EFFECTS OF NUTRIENT ADDITION ON FOLIAR TRAITS, FOLIAR AND LITTER CHEMISTRY, AND RESORPTION EFFICIENCY IN NORTHERN HARDWOOD FORESTS

By

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ABSTRACT

J.M. Zukswert. Effects of Nutrient Addition on Foliar Traits, Foliar and Litter Chemistry, and Resorption Efficiency in Northern Hardwood Forests, 239 pages, 13 tables, 19 figures, 2023. Springer Basic style guide used.

Long thought to be nitrogen (N)-limited, temperate forests in northeastern North America respond to the addition of other elements such as phosphorus (P) and calcium (Ca), suggesting multiple element limitation. Foliage serves a significant role in forest nutrient cycling and is often used to indicate nutrient availability and limitation. This dissertation explored characteristics of foliage and litter in a long-term, fully factorial nitrogen and phosphorus addition study to better understand the ways in which foliage responds to and indicates nutrient limitation; the study also included a more limited set of calcium addition plots Tree diameter growth in these forests was recently found to respond more to the addition of both N and P than either N or P alone, indicating that these trees are co-limited by N and P. In the first chapter, I provide comprehensive background for the dissertation. The second chapter reports the responses of five foliar traits to N and P addition in six northern hardwood species and at the community level. The third chapter reports responses of gas-exchange traits in *Betula alleghaniensis* (yellow birch) and *Acer saccharum* (sugar maple), specifically stomatal density, stomatal length, and carbon isotope composition, to N, P, and calcium silicate ($CaSiO_3$) addition. The fourth chapter reports changes in foliar and litter nutrient concentrations and resorption efficiency at the community level after three to five and nine to ten years of treatment. All these studies demonstrate effects of both N and P addition on foliar or litter characteristics, and the directions of these effects are consistent with N and P co-limitation in these forests. Foliar and litter N and P results suggest that N addition might increase P bioavailability, but P addition does not seem to increase N bioavailability. At least one response to N, P, or $CaSiO_3$ was detected in most traits or characteristics, and species varied in their responses to nutrient addition. The biological mechanisms suggested by these results can help predict the effects of changes in nutrient availability in light of nutrient limitation.

Key words: foliage, leaf litter, nutrients, traits, resorption, limitation

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CHAPTER 1: NUTRIENT LIMITATION AND THE ROLE OF LEAVES

Foliage plays a significant role in forest nutrient cycling despite its small biomass. The chemical and physical characteristics of leaves and the extent to which leaves resorb nutrients during senescence all contribute to the rates of nutrient cycling and ultimately to soil formation in forests. Nutrient availability influences how well plants grow, but plants can also alter their environment through a variety of mechanisms, including through the production of foliage and litter, potentially modifying their environment through positive feedback loops to produce more of the nutrient conditions in which they thrive (Hobbie 2015). Foliage and litter are also useful for understanding nutrient limitation of individual plants, species, and communities in that plants may resorb proportionally more of a limiting nutrient to increase efficiency and resorb less when the nutrient limitation is alleviated (Killingbeck 2004), though evidence for this is mixed. Characteristics of foliage and litter differ by species, meaning that processes influencing the relative species abundance of plant communities, such as succession and nutrient addition, can subsequently influence nutrient cycling in forest ecosystems. My dissertation examines characteristics of foliage and litter in a long-term, fully factorial nitrogen and phosphorus addition study, with extra calcium-addition plots on site, to better understand the ways in which foliage respond to and indicate nutrient limitation and alleviations thereof.

Nutrient Limitation

Nutrients are considered “limiting” when adding them increases the rate of a biological process or the size of an ecosystem component (Vitousek et al. 2010). Plants, for example, are “limited” by nutrients if adding that nutrient increases their growth and consequently their biomass. Limiting nutrients can be “ultimate”, meaning that the addition of this nutrient can transform ecosystems, or “proximate”, meaning that the addition of this nutrient can produce a short-term increase in plant growth (Chapin et al. 2011). Nitrogen (N) and phosphorus (P) are commonly limiting nutrients, both ultimate and proximate, in many terrestrial ecosystems.

Nitrogen and phosphorus are essential macronutrients in plant metabolism, which helps explain why they are so often limiting. Nitrogen is a necessary component of amino acids and nucleic acids and is required in relatively large amounts; of all plant macronutrients, N is required in the greatest supply (Taiz et al. 2015). Nitrogen is a critical component of the enzyme RuBisCO, which is crucial for photosynthesis. It is also found in chlorophyll; between 50 to 80% of foliar N is found in chloroplasts (Makino and Osmond 1991; Estiarte et al. 2022). Phosphorus is a necessary component of nucleic acids and adenosine triphosphate (ATP), and it is found in the phospholipids that compose all cell and organelle membranes (Schlesinger and Bernhardt 2013). A significant portion of P in cells is inorganic, and the chemical forms and distribution of P in cells can be more variable than that of N (Estiarte et al. 2022). Phosphorus in forests originates from rocks and minerals and is then recycled through the forest floor and soil, released primarily by phosphatase enzymes (Chapin et al. 2011; Binkley and Fisher 2013). This pool of recycled P is the main P source for plants and other biota, as atmospheric P deposition and weathering inputs account for a small fraction of the P cycle (Schlesinger and Bernhardt 2013).

Temperate forests at high latitudes are often assumed to be N-limited and tropical forests at low latitudes assumed to be P-limited (Vitousek et al. 2010). This assumption is informed by Walker and Syers's (1976) study of P along a soil chronosequence, in which they explained that P was in high supply in relatively young soils, but slowly becomes depleted over time as rocks and minerals weather. Meanwhile, the buildup of N in ecosystems can take a long time, so many young soils have relatively high levels of P but are limited by N (Walker and Syers 1976). We now know that these latitudinal patterns do not always hold and that temperate forests can be P-limited due to parent material having naturally low P, soil layers that prevent roots from accessing P, loss of P through leaching, and anthropogenic causes, such as N deposition (Vitousek et al. 2010).

