis and relative growth (Perez-Harguindeguy et al. 2013), so it might be expected that N concentrations would also be higher in a treatment that results in higher SLA. Yet in this study, foliar N concentrations were lower in all three species in the Ca treatment plots, despite the increased leaf surface area available for C capture in beech and red maple. Although consistent with other studies (Long et al. 1997; Juice et al. 2006; Littke and Zabowski 2007) and possibly attributable to reduced net N mineralization in the Ca treatment plots (Groffman et al. 2006), it may also simply be a dilution effect. The aforementioned studies did not report N content per leaf, but among my sampled leaves, N content was higher in the Ca plots. In each treatment, N concentrations were similar in the maple congeners, and were lower than those of beech. Mader et al. (1969) linked sugar maple decline to N deficiency in western Massachusetts, but sampled sugar maples in this study had N concentrations that spanned nearly the exact range of healthy values suggested by Kolb and McCormick (1993). Since foliar concentrations of N and S tend to be positively correlated (Perakis et al. 2006), it was not surprising that, among all species, green leaf S was also lower in the Ca-treated plots.

If acidification in the study region had led to increased labile Al and Mn, higher foliar concentrations of these elements should occur in the control plots, yet treatment effects on green leaf concentrations were negligible. Manganese concentrations were about one-third lower in my plots than those reported by Juice et al. (2006), and were more similar to those in maples on buffered soils on lower slopes of Pennsylvania's Allegheny Plateau than those on unbuffered acidified soils upslope (Fernando et al. 2016). Soils at higher elevations in the study area have experienced more severe cation depletion due to acidic deposition (Likens et al. 1996), but an effect of elevation on Mn or Al concentrations was not evident in the present study. In fact, the highest Mn concentrations were in the lowest elevation stand (C8), and the lowest concentrations were in one of the highest elevation stands studied, 400 m higher (JBM). This may be attributable to other factors, like substrate differences between sites.

It was especially surprising that trees in the control plots accreted 67% more Mn into senescing leaves than those in the Ca-treated plots, even though green leaf concentrations differed by only 2.5%. Though Mn is toxic at high levels, it is an essential micronutrient. Thus, if Ca treatment favors internal recycling of Mn, this effect could be stemming from a reduction in available soil Mn or from enhanced Mn requirements in the Ca plots, possibly owing to the role of Mn in photosynthesis. Other Ca addition studies have found positive relationships between increased available Ca and photosynthesis (St. Clair and Lynch 2004; Halman et al. 2008). Increased availability of Mn and Al and reductions in Ca availability in acidified soils are mirrored in reduced Ca:Mn and Ca:Al ratios in both soil and foliage (Cronan and Grigal 2005; Watmough et al. 2007). These ratios were higher for all species in the Ca treatment plots, with the exception of the Ca:Mn ratio in sugar maple. If higher Ca:Mn ratios reflect reduced bioavailability of Mn in the soil, this may also promote enhanced Mn conservation in the tree.

Increasing availability of Mn and Al due to soil acidification has been strongly implicated in the decline complex of sugar maple in the northeastern United States. Calcium addition did not affect the concentration or resorption of either element in sugar maple in the present study. Decline in sugar maple trees has been associated with foliar Mn concentrations greater than 2.0 mg g^{-1} (Horsley et al. 2002), but sampled sugar maple trees in the present study had concentrations less than a third of this value (0.62 mg g^{-1}). The mean Al and Mn concentrations in sugar maple in the study sites were nearly one-half and one-third, respectively, of those reported for sugar maple trees in Pennsylvania, and fell within or below the reported healthy ranges for sugar maple (Kolb and McCormick 1993; Horsley et al. 2000).

Ratios of Ca:Mn and Ca:Al in sugar maple were well above other reported ranges. Horsley et al. (2000) gave a range of 1.8-26.9 for Ca:Mn and 54-463 for Ca:Al; the measured Ca:Mn and Ca:Al in this study ranged between 7.2-55.4 and 258-876, respectively. Cronan and Grigal (1995) found that a foliar Ca:Al ratio of 12.5 or less was correlated with a 50% risk for aluminum stress. However, Long et al. (1997) suggest that a ratio of 110 or less is associated with declining sugar maple in field studies. In the present study, computed Ca:Al ratios were well above this threshold. Furthermore, the Ca:Al ratios were also higher than those reported for declining sugar maple in southern Quebec (Bernier and Brazeau 1988b), both limed and unlimed plots in Pennsylvania (Long et al. 1997), and both low and high quality sites in Vermont (Wilmot et al. 1995).

Both Al and Mn can act as phytotoxins, with toxic levels initially manifesting as stunted roots and thus, reduced nutrient uptake (Mossor-Pietraszewska 2001). Any reduction in uptake capacity in my sampled trees should be reflected in lower foliar concentrations of a variety of nutrients. Reflecting the lack of treatment effects on foliar Al or Mn, however, treatment effects on the concentrations of most other foliar nutrients were also not evident. The concentrations of the other base cations analyzed, Mg and K, were the same or lower in green leaves in the Ca-treated plots compared to the control plots. A similar response has been observed by others, and may be attributable to competition for exchange sites and antagonism among the base cations (Schneider and Zech 1991; Côté et al. 1995; Long et al. 1997; Huber et al. 2006). Lower concentrations may also be due to dilution since sampled leaves were larger in the Ca plots and K and Mg content per leaf was higher in the Ca plots.

Researchers have hypothesized that depletion of nutrient cations and the concurrent mobilization of Al and Mn in the soil may drive sugar maple decline. The proficiency and efficiency with which sugar maple, in particular, resorbed nutrient cations in the present study was not significantly affected by calcium addition. Foliar Mg was significantly lower in sugar maple trees treated with Ca, and trees in both treated and untreated plots had foliar Mg concentrations below the optimum for sugar maple (Côté et al. 1993). The range of concentrations observed (0.85-1.25 mg g^{-1}) was close to ranges reported in sugar maple in Pennsylvania (Horsley et al. 2000) and in southern Quebec (Ouimet and Fortin 1992; Côté et al. 1995) but at the low end or below the presumed healthy range of foliar Mg for sugar maple (Kolb and McCormick 1993). Sugar maple trees in declining stands in Pennsylvania tended to have foliar Mg levels of 0.7 mg g^{-1} or less (Horsley et al. 2002), while sugar maple trees exhibiting signs of Mg deficiency had fresh leaf concentrations of 0.3-0.9 mg g^{-1} (Bernier and Brazeau 1988b). The majority of my sampled sugar maple trees had concentrations above these thresholds, indicating sufficient levels of Mg in the green leaves.

Sugar maple K concentrations were slightly lower in the Ca treatments compared to the controls, an observation consistent with other Ca addition studies (Côté et al. 1995; Long et al. 1997). Potassium concentrations of just 2.0-3.0 mg g^{-1} have been observed in declining sugar maple stands (Bernier and Brazeau 1988c), while most stands studied in the Quebec Appalachians with a foliar K concentration of 5.5 mg g^{-1} or above exhibited low levels of decline symptoms (Bernier and Brazeau 1988a). At the sites in the present study, the ranges of K concentrations in both control (7.51-8.98 mg g^{-1}) and treated (6.58-7.75 mg g^{-1}) plots were within the healthy range suggested by Kolb and McCormick (1993) and close to the optimum K concentration proposed by Côté et al. (1993).

Foliar P concentrations were slightly higher and both proficiency and efficiency of resorption declined with Ca treatment, which could reflect elevated soil P availability and accelerated P cycling (Fiorentino et al. 2003). One theory in nutrient cycling is that, with increasing stand age or multiple harvests, Ca will become more limiting; thus, a greater response to Ca addition in older stands should occur. If vegetation demand for Ca increased, uptake of N and P would likely also increase, unless these elements are also limiting. Yet green leaf N concentrations were lower and P resorption efficiency and proficiency declined when Ca was added to the older stands. The 9% decrease in the N:P ratio in the Ca-treated plots across both age classes was consistent with the lower foliar N and higher foliar P concentrations. The N:P ratio in the control plots was 20.3, indicative of P limitation; in the plots receiving Ca additions, the N:P ratio was 18.0 and closer to the range of co-limitation by N and P (Güsewell 2004; Koerselman and Meuleman 1996).

Of the three species, sugar maple had the lowest P concentrations, regardless of treatment, though these concentrations were within the healthy range for sugar maple (Erdmann et al. 1979; Kolb and McCormick 1993), and above that associated with decline in Quebec (Bernier and Brazeau 1988c). The average green leaf P concentration in the control plots was the same as that observed in sugar maple trees in Pennsylvania (Long et al. 1997). Unlike Côté et al. (1995), I found that Ca additions were linked with higher foliar P concentrations in sugar maple. The observed reduction in foliar N:P in sugar maple in Ca treatments was consistent with higher foliar P concentrations in the Ca treatment plots.

Conclusions

There do not appear to have been any age-specific improvements in foliar nutrition due to Ca addition, and overall, sugar maple did not exhibit a greater response to Ca treatment than the other two study species. In fact, most of the significant interactive effects between species and treatment were due to treatment effects in beech and red maple. Though sugar maple decline has been reported in patches throughout this region in the past, it is possible that the sampled trees were not affected or are currently in a recovering state, thus highlighting the mercurial nature and complex forest dynamics associated with this decline syndrome.

Some of the most notable effects of Ca amendment in this study were on foliar physical characteristics, which can ultimately affect ecosystem-level processes such as C storage, forest growth and regeneration, and rates of nutrient cycling. These potential effects warrant further study. The observed increases in leaf mass and area following Ca addition also highlight the importance of considering nutrient content per leaf when interpreting nutrient concentration results. For example, previous studies have reported lower foliar N concentrations following Ca treatment, but it is not clear whether this is related to shifts in biogeochemical cycles or simply due to a dilution effect associated with larger, heavier leaves following Ca treatment. Future studies should take into account both concentration and content when leaf physical characteristics are affected by treatment.

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Chapter 4: Conclusions

Human activities have the potential to affect nutrient cycling in natural forest ecosystems, with further implications for plant internal nutrient dynamics. In a way, the calcium, control, and N/P plots that have been established in the forests in the White Mountains of New Hampshire could represent past, present, and future statuses of these forest stands. Over the past several decades, calcium depletion concurrent with acidic deposition and aging stands may have altered foliar physical characteristics. This could have further implications for decomposition, herbivory, and nutrient use efficiency. As the cycles of nitrogen and phosphorus continue to be altered by anthropogenic activities, shifts in relative limitation status may occur. To maintain stoichiometric balance between N and P, it is unclear whether tree internal nutrient conservation will increase, reflected in enhanced resorption efficiency and proficiency, or if external nutrient cycling will accelerate via, for example, higher litter nutrient concentrations. The results presented here show surprisingly little effect of N additions on P dynamics and vice versa. The effects of N or P addition on N and P separately are similar to the results of others, but the lack of evidence for multiple element resorption controls in these relatively recently fertilized, mid-aged to mature forest stands raises questions of the influence of stand age class or successional stage on resorption dynamics. The influence of the relative degree of limitation and the time interval of that limitation also warrant future study. Examination of species plasticity in responsiveness to such shifts in limitation and nutrient availability may also help better inform predictions of forest response to changing nutrient cycles. Methodologically, future studies should also consider variation in nutrient concentrations and resorption throughout entire seasons, especially if fertilization is a part of the study design.

Table 2.1. Examples of previously published studies relating availability of nitrogen or phosphorus to resorption efficiency of the respective nutrient.