Forests in northeastern North America have had significant N deposition over time as a result of fertilizer application and fossil fuel combustion (Driscoll et al. 2003); the dissolution of NO_x emissions in precipitation contributes to the phenomenon of acid rain (Schlesinger and Bernhardt 2013). The greater inputs of N relative to P over time have been shifting N:P ratios in organisms in many locations, which can exacerbate P limitation, reducing photosynthesis rates and carbon storage (Peñuelas et al. 2013). While N deposition is widespread across many areas across the globe, N deposition has been decreasing in recent years in the United States as a result of the Clean Air Act Amendments of 1991, particularly in the eastern United States (Lloret and Valiela 2016). Recent studies have shown that the effects of anthropogenic N deposition are highly spatially heterogeneous, and that some areas are N limited and going through a reverse eutrophication process, or "oligotrophication" (Groffman et al. 2018; Mason et al. 2022). Climate change is also expected to exacerbate N limitation, as the availability of CO_2 is increasing; observations of decreasing foliar N concentrations over time provide evidence for this process (Wang et al. 2020; Mason et al. 2022). The status of N and P availability and limitation in northern forest ecosystems are therefore important to characterize spatially, temporally, and in light of climatic and anthropogenic changes.

While N and P are the most commonly limiting nutrients, plants require many other macro- and micronutrients, which can also be limiting in certain circumstances. Two other nutrients that can be limiting include potassium (K) and calcium (Ca). K is involved in regulating the opening and closing of stomata and serves as a cofactor for many enzymes (Taiz et al. 2015). Because it has one valence electron and does not form covalent bonds, K is easily leached from leaf litter, often before the litter falls (Binkley and Fisher 2013). K is not studied as often in the context of limitation in ecosystem ecology, , not nearly as often as N and P, but in many cases does increase aboveground productivity when added, suggesting potential limitation (Tripler et al. 2006; Sardans and Peñuelas 2015). Sugar maple (*Acer saccharum* Marsh.) can be limited by K, particularly when magnesium (Mg) levels are high relative to K (Ouimet and Camiré 1995). Potassium can also limit tree growth in soils that are sandy, organic, or old (Binkley and Fisher 2013).

Calcium serves a structural role within plants by cross-linking pectins, thereby providing stability to cells and cell membranes, and serves a signaling role in response to environmental stimuli (Schaberg et al. 2001; Taiz et al. 2015). Concern around the availability of Ca in northeastern forests heightened in the late 20th and early 21st century due the effects of acidic deposition on soil chemistry (Likens et al. 1996), which, with loss of Ca due to forest harvesting, might increase Ca deficiency in trees (Federer et al. 1989). Calcium deficiency can have far-reaching effects on overall tree and thereby forest health (Schaberg et al. 2001). Indeed, Vadeboncoeur (2010) documented Ca limitation in forests of northeastern North America, in addition to N and P, from a meta-analysis of nutrient-addition studies, though he indicated that Ca limitation itself may be difficult to determine due to the effect that most Ca fertilizers concurrently have on soil pH.

Single vs. Multiple Resource Limitation and Coupled Nutrient Cycles

Conceptualizing plants or communities as being limited by a single nutrient, such as N or P, has been a common paradigm in ecology, reflected in Liebig's Law of the Minimum. According to the Law of the Minimum, only one nutrient is limiting at a time, with the single nutrient that is present in the lowest supply relative to its demand being the limiting one (Chapin et al. 2011). We now know, however, that biogeochemical cycles of different elements are interconnected and accordingly that plants and ecosystems can be limited by multiple resources, or co-limited. Limitation by both N and P may be more common than previously thought (Elser et al. 2007). In northeastern North American forests in particular, there is evidence of multiple element limitation by N, P, and Ca (Vadeboncoeur 2010). These recent findings challenge Walker and Syers's (1976) conceptualization of N and P limitation and the Law of Minimum.

Hypotheses surrounding multiple resource limitation date back to at least the 1980s, when authors such as Bloom et al. (1985) and Chapin et al. (1987) employed a cost-benefit analysis approach to studying plant growth. In their economic analogy, Bloom et al. (1985) liken a plant to a business firm, acknowledging that plants acquire resources (such as water, nutrients, and carbon) from the environment that they can either "save" or "spend" to construct a "product" (such as leaves, stems, and roots) that can in turn assist the plants in acquiring additional resources. One of the major points in their economic analogy is that the relative costs of resources can be more important than the absolute costs. They concluded that plants optimize their allocation of resources such that the benefit-to-cost ratio of each resource is similar. They do this by adjusting how they allocate their "internal reserves" (e.g., carbon, nutrients, and water in plant biomass) to acquire scarce resources or reduce demand for other resources. In this way, limitation is more complex than the Law of the Minimum implies, suggesting that plants are simultaneously limited by multiple resources. Chapin et al. (1987) provided more specific examples of this analogy, illustrating how the internal balance of carbon and N relative to the environmental supply could influence how plants acquire and assimilate N. Following these economic analogies, Rastetter and Shaver (1992) developed a theoretical model to explain and predict multiple element limitation of primary production and plant biomass accumulation, initially simulating the behavior of two unspecified elements (E_1 and E_2, which could be C and N, or N and P, etc.) in their model. This simulation model is now referred to as the MEL model. More recent versions of the MEL model include multiple limiting resources (C, N, P, light, and water) and can be used to predict changes in nutrient limitation following disturbance, such as forest harvesting (Rastetter et al. 2013) and climate change (Rastetter et al. 2022).

Around the time that economic analogies of plant growth and biomass allocation began to emerge, and the MEL model was published, existing generalizations about the primary limiting nutrients in terrestrial and aquatic ecosystems were coming into question. While it was assumed that N was the primary limiting nutrient in terrestrial and marine ecosystems (Vitousek & Howarth, 1991) and P was the primary limiting nutrient in lakes (Schindler 1977), research began to show that N and P could be equally limiting of primary production in lakes and streams (Elser et al. 1990; Francoeur 2001). These new findings led Elser et al. (2007) to conduct a meta-analysis on N and P addition studies across ecosystems types to quantify how common co-limitation is. They found that co-limitation is much more prevalent across ecosystems than previously thought, and that N and P are equally limiting in freshwater, marine, and terrestrial environments.

Elser et al. (2007) defined co-limitation operationally as the synergistic effect of both N and P addition, as compared to N and P addition alone. Since then, others have delved deeper into possible mechanisms of co-limitation, types of co-limitation, and potential outcomes of limitation. Davidson and Howarth (2007) responded to the Elser et al. (2007) meta-analysis by calling for the need to better understand mechanisms underlying co-limitation as well as clarification around dosages applied. For example, adding too much of both nutrients might indicate co-limitation but might also just mean that the first nutrient limitation is alleviated and then the second, which is still consistent with Liebig's Law of Minimum and would not suggest co-limitation.

Harpole et al. (2011) responded to Davidson and Howarth (2007)'s call for more research on co-limitation mechanisms with an updated meta-analysis of global N and P addition studies, defining several types of co-limitation and searching for evidence of them in a global dataset. Harpole et al. (2011) define co-limitation as *simultaneous*, which is when an increase in biomass or productivity occurs only when both co-limiting nutrients are added together, or *independent*, which occurs when biomass increases when nutrients are added independently. The addition of both co-limiting nutrients can be super-additive, additive, or sub-additive. They also acknowledge *synergistic* co-limitation, which more closely matches the definition of co-limitation put forth by Elser et al. (2007), in which the addition of both resources results in an interactive increase in biomass or production. This could occur by simultaneous co-limitation or by super-additive independent co-limitation. It could also occur by *serial* limitation, in which Liebig's Law of Minimum holds true and an increase in biomass with the addition of both nutrients is due to sequential alleviation of nutrient limitation. Harpole et al. (2011) found that 28% of the studies in their meta-analysis exhibited signs of simultaneous or independent co-limitation, while 22% showed evidence of serial limitation. In this way, they suggest that strictly defined co-limitation may be less common than Elser et al. (2007) predicted, and that more studies were needed to address co-limitation mechanisms.

Effects of Nutrient Limitation and Availability on Foliar Traits

The relative availability of resources in the environment can influence plant physiology and morphology. These influences may be expressed as morphological and physiological differences among tree species (e.g., differences in leaf size and shape), but can also be expressed as differences within species or individuals found in various habitats. Some differences in plant physiology and morphology that relate to resource availability are genetic, but changes in plant morphology and physiology due to changes in relative resource availability can also occur during the lifetime of a tree in response to changes in the environment. Changes in a tree's physiology in response to environmental changes can result in morphological changes, some of which may be adaptive; this phenomenon is called plasticity (Bradshaw 1965). Foliar traits, in this way, can be useful for understanding differences among, and changes within, species and individuals in response to a variety of environmental conditions.

Traits are measurable characteristics of organisms that provide an indication of that organism's fitness or performance. Many foliar traits, in particular foliar "functional" traits, are thought to influence plant growth, survival or reproduction (Violle et al. 2007). These foliar traits have been shown to reflect life-history strategies that occupy a spectrum from more "acquisitive," fast-growing plants with short-lived leaves (higher specific leaf area, higher foliar nitrogen and phosphorus concentrations, greater photosynthetic capacity) to more "conservative," slow-growing plants with long-lived leaves (lower specific leaf area, lower foliar nutrient concentrations, lower photosynthetic capacity; Diaz et al. 2004). These relationships are now referred to as the "leaf economics spectrum" (Wright et al. 2004). The original foliar traits included in this spectrum were specific leaf area (SLA), foliar N, foliar P, leaf lifespan, leaf dark respiration, and photosynthetic capacity, and subsequent studies have identified that leaf dry matter content (LDMC) falls along this spectrum as well (e.g., Smart et al. 2017) . This spectrum, and other similar economic spectra for wood and stem traits (Chave et al. 2009; Baraloto et al. 2010) and for whole-plant traits (Reich 2014; Díaz et al. 2016), capture the concept that that trait values tend to co-vary in ways that reflect the tradeoffs plants face between devoting resources to acquiring nutrients and growing quickly versus conserving nutrients and persisting longer.

Higher foliar N concentrations in leaves tends to be associated with plants that have a more "acquisitive" strategy, with faster growth rates and higher photosynthetic capacity. In turn, this tends to correspond with faster N mineralization rates (Ollinger et al. 2002). This association of high foliar N with an acquisitive resource strategy is partly because most N in foliar is associated with chloroplasts and other molecules associated with photosynthesis, namely RuBisCO and chlorophyll (Makino and Osmond 1991). In many cases, as expected, foliar N increases with N addition, particularly if N is limiting (e.g., (Diekmann and Falkengren-Grerup 2002). Nitrogen addition might lead to the production of more photosynthetic enzymes and pigments, which in turn increases the capacity for growth and photosynthesis (Zhang et al. 2018a). In areas where N is not strongly limiting, however, all tree species may not respond in the same way. In the Adirondacks, for example, only *A. saccharum* (sugar maple) and *Betula alleghaniensis* Britton (yellow birch) responded to N addition with foliar N increase (Lovett et al. 2013). A lack of foliar N response to N addition can also be observed in nutrient-poor environments where species tend to exhibit more conservative strategies and resist changes in the environment (e.g., Scalon et al. 2017). Foliar N concentrations can decrease with P addition if P is limiting in an ecosystem, though if trees are co-limited, it is possible that foliar N could either not change or increase in response to P (Bracken et al. 2015). Co-limitation is possible at the community level if some species are limited by N and some are not, such as if some species respond to P addition with a decrease in foliar N, and to N addition with a decrease in foliar P (Bracken et al. 2015).

High foliar P also tends to correspond with a more acquisitive strategy, as it often correlates with foliar N, SLA, and photosynthetic capacity along the leaf economics spectrum (Wright et al. 2004). Foliar P is an important component of nucleic acids such as DNA and RNA, lipid membranes (e.g., phospholipid bilayers), and energy molecules such as ATP. Unlike N, where most N in foliage is found in proteins, P is stored in a greater variety of molecule types, with 58% stored as inorganic P stored in the cytoplasm and vacuoles (Veneklaas et al. 2012) and the remaining organic P divided into nucleic acids, lipids, and esters (Estiarte et al. 2022).