* indicates green leaf nitrogen concentrations used instead of soil nutrient availability, with the assumption that leaf nutrient status reflects soil availability
□ indicates both leaf and soil nutrient availability considered
§ indicates both leaf and soil nitrogen were considered, but for phosphorus, only soil availability was reported
† indicates both leaf and soil phosphorus were considered, but for nitrogen, only soil availability was reported
ǂ indicates the same species were not analyzed in every site
□ as calculated by Chapin and Moilanen 1991
[1] indicates a study without fertilization, but observing differences in sites of contrasting fertility or natural nutrient gradients along a chronosequence, for example.
[2] indicates a fertilization study
[3] indicates a meta-analysis or literature review

Source	Relation between N status and NRE	Relation between P status and PRE	Plant type and location
[1]Boerner 1984	Negative □	Negative □	Deciduous trees, southern Ohio
[1]Kost and Boerner 1985	Negative	Negative	Deciduous tree, southern Ohio
[1]Côté et al. 2002	Negative *	Negative *	Deciduous trees, southern Quebec
[1]Lin and Sternberg 2007		Negative	Mangrove tree, Florida
[1]Enoki and Kawaguchi 1999	Negative		Conifer
[1]Pugnaire and Chapin 1993	Negative	No relation	Evergreen trees and shrubs, northern California
[1]Yuan et al. 2005	Negative		Shrub, northern China
[1]Shaver and Melillo 1984	Negative	Negative	Marsh graminoids, growth chamber
[1]Hayes et al. 2014	Negative ǂ	Negative †ǂ	Trees, shrubs, vines, perennial herbs, sedges, southwestern Australia
[1]Liu et al. 2014	Negative *	Negative *	Trees, shrubs, grasses, four vegetation types in karst region of southwestern China
[1]Chapin and Moilanen 1991	No relation	No relation	Deciduous tree, Alaska
[1]Del Arco et al. 1991	No relation §	No relation	Various woody species, central-western Spain
[1]Escudero et al. 1992	No relation		Various woody species, central-western Spain
[1]Wright and Westoby 2003	No relation	No relation	Evergreen species, eastern Australia
[1]Rentería et al. 2005	No relation □	No relation □	Leguminous and non-legume trees, tropical dry forest, Mexico
[1]Killingbeck and Costigan 1988	No relation	No relation	Understory shrubs, Rhode Island
[1]Walbridge 1991		No relation	Shrubs, North Carolina

[1]Cárdenas and Campo 2007	Positive	No relation	Nitrogen-fixing tree, tropical dry forest, Mexico
[1]Lal et al. 2001	Positive	Positive	Various trees, tropical dry forest
[2]Chapin and Moilanen 1991		Negative	Deciduous tree, Alaska
[2]Li et al. 2010	Negative		Deciduous trees, northeastern China
[2]Flanagan and Van Cleve 1983	Negative □	No relation □	Evergreen and deciduous trees, Alaskan taiga
[2]Cordell et al. 1991	No relation	Negative	Evergreen tree, Hawaii
[2]Van Heerwaarden et al. 2003	Negative		Woody deciduous and evergreen, graminoid, herb, bog in northern Sweden
[2]Soudzilovskaia et al. 2007	No relation	Negative	Sedges, graminoids, herbs, Russia
[2]Lu and Han 2010	Negative		Grassland species, northern China
[2]Lu et al. 2013	Negative		Graminoids, northern China
[2]May et al. 2005	Negative (2 sp.); no relation (1 sp.)		Deciduous trees, West Virginia
[2]Vitousek 1998	No relation	No relation/negative depending on initial limitation status	Evergreen tree, Hawaii
[2]Rejmánková 2001		No relation (1 sp.); negative (1 sp.)	Wetland sedge, Belize
[2]Kozovits et al. 2007	No relation	No relation	Woody species, tropical savannah, central Brazil
[2]Harrington et al. 2001	No relation	No relation	Evergreen tree, Hawaii
[2]Helmisaari 1992	No relation		Conifer tree, eastern Finland
[2]Lajtha and Klein 1988	No relation	No relation	Evergreen shrub, phytotron
[2]Chapin and Shaver 1989	No relation	No relation	Graminoids, forbs, deciduous shrubs, evergreen shrubs, Alaska
[2]Kazakou et al. 2007	No relation		Herbs, common garden, France
[2]Birk and Vitousek 1986	No relation (leaf level); positive (stand level)		Conifer tree, coastal South Carolina
[2]Lajtha 1987	Positive *	No relation *	Evergreen shrub, desert
[2]Turner and Olson 1976	Positive □		Evergreen conifer, western Washington
[2]Nambiar and Fife 1987	Positive	Positive	Conifer tree
[3]Kobe et al. 2005	Negative *	Negative *	Global dataset
[3]Vergutz et al.2012	Negative *	Negative *	Global dataset
[3]Yuan and Chen 2015	Negative	Negative	Global dataset
[3]Aerts1996	No relation *	No relation *	Global dataset
[3]Chapin and Kedrowski 1983	Positive *	No relation *	

Table 2.2. Descriptions of the eight sampled stands.

						Basal area (m^2 ha^{-1}) *						
Site	Stand	Last cut	Elevation (m)	Aspect	Slope (%)	BA	BE	PC	RM	SM	WB	YB
BEF	C4	1978	410	NE	20-25	7.2	1.4	0.5	2.0	0.03	10.2	0.9
	C6	1975	460	NNW	13-20	0.2	4.8	0.3	9.0	1.2	8.5	8.1
	C8	1883	330	NE	5-35		21.7		0.5	19.0	0.2	6.3
	C9	~1890	440	NE	10-35		8.6			16.6		5.9
HB	HBM	1971	500	S	10-25	0.3	1.9	0.1	1.4	1.6	2.4	9.5
	HBO	~1910	500	S	25-35		8.5			3.9		22.8
JB	JBM	1985	730	WNW	25-35	0.2	0.1	0.4		2.1	2.3	5.1
	JBO	~1900	730	WNW	30-40		1.7			35.1		7.6

* BA – bigtooth aspen; BE – American beech; PC – pin cherry; RM – red maple; SM – sugar maple; WB – white birch; YB – yellow birch

Table 2.3. Example ANOVA table for average surface area per green leaf using the model given in Equation 1.

Source	df	Mean Square	F value	p-value
Age	1	1659.76	17.29	0.0060
Stand(Age)	6	95.97	1.78	0.1261
N treatment	1	83.04	1.54	0.2212
P treatment	1	94.26	1.75	0.1930
N x P	1	532.46	9.89	0.0031
N treatment x Age	1	0.08	0.00	0.9697
P treatment x Age	1	12.57	0.23	0.6315
N x P x Age	1	38.80	0.72	0.4008
Species	2	65.24	1.21	0.3079
Species x N treatment	2	200.65	3.73	0.0324
Species x P treatment	2	24.08	0.45	0.6424
Species x N x P	2	80.95	1.50	0.2341
Error	42	53.85		

Table 2.4. Least squares means for green leaf physical characteristics. Standard errors are in parentheses. Significant interactions and main effects of age class and treatment are given for p < 0.10.

		Age Class	
Variable	Treatment	Successional	Mature
Surface area per leaf (cm^2)	Control	***46.82 (2.8)***	***36.77 (3.2)***
	N	***52.41 (2.8)***	***38.81 (3.2)***
	P	***58.70 (2.8)***	***42.81 (3.2)***
	NP	***47.96 (2.8)***	***35.96 (3.2)***
Mass per leaf (g)	Control	§ ***0.250 (0.02)***	§ ***0.202 (0.02)***
	N	§ ***0.268 (0.02)***	§ ***0.222 (0.02)***
	P	§ ***0.313 (0.02)***	§ ***0.239 (0.02)***
	NP	§ ***0.242 (0.02)***	§ ***0.180 (0.02)***
Specific leaf area (cm^2/g)	Control	† 13.72 (1.3)	† 13.99 (1.6)
	N	† 13.49 (1.3)	† 14.05 (1.6)
	P	† 16.85 (1.3)	† 15.41 (1.6)
	NP	† 15.44 (1.3)	† 15.12 (1.6)
LDMC	Control	§ 0.426 (0.01)	§ 0.442 (0.02)
	N	§ 0.427 (0.01)	§ 0.432 (0.02)
	P	§ 0.427 (0.01)	§ 0.450 (0.02)
	NP	§ 0.400 (0.01)	§ 0.411 (0.02)

† indicates significant main effect of P treatment

§ indicates significant main effect of N treatment

Bold type indicates significant interaction between N and P treatment

Italicized type indicates significant main effect of age class

* indicates significant interaction between age class and treatment

Table 2.5. Least squares means for foliar nutrient concentrations and resorption efficiency of N and P. Model included corresponding pre-treatment variable as a covariate. Standard errors are in parentheses. Significant interactions and main effects of age class and treatment are given for p < 0.10.

		Green		Senesced		RE	
		Age Class					
Element	Treatment	Successional	Mature	Successional	Mature	Successional	Mature
N	Control	†§22.37 (0.5)	†§21.66 (0.6)	§10.71 (0.8)	§9.83 (1.0)	51.9 (3.9)	54.0 (4.8)
	N	†§25.67 (0.4)	†§24.11 (0.7)	§12.59 (1.0)	§11.03 (1.0)	51.1 (5.2)	53.4 (4.7)
	P	†§23.01 (0.6)	†§20.51 (0.6)	§10.21 (1.0)	§12.27 (0.9)	58.9 (5.6)	46.5 (5.2)
	NP	†§24.56 (0.4)	†§22.79 (0.5)	§12.45 (0.7)	§11.89 (0.9)	47.7 (3.5)	47.3 (4.3)
P	Control	†1.09 (0.05)	†1.11 (0.06)	†§***0.39 (0.1)**	†§***0.35 (0.1)**	†***63.8 (4.5)**	†***67.8 (5.6)**
	N	†1.15 (0.05)	†1.08 (0.06)	†§***0.42 (0.1)**	†§***0.48 (0.1)**	†***65.3 (5.7)**	†***56.8 (5.5)**
	P	†1.48 (0.06)	†1.39 (0.07)	†§***0.82 (0.1)**	†§***1.07 (0.1)**	†***58.1 (6.7)**	†***27.7 (6.8)**
	NP	†1.43 (0.05)	†1.36 (0.06)	†§***0.73 (0.1)**	†§***0.65 (0.1)**	†***50.3 (4.3)**	†***53.4 (5.2)**
N:P	Control	†§20.3 (0.6)	†§20.0 (0.8)	30.3 (2.7)	27.2 (3.4)	†§****0.81 (0.2)***	†§****0.78 (0.2)***
	N	†§22.5 (0.6)	†§23.7 (1.1)	36.8 (3.4)	24.0 (3.3)	†§****0.80 (0.2)***	†§****0.97 (0.2)***
	P	†§15.7 (0.8)	†§15.5 (0.9)	15.5 (3.5)	15.1 (3.7)	†§****0.78 (0.2)***	†§****2.28 (0.3)***
	NP	†§17.7 (0.6)	†§17.1 (0.8)	21.6 (2.5)	23.5 (3.0)	†§****1.03 (0.2)***	†§****1.01 (0.2)***

† indicates significant main effect of P treatment

§ indicates significant main effect of N treatment

Bold type indicates significant interaction between N and P treatment

Italicized type indicates significant main effect of age class

* indicates significant interaction between age class and treatment

Table 3.1. Characteristics of sampled stands.

							Basal area (m^2 ha^{-1}) *					
Site	Stand	Last cut	Age class	Elevation (m)	Aspect	Slope (%)	BE	PC	RM	SM	WB	YB
BEF	C1	1990	Successional	570	Flat to SE	5-20	0.4	1.7	<0.1	<0.1	5.1	0.1
	C6	1975	Successional	460	NNW	13-20	4.8	0.8	9.0	1.2	8.5	8.1
	C8	1883	Mature	330	NE	5-35	21.7	0	0.5	19.0	0.2	6.3
HB	HBCa	~1910	Mature	500	SW	25-35	3.1	0	0.1	3.5	0.4	6.8
JB	JM	1985	Successional	730	WNW	25-35	0.1	1.1	0	2.1	2.3	5.1
	JO	~1900	Mature	730	WNW	30-40	1.7	0	0	35.1	0	7.6

*BE – American beech; PC – pin cherry; RM – red maple; SM – sugar maple; WB – white birch; YB – yellow birch

Table 3.2. Example ANOVA table for average surface area per green leaf to show model testing for main effects of age, treatment, and species, as well as the two interactions. Stand was nested within age.

Source	DF	Mean Square	F-value
Age	2	194.68	14.46
Stand(Age)	3	13.46	0.31
Age x Treatment	2	0.05	0.00
Treatment	1	217.24	5.04
Species	2	13.41	0.31
Treatment*Species	2	211.34	4.91
Error	11	43.08	

Table 3.3. Least squares means for green leaf physical characteristics. Standard errors are in parentheses. Bold type indicates a significant treatment main effect and italicized type indicates a significant main effect of age class. An asterisk indicates a significant interaction between age class and treatment (p<0.1).

		Age Class	
Variable	Treatment	Successional	Mature
Surface area per leaf (cm^2)	Control	***47.65 (2.7)***	***39.50 (3.3)***
	Calcium	***54.65 (2.7)***	***46.28 (3.3)***
Mass per leaf (g)	Control	***0.262 (0.02)***	***0.223 (0.03)***
	Calcium	***0.333 (0.02)***	***0.257 (0.03)***
Specific leaf area (cm^2/g)	Control	***13.29 (0.8)**	***11.90 (1.0)**
	Calcium	***13.10 (0.8)**	***15.05 (1.0)**
LDMC	Control	***0.419 (0.01)***	***0.440 (0.01)***
	Calcium	***0.447 (0.01)***	***0.449 (0.01)***

Table 3.4. Least squares means for green and senesced leaf element concentrations and resorption efficiency (RE). Standard errors are in parentheses. Bold type indicates a significant treatment main effect and italicized type indicates a significant main effect of age class. An asterisk indicates a significant interaction between age class and treatment (p<0.1). All concentrations are expressed in units of mg/g except for aluminum, which is in units of µg/g. Resorption efficiencies are percentages.