Typically, foliar P increases with P addition (Yuan and Chen 2015; You et al. 2021), as expected. Plants that are growing in more P-rich soils and have higher foliar P concentrations tend to have higher proportions of easily soluble metabolic P (e.g., inorganic P, ATP, sugar phosphates; Hidaka and Kitayama 2011; Tsujii et al. 2017). Increases in this P pool in foliar cells could lead to the increase of free and bound water in leaves (Singh et al. 2006). Increases in foliar P tend to be greater with P addition alone than with both P and N addition, indicating an interaction of N and P (You et al. 2021). As with N, responses of foliar P to N addition are variable and might relate to which nutrient is most limiting in those ecosystems. For example, a recent meta-analysis of nutrient-addition studies showed that foliar P decreased with N addition in conditions where only N was limiting, but increased with N addition in co-limiting conditions or conditions where P was limiting (Zhang et al. 2022). Mechanistically, foliar P can increase with N addition due increases in phosphatase production and activity with N addition, which increases P availability (Marklein and Houlton 2012). In the Adirondacks, N addition did not influence foliar P concentrations of several northern hardwood tree species (Weand et al. 2010), which could suggest in this case that N is not the primary limiting nutrient in these forests. Foliar P concentrations in woody plants did not change with N addition in woody plants in a study in Brazil, but foliar P did not change with P addition, either (Scalon et al. 2017);

the woody plants in the neotropical savanna ecosystem that was studied tend to have more “conservative” trait values, which suggests that the responsiveness of traits like foliar P to N and P addition could relate to the resource allocation strategy of the species studied. Species can also differ in their response of foliar P to nutrient addition based on their associated mycorrhizal associations; for example, foliar P of ectomycorrhizal-associated species has been shown to decrease with N addition, but a decrease was not observed in arbuscular mycorrhizal-associated species (Yang et al. 2019), which tend to be more effective at acquiring P (Lambers et al. 2008).

Specific leaf area (SLA) tends to co-vary with foliar N and P along the leaf economics spectrum (Wright et al. 2004). It therefore tends to represent an acquisitive strategy, with high SLA associated with greater photosynthetic capacity (Wright et al. 2004), faster relative growth rate, and greater N absorption rate in roots (Osone et al. 2008). If an increase in SLA within the lifetime of a plant corresponds with a faster relative growth rate, then we might predict the SLA would increase in response to the addition of a limiting nutrient.

SLA has been measured in several past nutrient-addition studies, mostly in response to N addition rather than P. In many cases, SLA did not respond to nutrient addition. For example, Zhang et al. (2018) failed to detect an effect of N addition on SLA in woody plants in their meta-analysis of N-addition studies. Specific leaf area did not respond to N or P addition in factorial N x P-addition studies in a woody savanna in Brazil (Scalon et al. 2017) or tropical forests in Hawaii (Ostertag 2010). In a factorial N x P-addition in southern China, however, P addition only increased SLA for one of the six studied species, and this species occurred in sites that were known to be phosphorus limited (Mo et al. 2020). This result suggests that species may vary in their response to nutrient addition and is consistent with SLA increasing in response to addition of a limiting nutrient, which is consistent with shift in trait values towards a more acquisitive strategy. Similarly, SLA increased with simultaneous N, P, and K addition in four species of alpine shrubs in a northern Siberian tundra (Iturrate-Garcia et al. 2020). These results collectively suggest that responses of SLA to nutrient addition are mixed, may vary by species, and, when there is a detectable effect, it is typically an increase in response to addition of a limiting nutrient.

Since the development of the leaf economics spectrum, more traits, such as leaf dry matter content (LDMC), have been found to co-vary along this spectrum. Leaf dry matter content is the oven-dry mass of a leaf divided by the water-saturated fresh mass and represents the average density of leaves (Pérez-Harguindeguy et al. 2013). It tends to negatively correlate with foliar P, N and SLA, and positively correlate with C:N, which suggests that high LDMC is more associated with a conservative resource strategy (Zukswert and Prescott 2017; Hecking et al. 2022).

Responses of LDMC to nutrient addition or soil fertility are mixed, but most studies suggest that low LDMC is associated with high soil fertility and that LDMC decreases with nutrient addition. Daou et al. (2021), for example, found that the community-weighted mean of LDMC decreased as soil fertility increased across 21 grassland sites in Quebec, Canada. In nutrient-addition studies involving herbaceous species, addition of N, P, and K together decreased LDMC in nine species in an old field ecosystem in central New York (Siefert and Ritchie 2016), while in an N x P x K factorial experiment in a semi-arid grassland in a Nutrient Network ("NutNet") site in Nebraska, only N influenced leaf functional traits, resulting in an 11% decrease in LDMC, with LDMC decreases evident within species (Tatarko and Knops 2018). Previous studies of nutrient effects on woody plants show mixed results. In four alpine shrub species, for example, LDMC decreased with simultaneous N, P and K addition (Iturrate-Garcia et al. 2020). In a coffee plantation, however, LDMC did not change with N addition (Buchanan et al. 2019). Interestingly, LDMC increased with N addition in P-limited Mongolian pine plantations (Zheng et al. 2017a). It could be, therefore, that LDMC decreases with the addition of limiting nutrients, and does not change or even increases with the addition of non-limiting nutrients.

Stomatal density is another trait that has been studied in an economics-spectrum context (Loranger and Shipley 2010). Stomata are the pores on leaf surfaces through which gases and water vapor pass during photosynthesis and transpiration. Both the density of stomata on leaf surfaces and the length of these stomata, which are negatively correlated, influence the capacity for gas exchange and transpiration (Hetherington and Woodward 2003). While they may not always correlate well with observed stomatal conductance, these traits can influence the maximum stomatal conductance, and thereby shed light on the capacity for trees to photosynthesize and transpire (Liu et al. 2018). At least in herbaceous plants, stomatal pore index, which is the product of density and length squared, is negatively related to SLA and area-based foliar N for many species (Bucher et al. 2016).

Stomatal density has a strong genetic component (Shimada et al. 2011) but is also influenced by environmental factors. Stomatal density tends to be higher with high light availability (Gay and Hurd 1975) and atmospheric CO_2 concentrations (Woodward and Kelly 1995) and lower with high soil moisture (Sun et al. 2014). Stomatal density can increase or decrease with increasing temperature (Beerling and Chaloner 1993; Hill et al. 2014). Previous studies on the effects of nutrient availability on stomatal density have produced inconsistent results

(Bertolino et al. 2019). Few of these studies examining the relationships between nutrient addition and stomatal density have investigated woody plants.

Responses of stomatal density and length to N addition are mixed but primarily show an increase in density and decrease in length. Stomatal density was higher in hybrid poplar (*Populus* x *euramericana* Guinier) clone cuttings grown with higher soil N in a controlled, growth chamber experiment (Siegwolf et al. 2001). Stomatal density in salmonberry (*Rubus spectabilis* Pursh) was also positively correlated with the density of salmon in rivers in British Columbia; the elevated N from the salmon carcasses could be related to the increase in stomatal density (van den Top et al. 2018). In an N x P-addition study of woody plants in a Brazilian savanna, stomatal pore index increased in response to nutrient addition, primarily due to a decrease in stomatal length, though species-specific responses varied (Costa et al. 2021). Under controlled laboratory conditions, stomatal density in *Arabidopsis thaliana* L. decreased with N addition and did not respond to P addition (Cai et al. 2017).

Several studies have been conducted to observe the effect of P addition on stomatal density, and most of these studies have occurred in nonwoody plants. These studies document an increase in stomatal density with P addition, but only under specific conditions. Stomatal density increased with P addition in cowpea (*Vigna sinensis* (L.) Savi ex Hassk.), but this P effect interacted with the effects of soil water and CO_2; in particular, elevated CO_2 increased stomatal density more under high soil P than low soil P conditions, but elevated soil water increased stomatal density under high soil P but not low soil P (Sekiya and Yano 2008). Similarly, stomatal density increased in chickpea (*Cicer arietinum* L.) under P addition and irrigation, suggesting that an increase in stomatal that an increase in stomatal density with P addition might require sufficient soil moisture (Chtouki et al. 2022). Phosphorus addition seems to increase the production of stomata per epidermal cell (Sekiya and Yano 2008), but to our knowledge, the exact mechanism behind this effect has not yet been elucidated.

Stomatal density may also change with the addition of other nutrients or elements. Upon observing an increase in transpiration with calcium silicate ($CaSiO_3$) addition at Hubbard Brook, for example, Green et al. (2013) hypothesized that changes in stomatal density or conductance might partly explain this observation. Response of stomatal density to Ca have been investigated in trees by Forey et al. (2015), studying *Fagus sylvatica* L. trees in Germany, but they did not observe a response to Ca in the form of calcium carbonate, 25 years after treatment. So far, there does not seem to be strong evidence that stomatal density increases with Ca addition.

Similar to stomatal density, carbon isotope composition, or $\delta^{13}C$, also relates to gas exchange. The formula defining $\delta^{13}C$ relates ^{13}C to ^{12}C in the sample through the following equation:

$$\delta 13C \ = \ (\frac{13C/12C\ sample}{13C/12C\ standard} \ - \ 1)\ x\ 1000$$

where 'sample' refers in this case to the ratio of ^{13}C to ^{12}C in foliage and the standard refers to an established reference material (Dawson et al. 2002). In general, the incorporation of carbon (C) into biomolecules, catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase (RuBisCO), tends to discriminate against ^{13}C and preferentially incorporate ^{12}C (Farquhar et al. 1982; Dawson et al. 2002). This occurs because the diffusivity of $^{13}CO_2$ in air is 4.4 ‰ less than that of $^{12}CO_2$, $^{13}CO_2$ being slightly heavier than $^{12}CO_2$, and because RuBisCO discriminates against ^{13}C (Farquhar et al. 1982). If stomata on leaf surfaces are more dense or open more often, allowing more CO_2 from the air (c_a) to diffuse into intercellular spaces, discrimination between ^{13}C and ^{12}C increases, which reduces the ^{13}C to ^{12}C ratio, thereby decreasing $\delta^{13}C$. The proportion of ^{13}C isotopes in plant biomass, which directly relates to $\delta^{13}C$, is correlated to c_i/c_a, which relates the supply of CO_2 to the leaf through open stomata (c_a) to the demand for CO_2 within the mesophyll (c_i). Nutrient availability can influence photosynthetic capacity, which influences c_a (Cernusak et al. 2013). If nutrient addition influences stomatal conductance, this could change c_i. In this way, $\delta^{13}C$ can provide insights into how nutrient addition might influence physiological function in a way that does not involve directly measuring gas exchange.

Carbon isotope composition is also often used to determine intrinsic water use efficiency, or iWUE, which is the ratio of net photosynthesis (A) to stomatal conductance (g_s). This can also be defined by the difference in c_a and c_i and therefore can be calculated using $\delta^{13}C$. Field iWUE measurements can be instantaneous, but calculations using $\delta^{13}C$ can represent a longer-term, time-integrated estimate of iWUE across leaf lifespans (Pérez-Harguindeguy et al. 2013). iWUE can be calculated from $\delta^{13}C$ using the following equation:

$$iWUE \ = \frac{Ca\ (b\ + \delta 13Cp\ - \ \delta 13Ca)}{1.6(b - a)}$$

where $\delta^{13}C_p$ is the isotopic signature of the plant, $\delta^{13}C_a$ is the isotopic signature of air, c_a is the concentration of CO_2 in the atmosphere, a relates to the diffusivity of ^{13}C (4.4 ‰), b is the fractionation against carboxylation of $^{13}CO_2$ by RuBisCO (27 ‰), and 1.6 is the ratio of diffusion rates of water vapor to CO_2 (Farquhar et al. 1982; Vadeboncoeur et al. 2020). Assuming the same c_a and $\delta^{13}C_a$, $\delta^{13}C$ of foliage thereby scales positively and linearly with $\delta^{13}C$. In this way, $\delta^{13}C$ can be used as a proxy for iWUE (Visakorpi et al. 2022).

Responses of $\delta^{13}C$ and iWUE to N addition have been studied more frequently than have responses to P or Ca addition (e.g., Raven et al. 2004). Most studies investigating the effect of N addition on $\delta^{13}C$ and iWUE document an increase in iWUE following an increase in $\delta^{13}C$, and thereby a decrease in the discrimination between ^{13}C and ^{12}C. This could occur if photosynthetic capacity increases following the alleviation of an N limitation (Raven et al. 2004; McNown and Sullivan 2013; Jennings et al. 2016). Indeed, a recent meta-analysis of N addition studies found a 3.1% increase in iWUE with N addition (Zhang et al. 2018). If P is primarily limiting or co-limiting, however, which has been increasingly observed in temperate ecosystems (Vadeboncoeur 2010; Goswami et al. 2018; Hou et al. 2020; Peng et al. 2021), we might expect an increase in $\delta^{13}C$ with P addition. Indeed, iWUE increased with P addition in multiple studies; for *Pennisetum glaucum* (L.) R. Br., P addition decreased iWUE when water was sufficient, but always increased iWUE when water was limiting (Raven et al. 2004). In a study that demonstrated an increase in stomatal density with P addition in cowpea, the discrimination between ^{13}C and ^{12}C was negatively correlated with stomatal density (Sekiya and Yano 2008). Since the discrimination between ^{13}C and ^{12}C is negatively correlated with $\delta^{13}C$, we could similarly expect to see a positive correlation between $\delta^{13}C$ and stomatal density, and thereby might also anticipate positive effects of P addition on $\delta^{13}C$. Previous studies have demonstrated links between Ca and iWUE derived from $\delta^{13}C$ as well, suggesting an increase in iWUE with greater concentrations of Ca in soil (Yin et al. 2022). These results collectively suggest that we might expect to see an increase in $\delta^{13}C$, and therefore iWUE, with addition of N, P, or Ca.