		Green		Senesced		RE	
		Age Class					
	Treatment	Successional	Mature	Successional	Mature	Successional	Mature
Al	Control	15.21 (0.9)	16.85 (1.1)	14.40 (2.0)	18.46 (2.5)	4.82 (10.2)	-14.66 (12.6)
	Calcium	13.86 (0.9)	17.10 (1.1)	13.52 (2.0)	14.50 (2.5)	-0.69 (10.2)	9.91 (12.6)
Sr	Control	*0.016(0.001)	*0.019(0.002)	*0.014 (0.003)*	*0.024 (0.004)*	*13.4 (16.0)	*-35.9 (19.7)
	Calcium	*0.015(0.001)	*0.022(0.002)	*0.017 (0.003)*	*0.022 (0.004)*	*-23.1 (16.0)	*0.02 (19.7)
Mn	Control	*0.422 (0.04)	*0.598 (0.05)	***0.994 (0.1)**	***0.864 (0.1)**	-130.7 (20.5)	-60.8 (25.3)
	Calcium	*0.344 (0.04)	*0.757 (0.05)	***0.640 (0.1)**	***0.862 (0.1)**	-82.7 (20.5)	-15.2 (25.3)
Ca	Control	7.75 (0.4)	6.05 (0.5)	8.05 (0.8)	8.39 (1.0)	*-5.00 (14.6)	*-40.31(18.0)
	Calcium	7.55 (0.4)	7.35 (0.5)	9.46 (0.8)	7.88 (1.0)	*-29.23 (14.6)	*-8.69 (18.0)
K	Control	***8.27 (0.4)***	***7.83 (0.5)***	**6.54 (0.5)**	**5.41 (0.7)**	19.90 (7.3)	29.82 (9.0)
	Calcium	***8.07 (0.4)***	***6.66 (0.5)***	**5.52 (0.5)**	**4.33 (0.7)**	29.51 (7.3)	35.81 (9.0)
Mg	Control	*1.31 (0.06)	*1.17 (0.07)	1.01 (0.2)	1.15 (0.2)	*23.70 (10.2)	*3.29 (12.6)
	Calcium	*1.21 (0.06)	*1.27 (0.07)	1.05 (0.2)	0.77 (0.2)	*15.51 (10.2)	*42.94 (12.6)
S	Control	**0.898 (0.07)**	**0.942 (0.09)**	0.470 (0.3)	1.185 (0.3)	44.0 (25.2)	-23.9 (31.0)
	Calcium	**0.816 (0.07)**	**0.759 (0.09)**	0.552 (0.3)	0.648 (0.3)	31.6 (25.2)	18.7 (31.0)
P	Control	1.12 (0.06)	1.14 (0.07)	**0.444 (0.07)*	**0.450 (0.08)*	**60.9 (4.3)*	**61.1 (5.3)*
	Calcium	1.11 (0.06)	1.32 (0.07)	**0.359 (0.07)*	**0.656 (0.08)*	**67.7 (4.3)*	**49.4 (5.3)*
N	Control	***23.72 (0.6)**	***22.18 (0.7)**	10.97 (0.4)	10.45 (0.4)	53.1 (2.0)	53.0 (2.4)
	Calcium	***23.48 (0.6)**	***19.43 (0.7)**	10.82 (0.4)	10.38 (0.4)	52.8 (2.0)	47.6 (2.4)
C	Control	**493.5 (2.2)**	**490.5 (2.7)**	*498.6 (3.2)	*499.5 (3.9)	-1.05 (0.8)	-1.85 (1.0)
	Calcium	**487.3 (2.2)**	**486.1 (2.7)**	*488.1 (3.2)	*501.0 (3.9)	-0.17 (0.8)	-3.13 (1.0)

Table 3.5. Least squares means for green and senesced leaf element content per leaf and resorption efficiency (RE). Standard errors are in parentheses. Bold type indicates a significant treatment main effect and italicized type indicates a significant main effect of age class. An asterisk indicates a significant interaction between age class and treatment (p<0.1). All content values are expressed in units of mg/leaf except for aluminum, which is in units of µg/leaf. Resorption efficiencies are percentages.

		Green		Senesced		RE	
		Age Class					
	Treatment	Successional	Mature	Successional	Mature	Successional	Mature
Al	Control	4.02 (0.4)	3.71 (0.5)	3.99 (0.5)	3.53 (0.6)	-2.55 (8.5)	-2.62 (10.4)
	Calcium	4.52 (0.4)	4.14 (0.5)	4.04 (0.5)	3.39 (0.6)	-0.71 (8.5)	10.49 (10.4)
Sr	Control	0.004(0.0004)	0.004(0.0005)	0.004 (0.001)	0.005 (0.001)	8.47 (11.9)	-21.85 (14.6)
	Calcium	0.005(0.0004)	0.005(0.0005)	0.006 (0.001)	0.005 (0.001)	-7.90 (11.9)	-4.97 (14.6)
Mn	Control	0.110 (0.01)	0.133 (0.02)	0.236 (0.03)	0.203 (0.03)	**-128.23 (19.6)**	**-74.88 (24.1)**
	Calcium	0.111 (0.01)	0.178 (0.02)	0.174 (0.03)	0.231 (0.03)	**-57.36 (19.6)**	**-21.75 (24.1)**
Ca	Control	***2.08 (0.3)***	***1.36 (0.3)***	2.32 (0.4)	1.78 (0.5)	-9.50 (12.0)	-29.77 (14.7)
	Calcium	***2.50 (0.3)***	***1.84 (0.3)***	3.01 (0.4)	1.89 (0.5)	-15.70 (12.0)	-12.15 (14.7)
K	Control	*2.14 (0.1)*	*1.72 (0.2)*	*1.80 (0.2)*	*1.24 (0.3)*	**15.96 (5.8)**	**30.03 (7.1)**
	Calcium	*2.61 (0.1)*	*1.72 (0.2)*	*1.79 (0.2)*	*1.08 (0.3)*	**30.15 (5.8)**	**36.77 (7.1)**
Mg	Control	**0.340 (0.03)**	**0.261 (0.04)**	*0.249 (0.03)	*0.252 (0.03)	**22.21 (9.1)**	**8.20 (11.3)**
	Calcium	**0.411 (0.03)**	**0.322 (0.04)**	*0.278 (0.03)	*0.177 (0.03)	**28.15 (9.1)**	**41.70 (11.3)**
S	Control	0.235 (0.02)	0.215 (0.03)	0.150 (0.04)	0.207 (0.04)	34.27 (12.7)	3.06 (15.6)
	Calcium	0.263 (0.02)	0.190 (0.03)	0.182 (0.04)	0.156 (0.04)	30.80 (12.7)	23.16 (15.6)
P	Control	**0.296 (0.02)**	**0.252 (0.03)**	0.123 (0.02)	0.104 (0.02)	*59.10 (4.9)	*61.44 (6.0)
	Calcium	**0.374 (0.02)**	**0.336 (0.03)**	0.122 (0.02)	0.154 (0.02)	*68.17 (4.9)	*51.38 (6.0)
N	Control	*6.19 (0.5)*	*4.86 (0.6)*	3.08 (0.4)	2.36 (0.4)	49.43 (3.2)	52.96 (3.9)
	Calcium	*7.64 (0.5)*	*4.98 (0.6)*	3.46 (0.4)	2.49 (0.4)	53.13 (3.2)	49.91 (3.9)
C	Control	***129.3 (11.3)***	***109.2 (14.0)***	138.4 (15.1)	113.6 (18.6)	-7.22 (6.0)	-1.53 (7.4)
	Calcium	***162.4 (11.3)***	***125.9 (14.0)***	153.8 (15.1)	119.7 (18.6)	2.34 (6.0)	-2.11 (7.4)

Table 3.6. Least squares means for element ratios with standard errors in parentheses. Bold type indicates a significant treatment main effect and italicized type indicates a significant main effect of age class. An asterisk indicates a significant interaction between age class and treatment (p<0.1).

		Age Class	
Ratio	Treatment	Successional	Mature
N:P	Control	***21.3 (0.8)***	***19.3 (1.0)***
	Calcium	***21.4 (0.8)***	***14.5 (1.0)***
Ca:Al	Control	513.5 (50)	386.3 (61)
	Calcium	574.7 (50)	478.9 (61)
Ca:Mn	Control	*23.3 (3.1)*	*9.9 (3.8)*
	Calcium	*25.0 (3.1)*	*11.2 (3.8)*

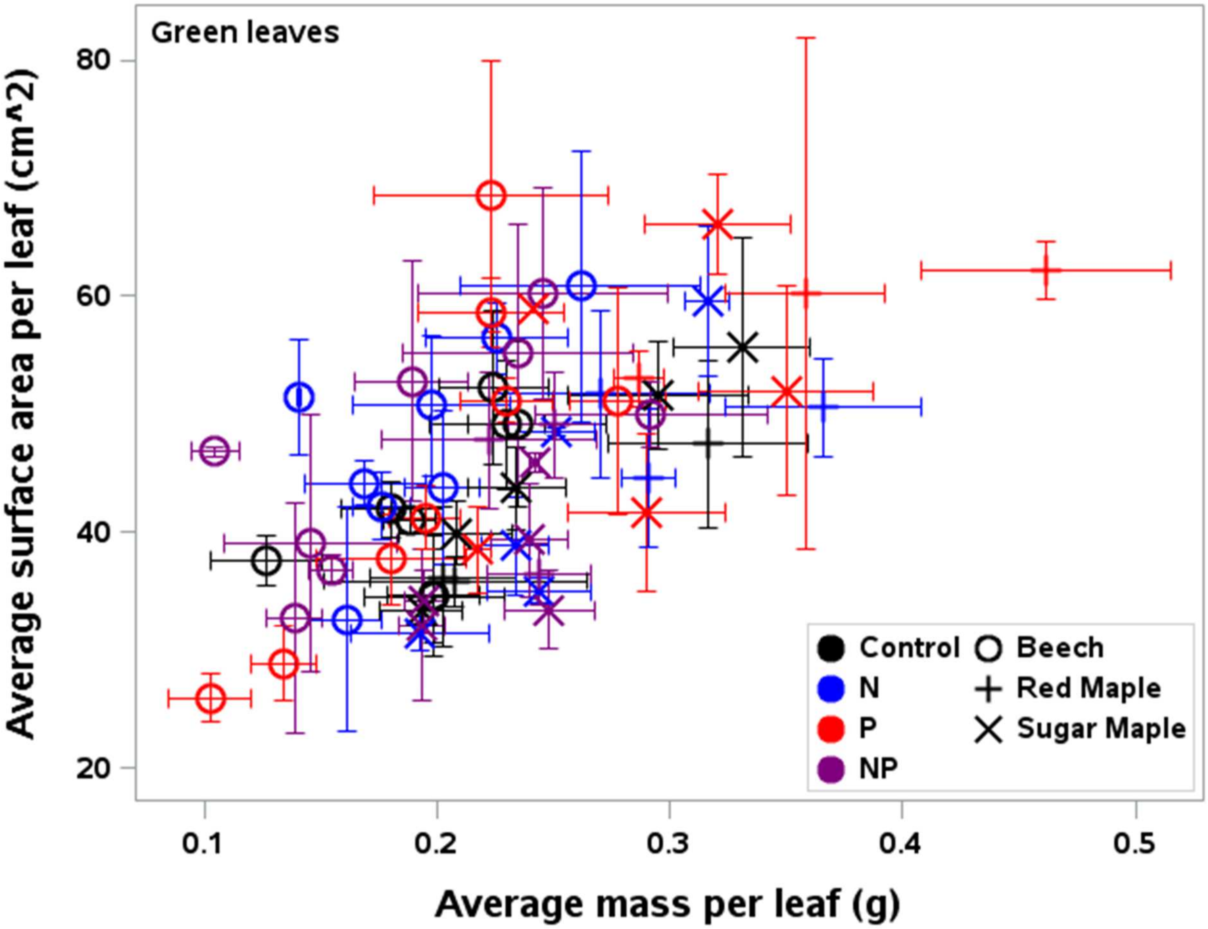


Figure 2.1. Average surface area and mass per leaf of green leaves by species and treatment. Each point represents the plot mean of 1-3 individuals. Error bars are standard errors of the plot mean.

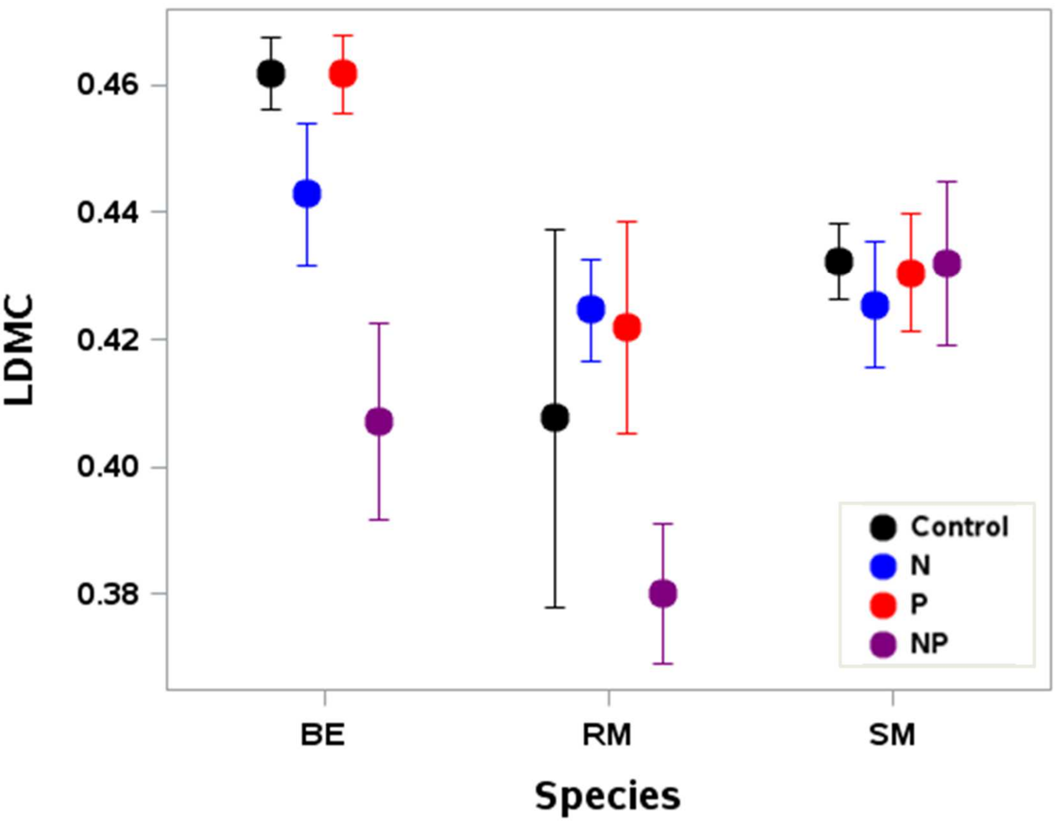


Figure 2.2. Leaf dry matter content of green leaves. Each point is the mean of each species and treatment combination (n=12).