Trait Terminology Disclaimer

I report foliar traits in all of my dissertation chapters. These traits include SLA, LDMC, foliar N, foliar P, $\delta^{13}C$, and stomatal density. While the first four traits, which belong to the leaf economics spectrum, are typically considered “functional traits” (Pérez-Harguindeguy et al. 2013), I acknowledge that my study will not enable strong demonstration of functional consequences of these traits. New perspectives have recently emerged claiming that *all* traits are functional in a way (Sobral 2021), however, while others demonstrate that many traits we consider to be “functional” may not actually correlate as well with tree growth as we might expect, and therefore may not be very functional (Rosas et al. 2021).

I intend to be consistent with the definition put forth by Violle et al. (2007) that functional traits indirectly influence fitness through effects on “growth, reproduction, and survival”. This functionality cannot be demonstrated in my dissertation, especially in terms of foliar N and P, in which increases could indicate luxury consumption that does not contribute to further biomass yield or functionality (van den Driessche 1974; Van Wijk et al. 2003). I will therefore refer to these traits as "foliar traits" as opposed to "functional traits".

Effects of Nutrient Availability on Foliar Chemistry and Resorption

With the development of the multiple resource limitation hypothesis near the end of the 20th century, and the discovery of the prevalence of co-limitation in the early 2000s, awareness of the interconnectedness, or "coupling", of nutrient cycles has increased and remains a pertinent research topic (e.g., Finzi et al. 2011). Coupling of nutrient cycles occurs when changes in the availability of one nutrient affects the availability of another, meaning that the biological and geochemical processes involving these nutrients are interdependent.

Nitrogen and phosphorus are biochemically interdependent, which could help explain widespread observations of N and P co-limitation (Marklein and Houlton 2012). They are both necessary components of nucleic acids such as DNA and RNA, and phosphatases are N-rich enzymes produced by plants and microbes that break down organic P into phosphate; in this way, plants and microbes require N in order to increase P availability. Marklein and Houlton (2012) explored the interdependence among N, P, and phosphatases in their meta-analysis of 34 N or P addition studies and found that N addition commonly stimulated phosphatase production and thereby increased the availability of P. In this meta-analysis, P addition suppressed phosphatase production, as did N + P addition, which could suggest that P availability has a stronger influence on phosphatase production than N availability (Marklein and Houlton 2012). These results highlight one potential mechanism for N and P co-limitation and illustrate how N and P cycles are coupled.

In addition to being coupled with N, P is coupled with Ca in part through effects of Ca on soil pH. At Hubbard Brook, where $CaSiO_3$ was added to an entire watershed to simulate recovery from acid deposition (Battles et al. 2014), foliar P concentrations increased and microbial biomass P decreased after the first year of treatment (Fiorentino et al. 2003). These results together suggest an acceleration of P cycling as a result of Ca addition, which could be due in part to the increase in soil pH with Ca addition. This increase in soil pH could increase the solubility of P by reducing the P occlusion through precipitation with iron and aluminum (Schlesinger and Bernhardt 2013). This increase in soil pH could also influence the solubility of organic matter or increase microbial activity by creating more favorable conditions for microbes (Marschner and Wilczynski 1991), both of which could accelerate decomposition and thereby P cycling.

Although no Ca-addition effects on N cycling were observed at Hubbard Brook in the first year of treatment (Fiorentino et al. 2003), effects of N addition have been observed on the cycling of Ca and other base cations in other studies, though these effects are also difficult to disentangle from effects of N addition on soil pH. In particular, N addition can lead to the loss of base cations, including Ca, Mg, and K in soils and foliage (Lucas et al. 2011). This decrease in Ca, Mg, and K is attributed to soil acidification that often accompanies N addition. Globally, N addition has been shown to reduce soil pH, and this is most prevalent in studies that use urea and ammonium nitrate and in studies that use rates greater than 50 kg N ha^{-1} yr^{-1} (Tian and Niu 2015). In these ways, N and P cycles are coupled with the cycles of other nutrients, and the ways in which these cycles interact are complex and often mediated by changes in soil pH.

Co-limitation and coupled nutrient cycles can be investigated through measuring nutrient concentrations in foliage and litter. Foliage and litter nutrient concentrations can serve as a tool for understanding responses of trees to nutrient availability in several ways. First, foliar chemistry is known to reflect soil nutrient availability (Parfitt et al. 2005; Lucash et al. 2012). In fact, foliar chemistry is sometimes considered more straightforward for use in diagnosing nutrient deficiencies than soil chemistry (Kopinga and van den Burg 1995). While concentrations of nutrients in foliage can reflect soil nutrient availability, concentrations of nutrients in senesced leaves, or leaf litter, can influence decomposition and soil nutrient availability (Hobbie 1992; Parton et al. 2007; Hobbie 2015) Together, concentrations of nutrients in foliage and litter can provide insights on soil nutrient availability, both in terms of the ability of plants to access nutrients as well as the contributions of plants to the process through senescence.

Foliar chemistry is also often used as an indirect indicator of nutrient limitation of growth and productivity. In their meta-analysis of autotrophic tissue concentration responses to nutrient limitation, Bracken et al. (2015) articulated several potential responses of tissue concentrations to nutrient addition in light of limitation. “Single-nutrient limitation,” by their definition, results when the addition of the limiting nutrient (N or P) produces a strong decrease in internal tissue concentrations of the other nutrient; for example, if N is limiting, N addition would result in decreases in P concentrations. “Community co-limitation” could occur if different species or individuals are limited by different nutrients, and overall, the community exhibits both lower P concentrations in response to N addition and lower N concentrations in response to P addition. If N and P exhibit biochemically dependent co-limitation, however, then the addition of one nutrient might result in no change in concentration, or even an increase in the concentrations of the other nutrient. In this way, a decrease, no change, or increase in the concentrations of one nutrient or the other could all potentially translate to interpretations of co-limitation, depending on the context.

Response ratios of foliar concentration (i.e., the ratio of foliar nutrient concentrations in control conditions to those in treated conditions) and ratios of foliar N to P can also be used to indicate nutrient limitation. In their review, Güsewell (2004) expressed that N:P ratios less than 10 typically indicated N-limited conditions and ratios greater than 20 indicate P-limited conditions. This range is an update from ratios defined by Koerselman and Meuleman (1996), who first proposed using an N:P ratio to indicate biomass limitation in plants and expressed that N:P ratios of 14 and lower as evidence of N limitation and N:P ratios of 16 and higher as evidence of P limitation. Güsewell (2004), however, clarifies that the interpretation of these ratios relates mainly to the results of short-term nutrient-addition. Meanwhile, Ostertag and DiManno (2016) conducted a meta-analysis of foliar chemistry responses to N, P, and N and P addition, using both foliar N:P ratios and response ratios (i.e., ratio of foliar chemistry in treated conditions to those in the control). While they did find the results of response ratios and N:P ratios to be similar, they discouraged the use of universal cutoff values (e.g., 10 and 20) and suggested defining cutoffs at levels specific to the site.

In addition to foliar nutrient concentrations, litter nutrient concentrations can also shed light on limitation and nutrient cycling more generally. The quantity and quality of leaf litter influences nutrient cycling through effects on nutrient availability and the composition of microbial and microfauna communities (Prescott 2002), and litter quality is both influenced by and affects site nutrient availability through feedback loops (Hobbie 1992), though these relationships are complex and require more research on the role of soil organic matter in this process (Hobbie 2015). Concentrations of nutrients in leaf litter can differ substantially by species (Prescott 2002) but are also influenced by environmental factors. For example, soil fertility can influence litter N concentrations in plant communities through environmental filtering, but litter N can simultaneously influence soil fertility through feedback effects (Laughlin et al. 2015). Litter N and P are also influenced by climate; globally, litter N concentrations tend to increase with increasing annual temperature and precipitation while P concentrations decrease with increasing temperature. Together, climate can account for about 22-32% of variation in litter N and P concentrations (Yuan and Chen 2009).

Research on the effects of nutrient addition on litter N has found that N addition increases litter N, but relationships between litter N and N availability are less clear. Many of these studies on litter N, and relationships between litter nutrient concentrations and nutrient availability, have been conducted in the context of decomposition. In their book on litter decomposition, which focuses mainly on conifer litter decomposition in temperate and boreal forests, Berg and McClaugherty (2008) wrote that there does not naturally seem to be a strong relationship between litter N and soil N, perhaps due to the fact that N tends to be limiting in temperate forest ecosystems. They observed that relationships among soil and litter chemistry tend to be stronger for Ca and Mg. Other studies, however, have demonstrated a relationship between soil fertility and litter N and P, suggesting that these concentrations increase with soil fertility (Richardson et al. 2005; Laughlin et al. 2015). Despite not finding a strong relationship between litter N and soil N, in response to N addition, Berg and McClaugherty (2008) found that litter N does tend to increase, as does litter P, K, and S to a small extent, in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.), while litter Ca decreases.

One might expect, then, that an increase in litter N concentrations with N addition might cause an increase in decomposition rate. Perhaps counterintuitively, many studies have observed a decrease in litter decomposition rate with N addition. In the Chronic Nitrogen Addition plots at the Harvard Forest in Massachusetts, for example, a study in which two levels of ammonium nitrate were annually added to a red pine plantation and mixed-wood forest, mass remaining of leaf litter from four tree species was significantly greater in forest plots that had been treated with N (Magill and Aber 1998). Subsequent work on this long-term study found that soil in fertilized plots exhibited reduced fungal biomass and greater accumulation of lignin than soil in unfertilized plots (Frey et al. 2014). Indeed, lignin concentrations tend to be higher in litter under N addition (Liu et al. 2016). Nitrogen addition can alter fungal community composition in ways that reduces the expression of lignolytic genes (Edwards et al. 2011), leading to a lower expression of enzymes like phenol oxidase (Carreiro et al. 2000). These observations underscore the need to disentangle the effects of N on litter substrates and effects of N on the surrounding environment when interpreting the effects of elevated N on litter decomposition and nutrient cycling (Hobbie 2005). In a reciprocal transplant study of sugar maple and black cherry (*Prunus serotina* Ehrh.), through which the effects of substrate N and environmental N can be disentangled, decomposition rate decreased with N addition and no differences in decomposition rate between the transferred litter and resident litter were detected (Bowden et al. 2019). These results suggest that the effects of N addition on the microbial community may influence decomposition rate more than the effects of N addition on litter chemistry.

Much less is known about the effects of P on litter chemistry. In a N x P-addition study conducted in northern hardwood forests, Gonzales et al. (2023) report a 130% increase in litter P with P addition, and 69% increase in litter P with N+P addition in beech (*Fagus*) and maple (*Acer*) species, which mirrors global observations in foliage that P tends to increase more with P addition alone than N+P addition (You et al. 2021). In an N x P-addition study in a subtropical forest in China, P addition led to an increase in litter P concentrations, which led to faster decomposition rates in litter sourced from the P and N+P treatments than in litter sourced from control plots (Zheng et al. 2017). Litter P concentrations also increased with P addition in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests in Oregon, USA, but the litter decomposition rate decreased (Van Huysen et al. 2016). Conversely, in a P-addition decomposition study in Ohio, P addition increased soil P but not litter P concentrations, and oak (*Quercus*) mass loss rate decreased (DeForest 2019). These results together suggest that relationships between litter P, P addition, and decomposition are not straightforward.