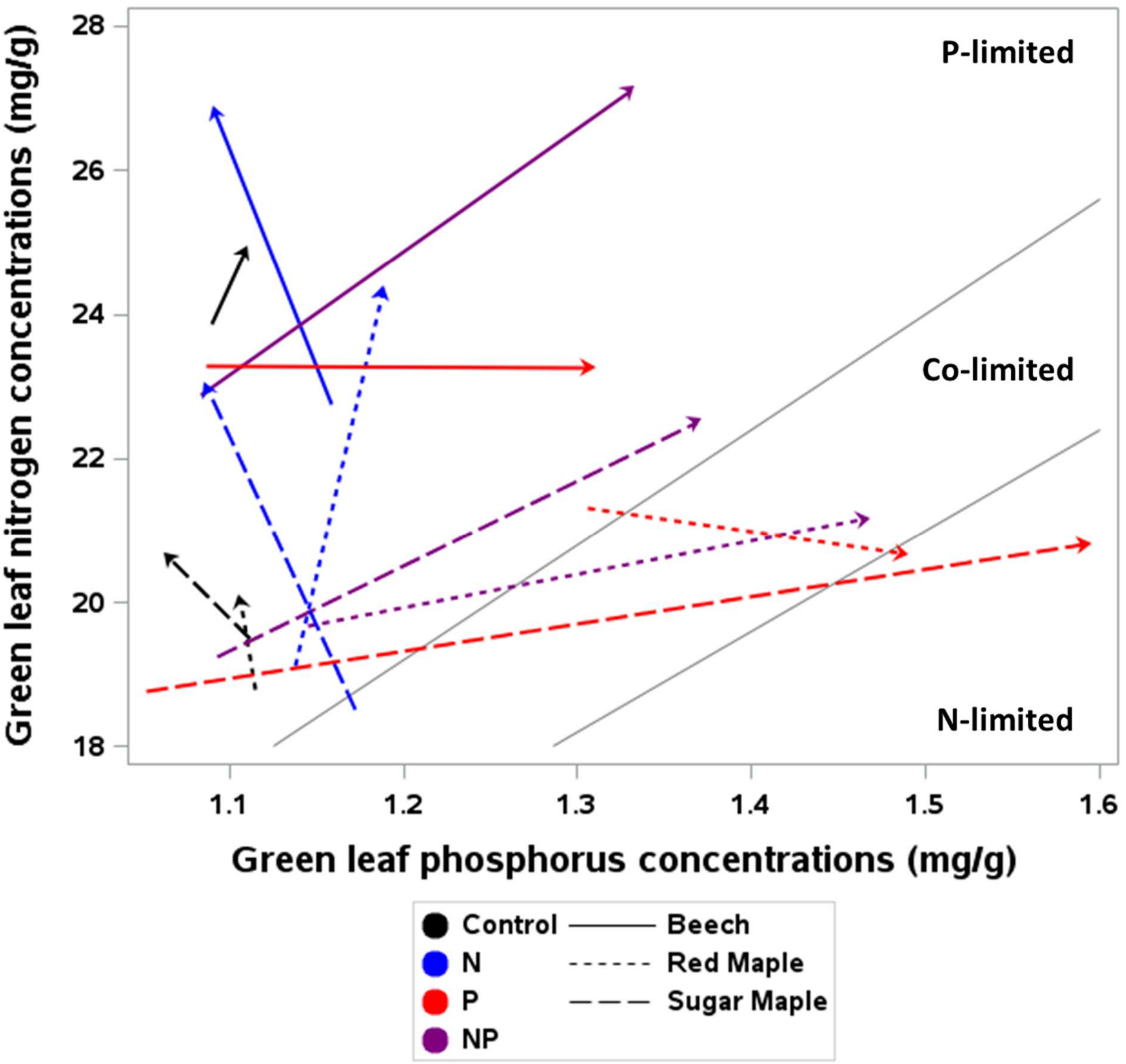


Figure 2.3. Vector plot of change in green leaf nitrogen and phosphorus concentrations from pre- to four years post-treatment for each species and treatment combination. The beginning of each arrow is the pre-treatment mean and the arrowhead is the post-treatment mean in 2015. The solid gray lines depict N:P ratios of 14 and 16, between which trees are considered co-limited.

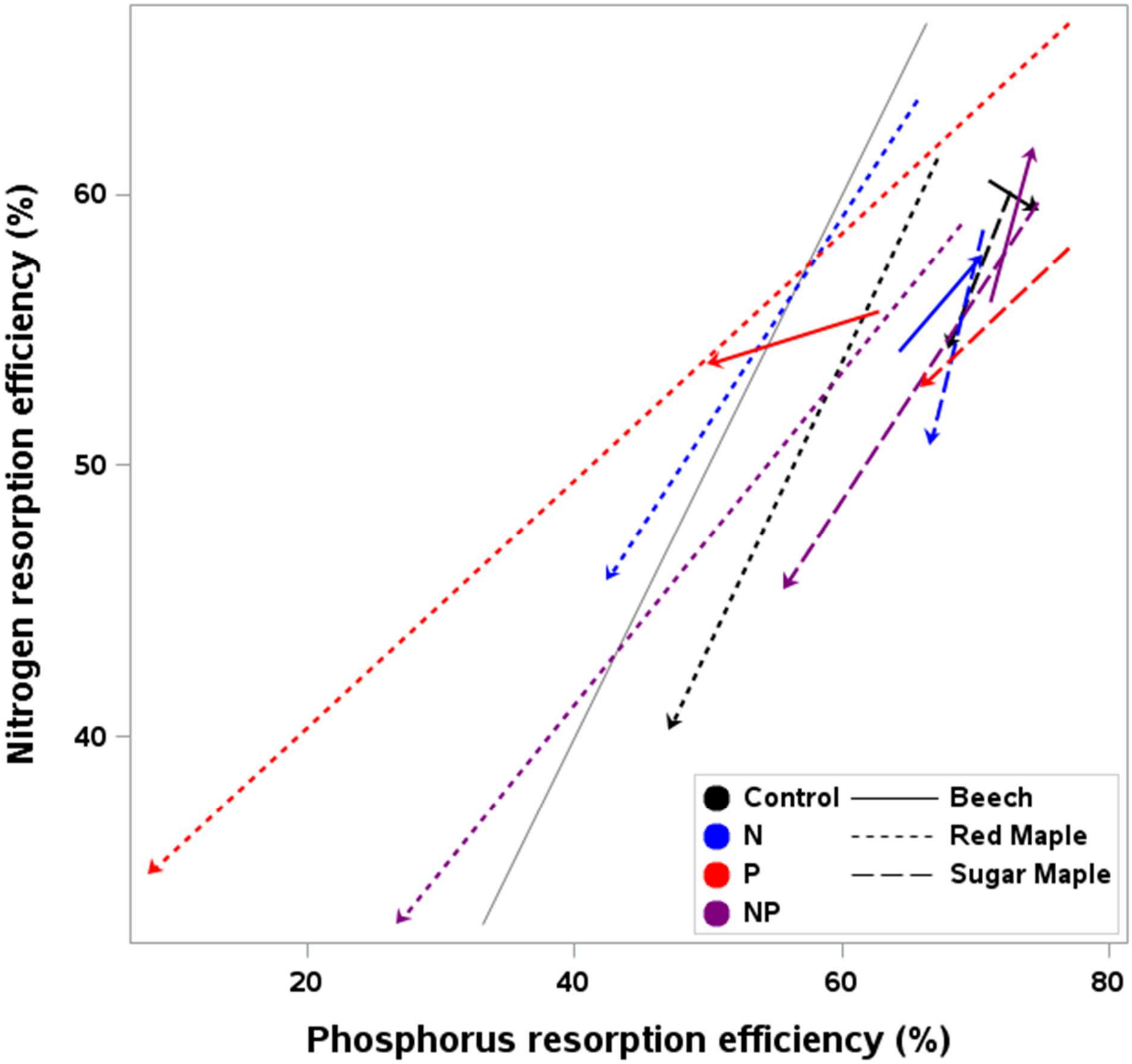


Figure 2.4. Vector plot of change in resorption efficiency from pre- to post-treatment. The beginning of each arrow is the pre-treatment mean, and the arrowhead represents the post-treatment mean for each combination of treatment and species. The solid gray line is a 1:1 line; points to the right of the line represent proportionally more phosphorus than nitrogen resorbed. Points to the left of the line indicate higher proportional resorption of nitrogen than phosphorus.

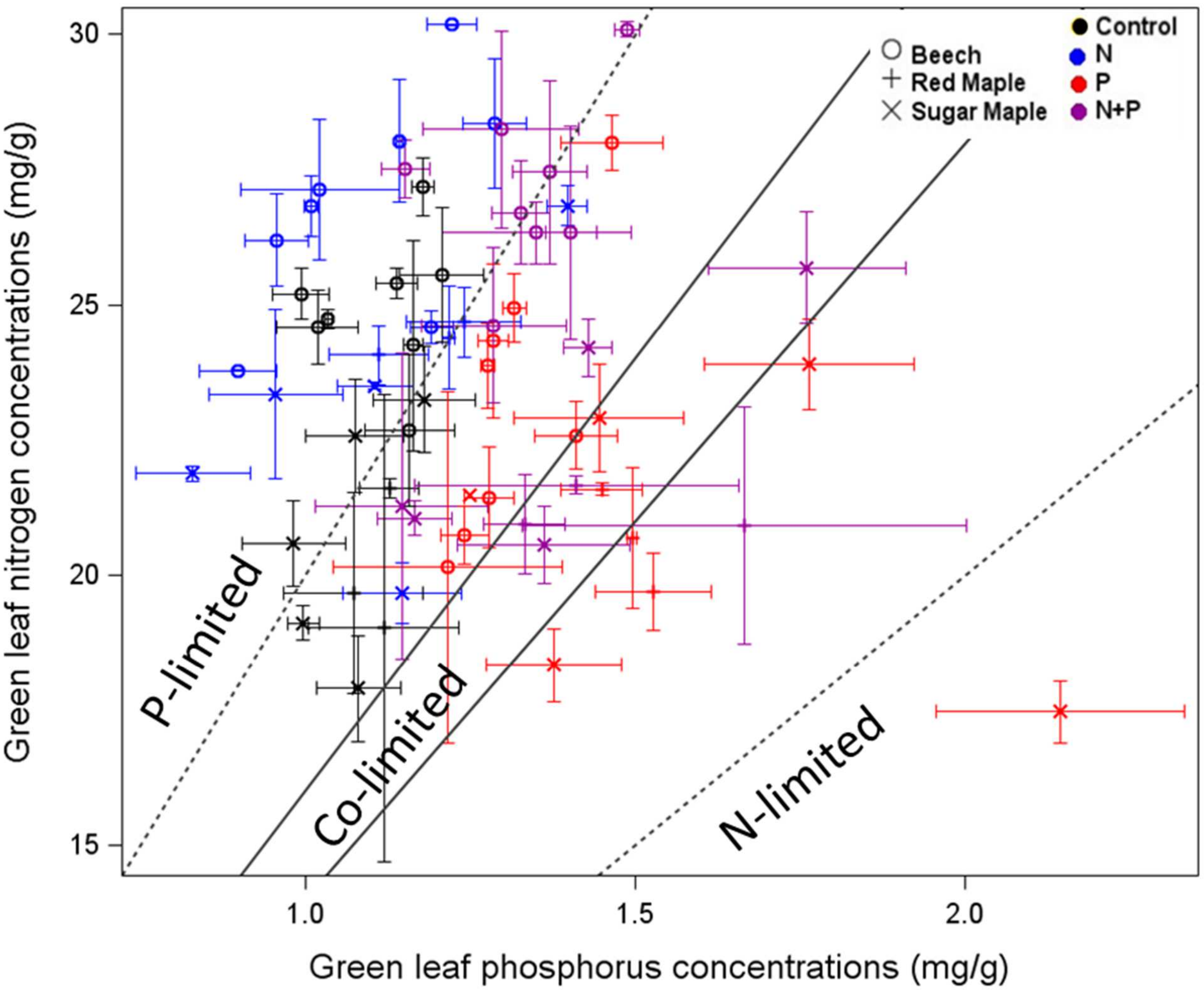


Figure 2.5. Scatterplot of green leaf phosphorus and nitrogen concentrations. Adding N increased green leaf N and pushed the P-limited stands in the study region further towards P limitation. When P was added, green leaf P increased, and green leaf N decreased, to the point of shifting towards co-limitation or even N limitation. Adding N and P together generally prompted a shift towards co-limitation. Even with P added, beech tended to remain in the P-limited zone, while the maples were able to accumulate more P in the green leaves. The solid and dotted lines delineate co-limitation between N:P of 14-16 and 10-20, respectively.

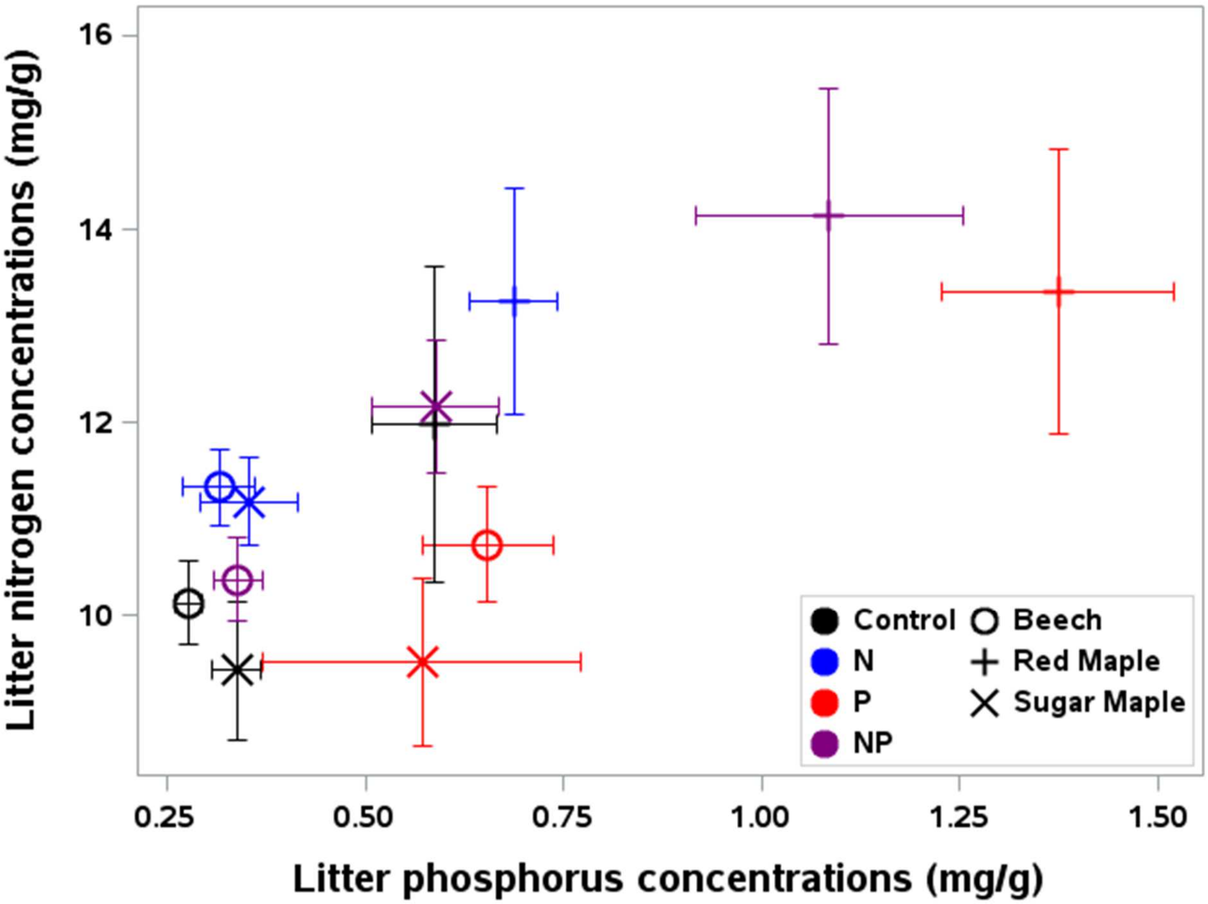


Figure 2.6. Litter nitrogen and phosphorus concentrations. Each point is the mean for each species and treatment combination.

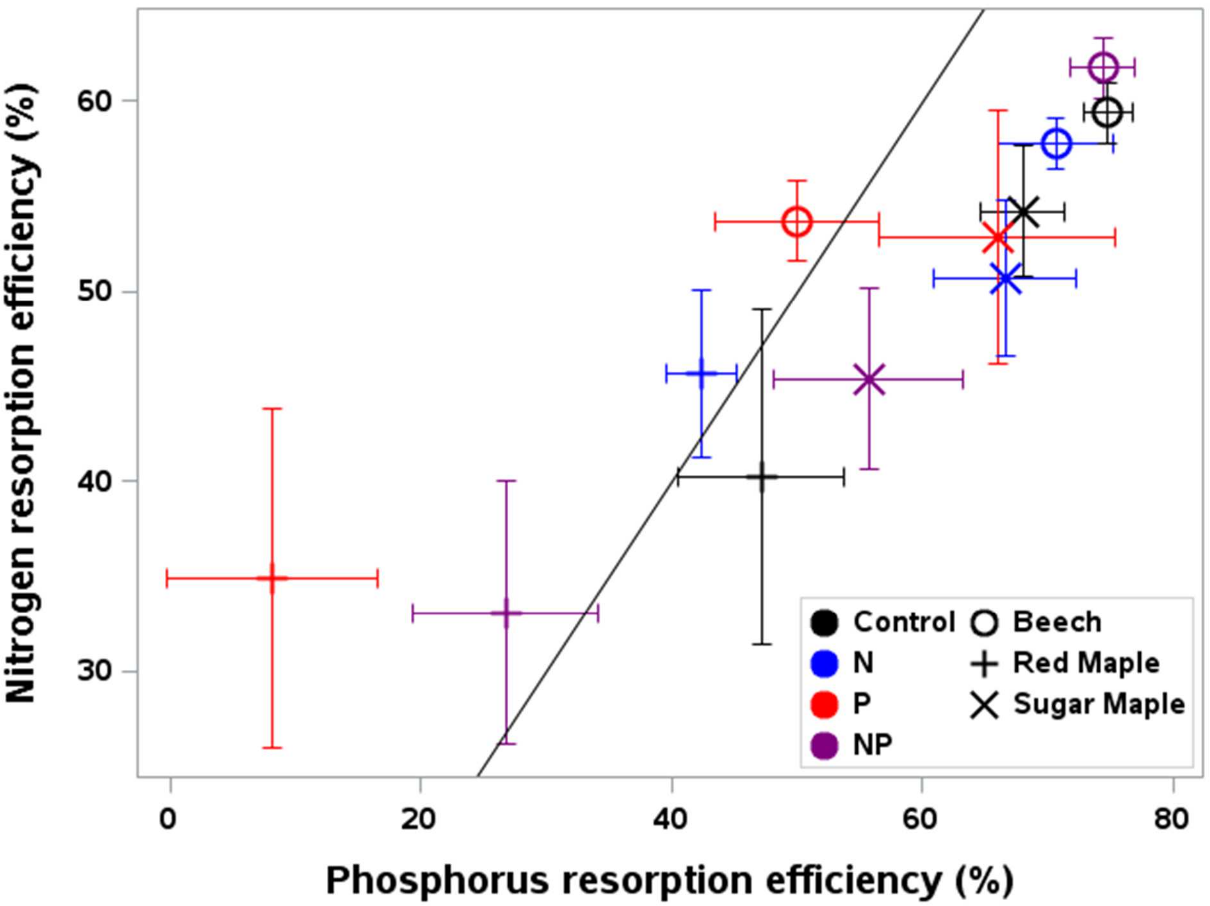


Figure 2.7. Resorption efficiency of nitrogen and phosphorus for three species among four treatments. Solid line is 1:1 line; a point on the line would indicate equal resorption of nitrogen and phosphorus.

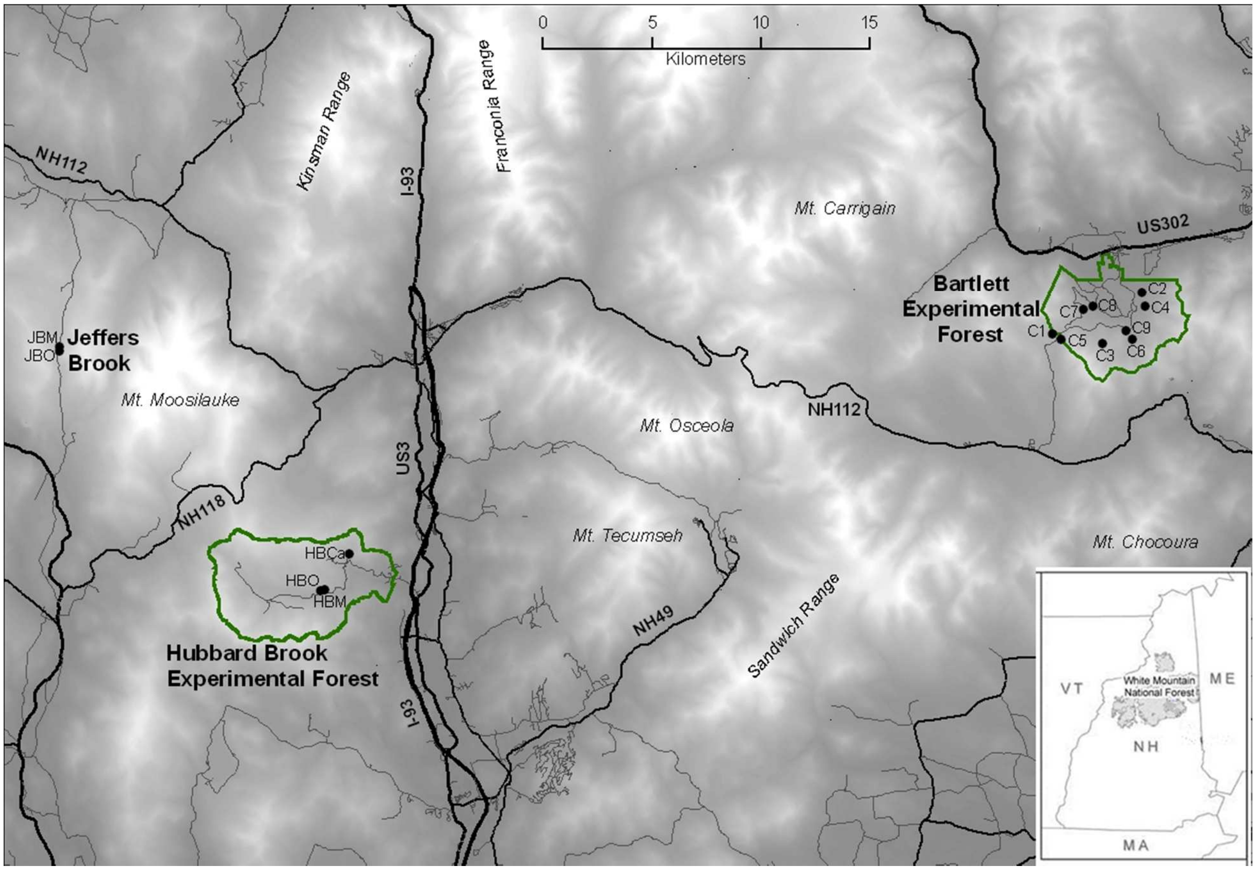


Figure 3.1. Map of sampling sites in the White Mountains of New Hampshire. Each sampled stand has one control plot and one plot that received a calcium amendment in 2011.

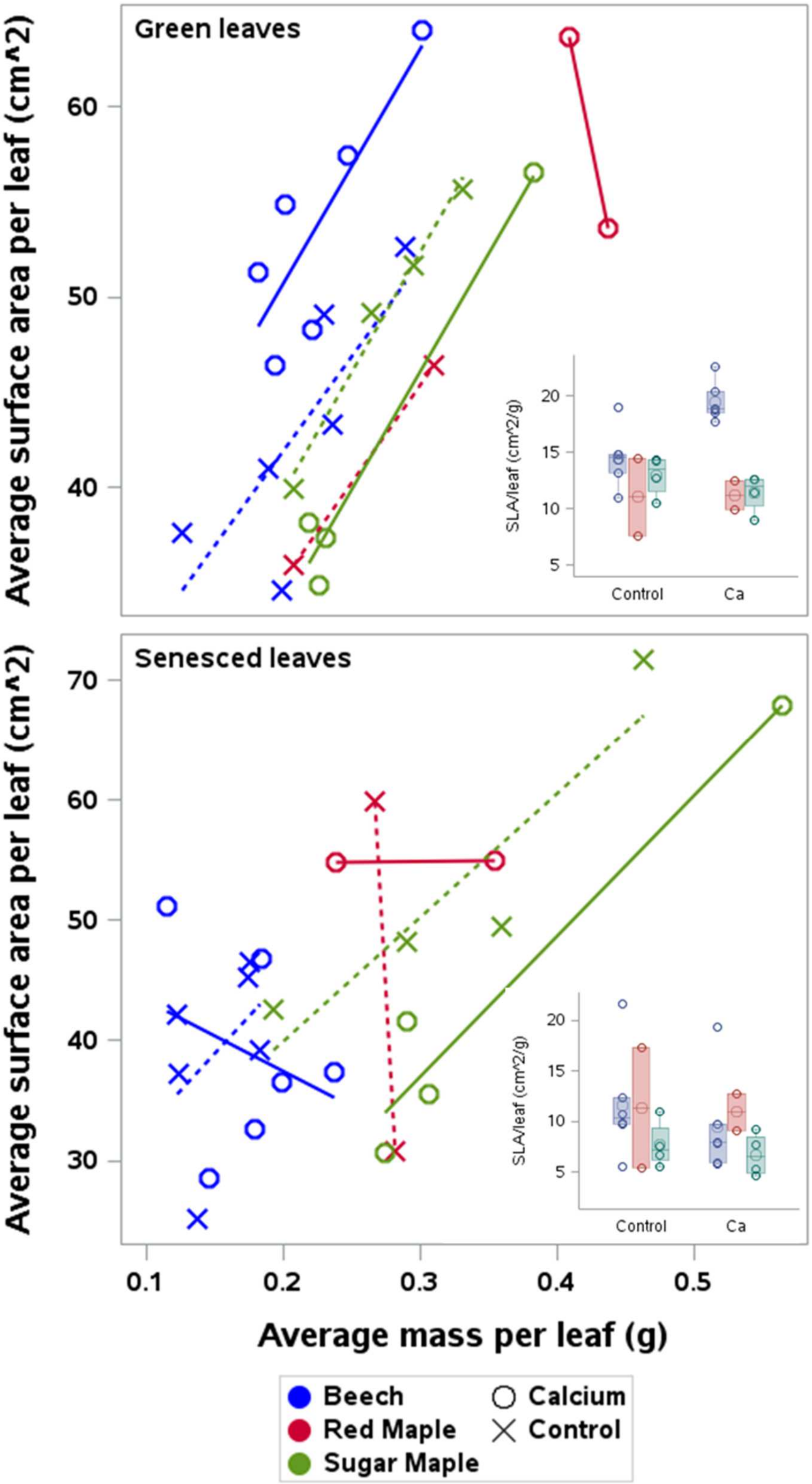


Figure 3.2. Average surface area and mass per leaf for green and senesced leaves by treatment and species. Inset boxplots depict specific leaf area.

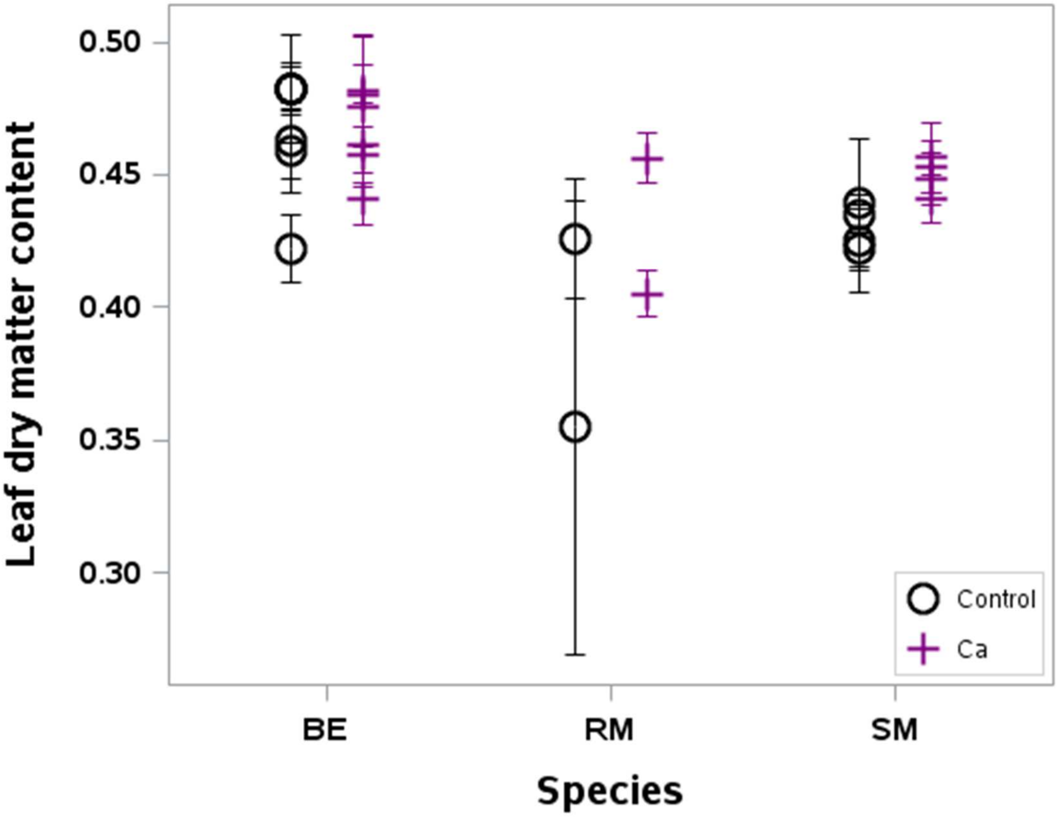


Figure 3.3. Leaf dry matter content of green leaves, calculated as the dry mass divided by the wet mass in grams. Markers represent plot means (n=3).

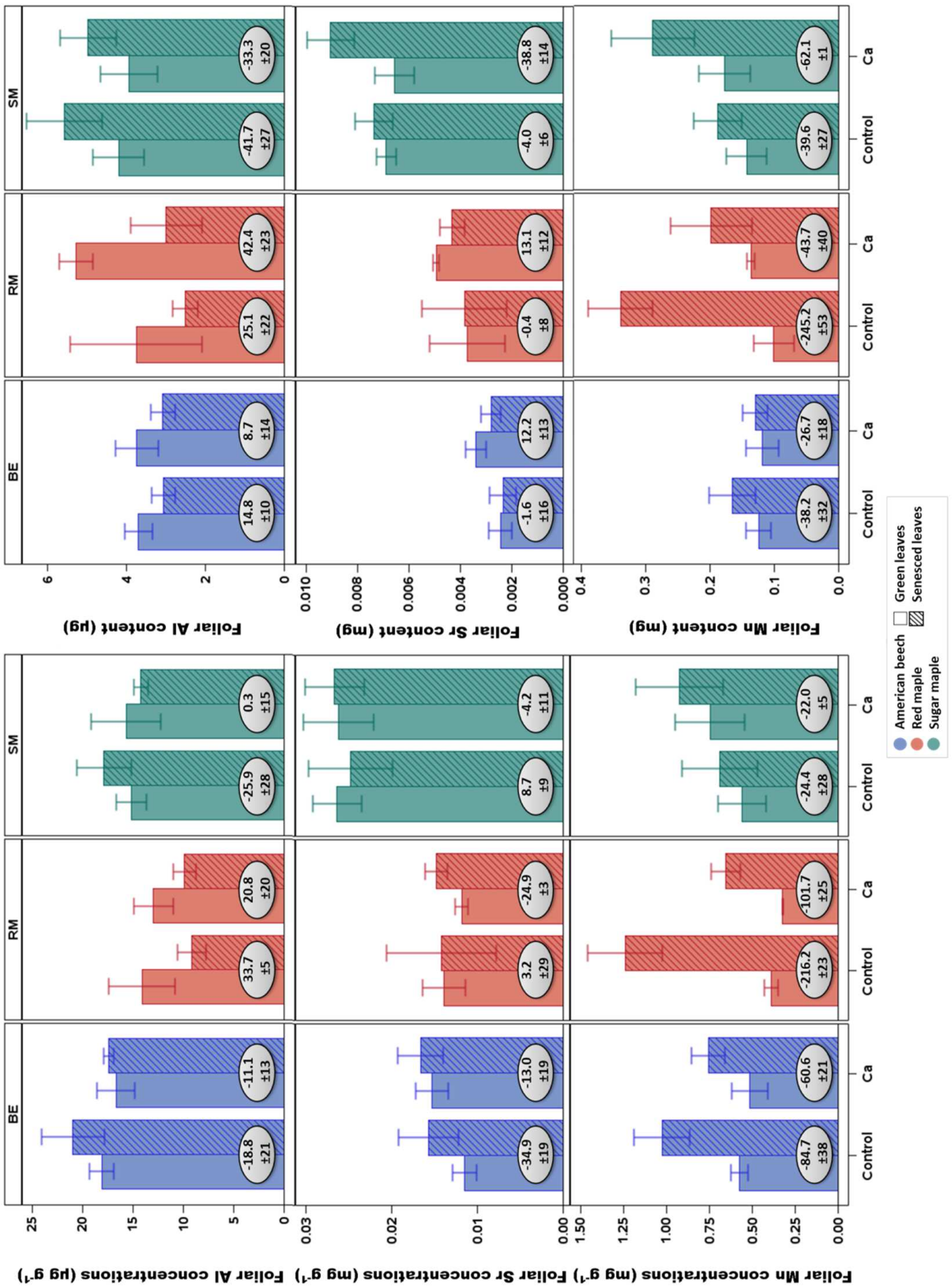


Figure 3.4. Foliar concentrations and content of metal cations in green and senesced leaves of beech (n=12), red maple (n=4), and sugar maple (n=8). Mean resorption efficiency and standard errors are in bubbles for each combination of species and treatment.

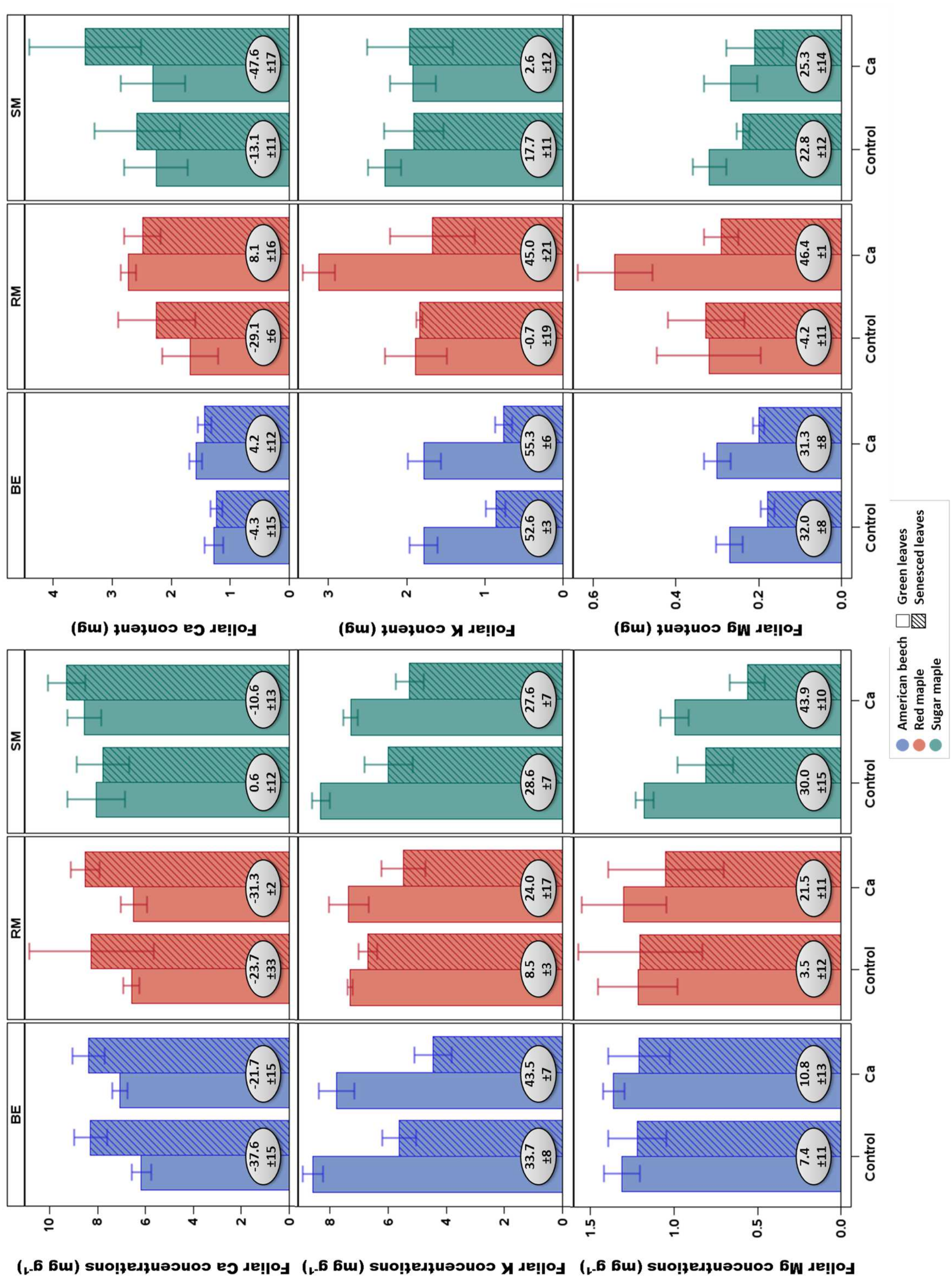


Figure 3.5. Foliar concentrations and content of base cations in green and senesced leaves of beech (n=12), red maple (n=4), and sugar maple (n=8). Mean resorption efficiency and standard errors are in bubbles for each combination of species and treatment.

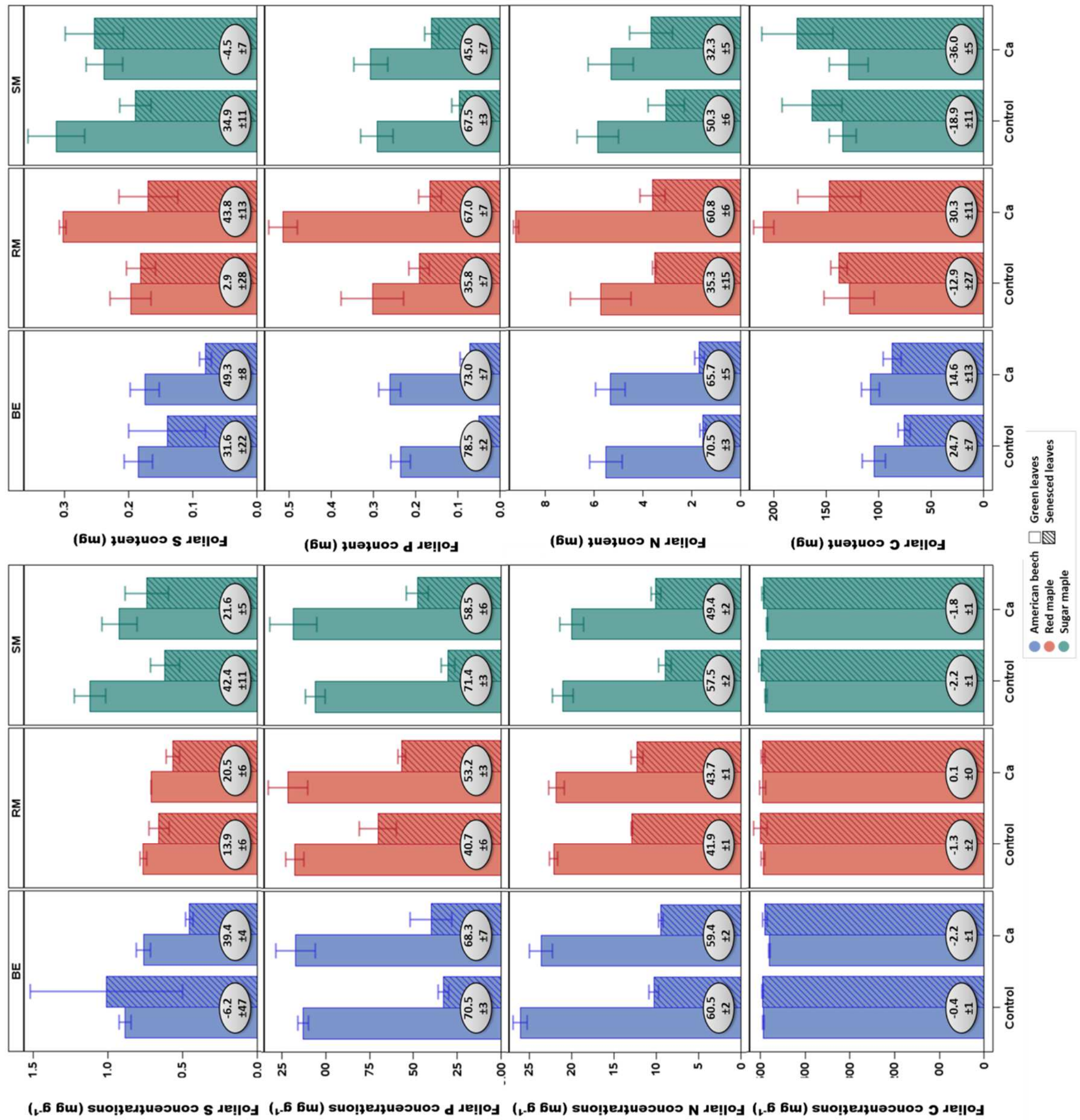


Figure 3.6. Foliar concentrations and content of other macronutrients in green and senesced leaves of beech (n=12), red maple (n=4), and sugar maple (n=8). Mean resorption efficiency and standard errors are in bubbles for each combination of species and treatment.

Appendix 1: Quality control

Table A1.1. Quality controls run during ashing and subsequent ICP for P. Blanks were less than 10% the lowest analyte value. Sample replicates were less than 15% relative percent deviation (RPD). Standard reference material (SRM) was within 10% of values given by the National Institutes of Standards and Technology. All values are in units of mg/g.

Ashing round	Blank	Rep	Sample replicates	SRM
`1	0.00	A	1.18	1.58
		B	1.16	1.57
2	0.00	A	1.06	1.57
		B	1.05	1.62
3	0.00	A	1.03	1.57
		B	0.98	1.55
4	0.0004	A	0.79	1.49
		B	0.91	NA
5	0.0006	A	0.96	1.50
		B	0.94	1.57
6	0.00005	A	1.03	1.55
		B	1.04	NA
7	0.00003	A	0.37	1.63
		B	0.38	1.54
8	0.00004	A	0.73	1.49
		B	0.72	1.53
9	0.00004	A	1.46	1.53
		B	1.50	1.50

Table A1.2. Quality control blanks and standards run for N analysis. Standards were within ≤ 10% of expected values.

Samples	Blank	Standard – aspartic acid	Standard – apple leaves	n (apple leaves)
1-88	0	10.49	2.37±0.02	4
89-141	0	10.44	2.33±0.05	5
142-204	0	10.45	2.31±0.04	6
205-229	0	NA	2.28±0.02	7
230-312	0	10.45	2.35±0.02	8

Appendix 2: Stand characteristics

During collection for green leaves in August, one observer estimated canopy dieback, defoliation, and transparency in 10% increments from the north and south aspects of each sampled individual, and then averaged these values for a whole tree estimate.

Chapter 2:

Dieback was highest in red maple and lowest in sugar maple ($p = 0.067$). Dieback was lower when N was added, especially in conjunction with P ($p = 0.098$). The mid-aged stands experienced 37% less dieback in plots fertilized with P ($p = 0.05$). Transparency, as might be expected, was 53% lower in the mid-aged stands than the older ones ($p = 0.08$) (Figure 2b). The main effects of N and P treatments on defoliation were significant, but not the interaction. Defoliation was 18% higher with N addition than without ($p = 0.05$), but 14% lower with P addition ($p=0.1$), ranging from 13.3% in the P plots to 18.0% in the N plots. The two maple species experienced more defoliation than beech ($p = 0.028$). Defoliation was negatively correlated with LDMC ($r = -0.31$) and positively correlated with foliar carbon ($r = 0.26$).

Chapter 3:

Average tree dieback and crown transparency were unaffected by treatment ($F_{1,12} < 0.38$, $p > 0.6$), but defoliation was higher in the control plots ($F_{1,11} = 4.26$, $p = 0.06$) (Figure 4), especially in the successional stands (age x treatment interaction, $F_{1,11} = 2.69$, $p = 0.1$). Red maple and beech experienced 65-75% less defoliation in the Ca plots compared to the control plots. Defoliation hovered at 15-16% in both treatments for sugar maple (species x treatment interaction, $F_{2,11} = 1.69$, $p = 0.2$).

Fine twig dieback in the canopy was not significantly affected by treatment. Despite numerous observations of relatively rapid (~ 3 y post-treatment) improvements in the crown condition of sugar maple in particular following Ca addition (Ouimet and Fortin 1992; Kolb and McCormick 1993; Wilmot et al. 1996; Long et al. 1997; Juice et al. 2006; Huggett et al. 2007; Moore et al. 2012), treatment effects on dieback or defoliation in this species four years after Ca addition were not evident in this study. The failure to detect differences in dieback could be related to high variability among individual trees. Alternatively, dieback and subsequent recovery may occur on timescales longer than this study, though effects of Ca treatment on crown condition have previously been observed only six years post-treatment (Juice et al. 2006). It seems noteworthy that, at the plot level, trees in the Ca treatments experienced less defoliation. This may be reflective of these trees having tougher leaves with lower foliar N, or may be related to a physiological response to defoliation initiated by Ca signaling (Schaberg et al. 2001).

		Age Class	
Variable	Treatment	Successional	Mature
Dieback (%)	Control	*21.01 (2.8)*	*9.38 (3.5)*
	Calcium	*20.43 (2.8)*	*13.70 (3.5)*
Defoliation (%)	Control	***17.65 (1.5)**	***9.54 (1.8)**
	Calcium	***11.53 (1.5)**	***9.00 (2.0)**
Transparency (%)	Control	20.24 (3.4)	27.67 (4.1)
	Calcium	17.21 (3.4)	27.26 (4.6)

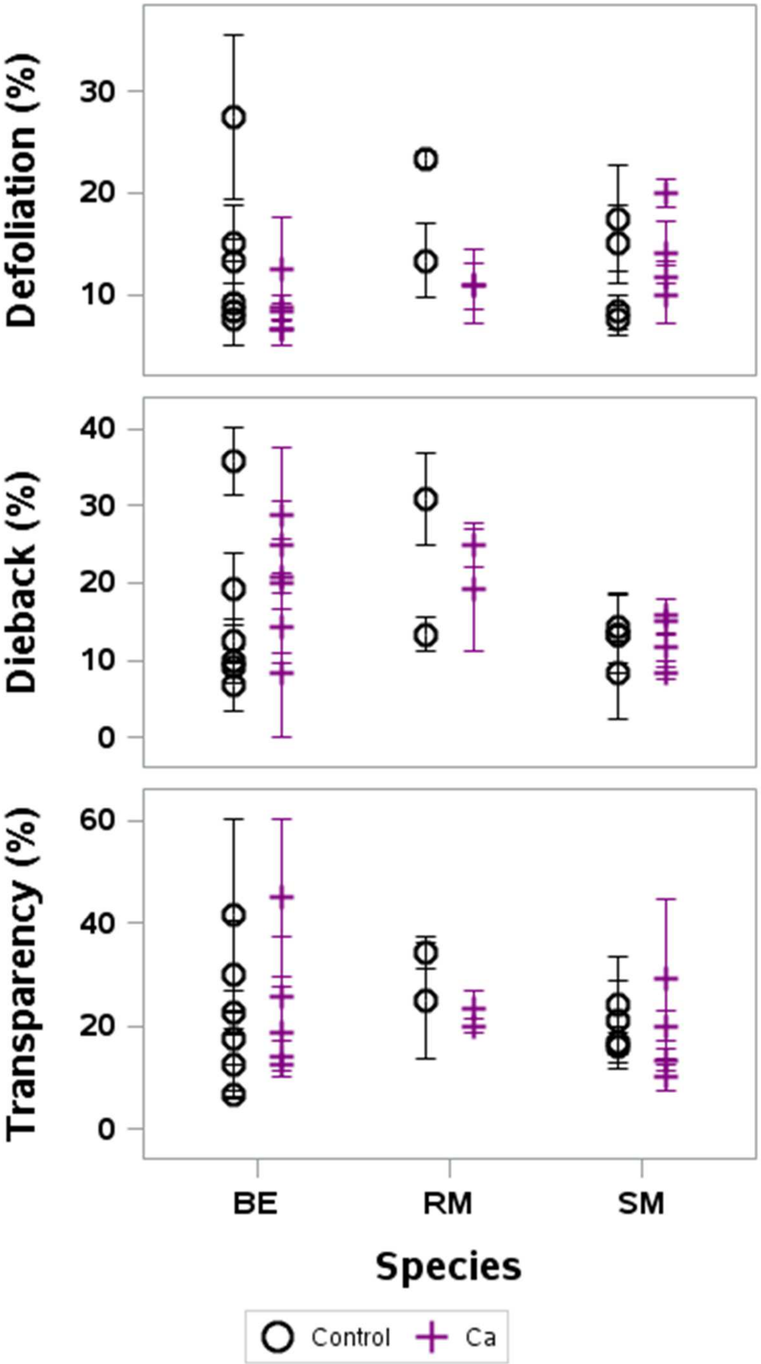
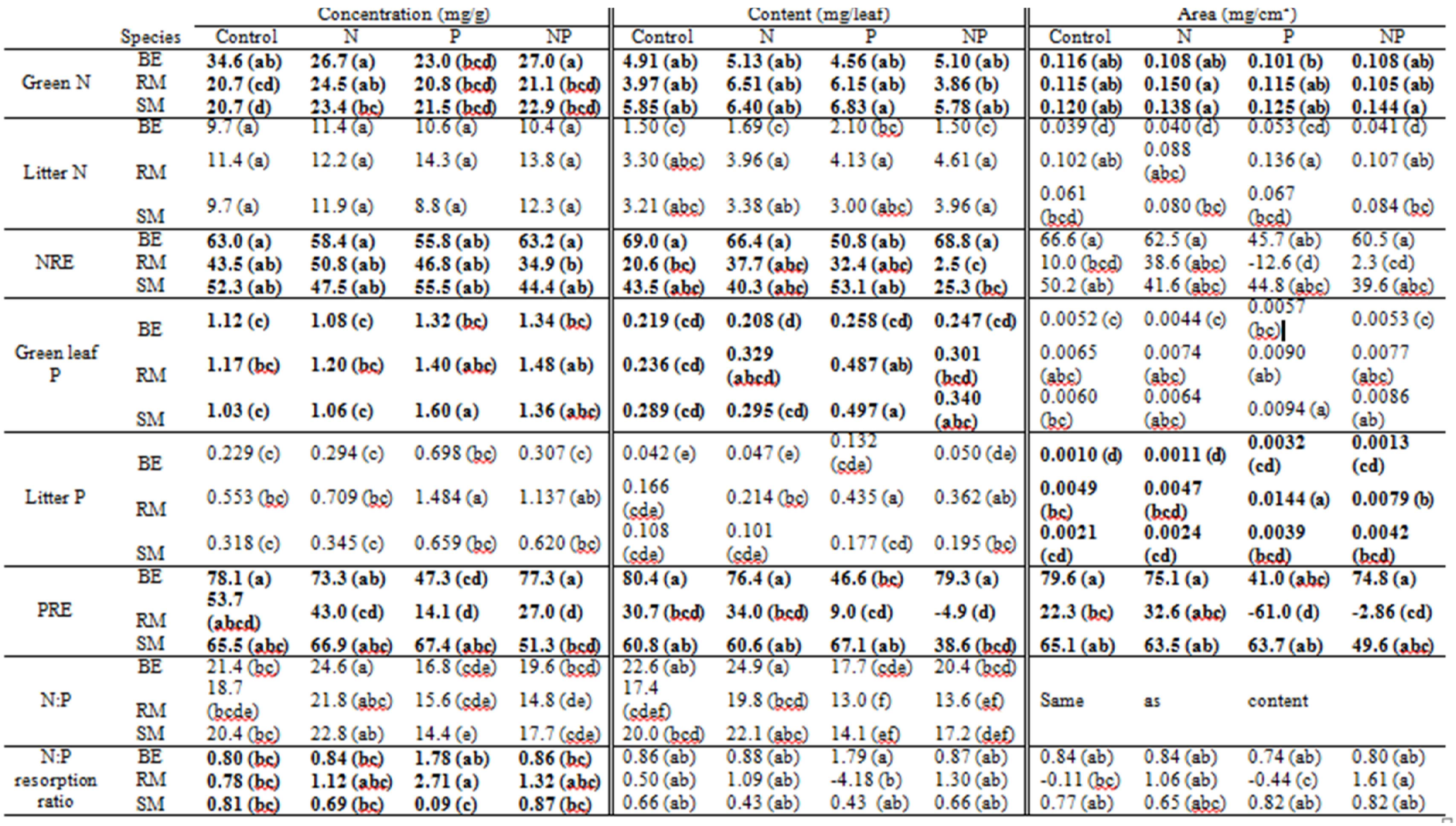


Figure A.1. Crown characteristics of sampled trees. Markers represent plot mean (n=3).

Appendix 3: Foliar nutrients and resorption as concentration, content, and area (Chapter 2)



		Concentration (mg/g)				Content (mg/leaf)				Area (mg/cm^2)			
	Species	Control	N	P	NP	Control	N	P	NP	Control	N	P	NP
Green N	BE	**34.6 (ab)**	**26.7 (a)**	**23.0 (bcd)**	**27.0 (a)**	**4.91 (ab)**	**5.13 (ab)**	**4.56 (ab)**	**5.10 (ab)**	**0.116 (ab)**	**0.108 (ab)**	**0.101 (b)**	**0.108 (ab)**
	RM	**20.7 (cd)**	**24.5 (ab)**	**20.8 (bcd)**	**21.1 (bcd)**	**3.97 (ab)**	**6.51 (ab)**	**6.15 (ab)**	**3.86 (b)**	**0.115 (ab)**	**0.150 (a)**	**0.115 (ab)**	**0.105 (ab)**
	SM	**20.7 (d)**	**23.4 (bc)**	**21.5 (bcd)**	**22.9 (bcd)**	**5.85 (ab)**	**6.40 (ab)**	**6.83 (a)**	**5.78 (ab)**	**0.120 (ab)**	**0.138 (a)**	**0.125 (ab)**	**0.144 (a)**
Litter N	BE	9.7 (a)	11.4 (a)	10.6 (a)	10.4 (a)	1.50 (c)	1.69 (c)	2.10 (bc)	1.50 (c)	0.039 (d)	0.040 (d)	0.053 (cd)	0.041 (d)
	RM	11.4 (a)	12.2 (a)	14.3 (a)	13.8 (a)	3.30 (abc)	3.96 (a)	4.13 (a)	4.61 (a)	0.102 (ab)	0.088 (abc)	0.136 (a)	0.107 (ab)
	SM	9.7 (a)	11.9 (a)	8.8 (a)	12.3 (a)	3.21 (abc)	3.38 (ab)	3.00 (abc)	3.96 (a)	0.061 (bcd)	0.080 (bc)	0.067 (bcd)	0.084 (bc)
NRE	BE	**63.0 (a)**	**58.4 (a)**	**55.8 (ab)**	**63.2 (a)**	**69.0 (a)**	**66.4 (a)**	**50.8 (ab)**	**68.8 (a)**	66.6 (a)	62.5 (a)	45.7 (ab)	60.5 (a)
	RM	**43.5 (ab)**	**50.8 (ab)**	**46.8 (ab)**	**34.9 (b)**	**20.6 (bc)**	**37.7 (abc)**	**32.4 (abc)**	**2.5 (c)**	10.0 (bcd)	38.6 (abc)	-12.6 (d)	2.3 (cd)
	SM	**52.3 (ab)**	**47.5 (ab)**	**55.5 (ab)**	**44.4 (ab)**	**43.5 (abc)**	**40.3 (abc)**	**53.1 (ab)**	**25.3 (bc)**	50.2 (ab)	41.6 (abc)	44.8 (abc)	39.6 (abc)
Green leaf P	BE	**1.12 (c)**	**1.08 (c)**	**1.32 (bc)**	**1.34 (bc)**	**0.219 (cd)**	**0.208 (d)**	**0.258 (cd)**	**0.247 (cd)**	0.0052 (c)	0.0044 (c)	0.0057 (bc)	0.0053 (c)
	RM	**1.17 (bc)**	**1.20 (bc)**	**1.40 (abc)**	**1.48 (ab)**	**0.236 (cd)**	**0.329 (abcd)**	**0.487 (ab)**	**0.301 (bcd)**	0.0065 (abc)	0.0074 (abc)	0.0090 (ab)	0.0077 (abc)
	SM	**1.03 (c)**	**1.06 (c)**	**1.60 (a)**	**1.36 (abc)**	**0.289 (cd)**	**0.295 (cd)**	**0.497 (a)**	**0.340 (abc)**	0.0060 (bc)	0.0064 (abc)	0.0094 (a)	0.0086 (ab)
Litter P	BE	0.229 (c)	0.294 (c)	0.698 (bc)	0.307 (c)	0.042 (e)	0.047 (e)	0.132 (cde)	0.050 (de)	**0.0010 (d)**	**0.0011 (d)**	**0.0032 (cd)**	**0.0013 (cd)**
	RM	0.553 (bc)	0.709 (bc)	1.484 (a)	1.137 (ab)	0.166 (cde)	0.214 (bc)	0.435 (a)	0.362 (ab)	**0.0049 (bc)**	**0.0047 (bcd)**	**0.0144 (a)**	**0.0079 (b)**
	SM	0.318 (c)	0.345 (c)	0.659 (bc)	0.620 (bc)	0.108 (cde)	0.101 (cde)	0.177 (cd)	0.195 (bc)	**0.0021 (cd)**	**0.0024 (cd)**	**0.0039 (bcd)**	**0.0042 (bcd)**
PRE	BE	**78.1 (a)**	**73.3 (ab)**	**47.3 (cd)**	**77.3 (a)**	**80.4 (a)**	**76.4 (a)**	**46.6 (bc)**	**79.3 (a)**	**79.6 (a)**	**75.1 (a)**	**41.0 (abc)**	**74.8 (a)**
	RM	**53.7 (abcd)**	**43.0 (cd)**	**14.1 (d)**	**27.0 (d)**	**30.7 (bcd)**	**34.0 (bcd)**	**9.0 (cd)**	**-4.9 (d)**	**22.3 (bc)**	**32.6 (abc)**	**-61.0 (d)**	**-2.86 (cd)**
	SM	**65.5 (abc)**	**66.9 (abc)**	**67.4 (abc)**	**51.3 (bcd)**	**60.8 (ab)**	**60.6 (ab)**	**67.1 (ab)**	**38.6 (bcd)**	**65.1 (ab)**	**63.5 (ab)**	**63.7 (ab)**	**49.6 (abc)**
N:P	BE	21.4 (bc)	24.6 (a)	16.8 (cde)	19.6 (bcd)	22.6 (ab)	24.9 (a)	17.7 (cde)	20.4 (bcd)				
	RM	18.7 (bcde)	21.8 (abc)	15.6 (cde)	14.8 (de)	17.4 (cdef)	19.8 (bcd)	13.0 (f)	13.6 (ef)	Same	as	content	
	SM	20.4 (bc)	22.8 (ab)	14.4 (e)	17.7 (cde)	20.0 (bcd)	22.1 (abc)	14.1 (ef)	17.2 (def)				
N:P resorption ratio	BE	**0.80 (bc)**	**0.84 (bc)**	**1.78 (ab)**	**0.86 (bc)**	0.86 (ab)	0.88 (ab)	1.79 (a)	0.87 (ab)	0.84 (ab)	0.84 (ab)	0.74 (ab)	0.80 (ab)
	RM	**0.78 (bc)**	**1.12 (abc)**	**2.71 (a)**	**1.32 (abc)**	0.50 (ab)	1.09 (ab)	-4.18 (b)	1.30 (ab)	-0.11 (bc)	1.06 (ab)	-0.44 (c)	1.61 (a)
	SM	**0.81 (bc)**	**0.69 (bc)**	**0.09 (c)**	**0.87 (bc)**	0.66 (ab)	0.43 (ab)	0.43 (ab)	0.66 (ab)	0.77 (ab)	0.65 (abc)	0.82 (ab)	0.82 (ab)

Table A3.1. Means for concentration are least squares means for full model with pre-treatment covariate included. Means for content and area are least squares means for the full model without a covariate. Bold type indicates a significant interaction present (α<0.1). Within each group, means with the same letter are not significantly different.

Curriculum Vita
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Education:

Jan. 2017	M.S. Environmental Science SUNY College of Environmental Science and Forestry, Syracuse, NY
Dec. 2014	B.S., Environmental Forestry and Biology SUNY College of Environmental Science and Forestry, Syracuse, NY

Teaching Experience:

2016	Graduate Assistant, SUNY ESF FOR 205, Accounting, Mr. Robert Kawa
2015	Graduate Assistant, SUNY ESF EFB 120, Global Environment, Dr. Rick Beal
2015	Graduate Assistant, SUNY ESF FOR 454, Renewable Energy Finance and Analysis, Mr. Michael Kelleher
2015	Graduate Assistant, SUNY ESF FOR 205, Accounting, Mr. James Fletcher
2010	Undergraduate Teaching Assistant, SUNY ESF EFB 120, Global Environment, Dr. Charles A.S. Hall

Research Experience:

2015-2016	Master's Thesis Research, SUNY ESF Graduate Program of Environmental Sciences, Dr. Dylan Parry and Dr. Ruth Yanai
2015-2016	Research Fellowship, SUNY ESF Department of Forestry and Natural Resources, Dr. Ruth Yanai
2014	Undergraduate Independent Study, SUNY ESF Department of Forestry and Natural Resources, Dr. Ruth Yanai

Presentations:

2016	Lasser GA, Hong DS, Yang Y, Gonzales KE, Yanai RD. Effects of nitrogen, phosphorus, and calcium on foliar characteristics of pin cherry, American beech, yellow birch, and white birch. Rochester Academy of Sciences. Rochester, NY. Poster presentation.
2016	Gonzales KE. Phosphorus biogeochemistry and biodiversity: Venturing into the unknown. Hubbard Brook Committee of Scientists Meeting. Millbrook, NY. Oral presentation.

2016	Phelps KE and RD Yanai. Interconnected cycles of N and P: resorption in a northern hardwood forest. Ecological Society of America 101st Annual Meeting. Fort Lauderdale, FL. Poster presentation. Travel funded by travel grants from the Graduate Student Association and the Office of Graduate Studies, SUNY-ESF.
2016	Phelps KE. Foliar nutrient resorption reflects interactions of N and P availability. Hubbard Brook Annual Cooperator’s Meeting. Woodstock, NH. Oral presentation.
2016	Phelps KE, Yanai RD, and CR See. Interconnected N and P cycles in a northern hardwood forest. SUNY Spotlight on Student Research. Syracuse, NY. Poster presentation.
2015	Phelps KE, Yanai RD, and CR See. Interconnected N and P cycles in a northern hardwood forest. Rochester Academy of Sciences. Rochester, NY. Poster presentation.
2015	Hubbard Brook Annual Cooperator’s Meeting. Woodstock, NH. Oral presentation.

Outreach:

2016	Volunteer, Helping Hounds Dog Rescue
2015-16	GPES Representative, Graduate Student Association
2015	Volunteer Judge, Central New York Science and Engineering Fair

Technical and Computer Experience:

- Microsoft Office Suite
- SAS 9.4
- Minitab
- ArcGIS
- 80-90 wpm typing
- Microsoft Front Page
- Adobe programs, including Contribute
- Stella
- ImageJ

Foreign Language Experience:

Fluent:	Spanish
Familiar:	French Portuguese Italian

Professional Membership:

2015-present	New York Academy of Science

Other Work Experience:

2014	Contact Paving R Way Summer Bridge Program – Teacher
2014	Ryan Painting and Renovations – Painter
2013	Redhouse Café – Barista
2012-13	Starbucks – Barista