When studying the effects of nutrient availability on foliar and litter nutrients, it is important to consider resorption, which incorporates the effects on foliage and litter together. Resorption is an active and adaptive physiological process that reduces the dependence of trees on their environment for nutrient uptake, buffering against potential changes in fertility and thereby reducing nutrient loss (Aerts 1996; Brant and Chen 2015). Using resorbed nutrients to build new tissues reduces resource and energy costs for plants (Wright and Westoby 2003). This may be particularly important in cool climates, where nutrient availability tends to be lower due to slower decomposition rates. For example, higher N resorption rates of red maple (*Acer rubrum* L.) have been documented in colder climates in eastern North America than in warmer climates (Gougherty et al. 2023), though this study did not explore whether this correlation was related to differences in soil nutrient availability. Plants typically resorb N, P, K, and sulfur (S), resulting in lower concentrations of these elements in litter than observed in foliage (Berg and McClaugherty 2008). Ca, Mg, and manganese, however, are not resorbed to the same extent, if resorbed at all, which means that these concentrations tend to be higher in leaf litter than in green foliage (Berg and McClaugherty 2008) due to mass loss during senescence. Resorption of P tends to be more variable and more responsive to environmental factors than N resorption, perhaps in part because P is used in more diverse ways than N in plant metabolism (Tsujii et al. 2017; Drenovsky et al. 2019; Estiarte et al. 2022)

In theory, trees are expected to increase their resorption efficiency (i.e., proportion of green leaf nutrients resorbed) in areas of low soil nutrient availability, which would indicate a possible adaptation to low nutrient availability. Evidence to support this expectation is mixed (Killingbeck 2004). Higher resorption efficiency in areas with lower site fertility was not observed in a 1996 meta-analysis of woody plants from North American and Europe (Aerts 1996), nor in white birch (*Betula papyrifera*) across a soil fertility gradient (Chapin and Moilanen 1991) but was observed in a 2012 global meta-analysis of woody and herbaceous plants (Vergutz et al. 2012). A 2018 meta-analysis that incorporated soil nutrients did find evidence of plants grown in low-fertility habitats exhibiting higher nutrient resorption efficiency rates than those grown in high soil nutrient habitats (Yan et al. 2018). Resorption efficiency has also been shown to decrease in response to N and P addition (Yuan and Chen 2015). To explain these inconsistent trends in resorption efficiency, it may be that resorption proficiency (i.e., the concentration of nutrients in leaf litter) is more strongly influenced by soil nutrient availability than resorption efficiency, meaning that the concentration of nutrients in litter is more directly correlated with nutrient availability of the site. It could be that the concentrations of nutrients in litter (proficiency) can be more directly acted upon by natural selection than the proportion of nutrients resorbed (efficiency; Killingbeck 1996; Wright and Westoby, 2003). Therefore, while evidence is mixed, more recent, direct evidence suggests increased resorption efficiency and resorption proficiency with decreased soil nutrient availability.

Recently, resorption has been studied in the context of limiting nutrients in order to better understand the relationship between resorption and soil nutrient availability in a relative sense; how do plants conserve nutrients in light of nutrient limitation? The “relative resorption hypothesis” suggests that plants resorb relatively more of a limiting nutrient. In a global meta-analysis using universal N:P ratio cutoffs to indicate nutrient limitation, the difference between NRE and PRE was greatest at high and low N:P values and reached zero around 15 (Han et al. 2013), which could indicate co-limitation if using the cutoffs of 10 and 20 to indicate N and P limitation (Güsewell 2004). NRE was greater than PRE in plants with lower N:P values and PRE was greater than NRE in plants with higher N:P values, indicating that resorption efficiency is higher in response to nutrient limitation (Han et al. 2013). In line with this hypothesis, fertilizing with a limiting nutrient and thereby alleviating the limitation should decrease resorption efficiency.

Resorption has been studied in the context of nutrient-addition experiments to illustrate the effects of soil fertility and nutrient limitation on resorption. Most of these studies have demonstrated a decrease in resorption efficiency and proficiency with an increase in soil fertility (Brant and Chen, 2015; Yuan and Chen, 2015). Globally, N resorption efficiency has been found to decrease with N addition alone and with N and P addition, but no change in N resorption efficiency with P addition was detected (Yuan and Chen 2015). Similarly, P resorption efficiency has been found to decrease with both P addition and N+P addition (Yuan and Chen 2015). Exact patterns, however, may differ with site conditions and species. For example, differences in P resorption efficiency were observed among tree species in the Adirondacks of New York, USA (high resorption efficiency for sugar maple, low resorption efficiency for oak), but effects of N addition on P resorption efficiency were not observed, suggesting that N deposition in that region was not causing substantial P limitation, as might have been expected (Weand et al. 2010). Nearby, in New Hampshire, P addition reduced foliar N concentrations in several young stands in a long-term N x P factorial study, suggesting P limitation (Gonzales and Yanai 2019). Across more of these same forest stands, resorption of sugar maple and American beech (*Fagus grandifolia* Ehrh.) decreased with P addition, but did not exhibit differences with N addition, which could reflect P limitation, or greater plasticity in P resorption than N (Gonzales et al. 2023).

Nutrient Limitation at the Individual, Species, and Community Level

Nutrient limitation can be conceptualized and expressed at different scales, including within an individual, within species and at the community level. Within an individual, nutrient limitation can change over time as trees grow larger. Small, young trees (less than 15 cm in diameter at breast height) were more strongly limited by P than large, mature trees across subtropical, warm-temperate, and cold-temperate forests in China, perhaps due increased use of P to enhance growth in young trees and more conservative P use in mature trees (Li et al. 2018). This observation is consistent with results from the Multiple Element Limitation in Northern Hardwood Ecosystems study in the northeastern United States, which documented P limitation in diameter growth of the average tree four years after factorial addition of N and P (Goswami et al. 2018), but N limitation in the largest trees (Hong et al. 2022).

Though limitation may change from P to N as trees become larger, changes in nutrient availability may shift forests from N limitation to P limitation and co-limitation over time following clearcutting. For example, a MEL model simulation of a bole-only, clearcut harvest that removed 9% of plant N and 5% of plant P resulted in a shift in soil N:P such that there was more soil organic P than N compared to pre-harvest soils due to the lower N:P of postharvest residues compared to soil organic matter (Rastetter et al. 2013). After 25 years, however, the stand shifted to needing more P than N (Rastetter et al. 2013). It is therefore important when interpreting results of nutrient-limitation studies to consider both the growth stage of the trees and development of the stands.

Nutrient limitation can differ by species, given that species differ in their morphological and physiological traits and therefore differ in their nutrient requirements. In northern hardwood forests, for example, birch (*Betula*) species have been observed to respond more to N addition than American beech (Crowley et al. 2012)**.** Three other northeastern tree species– balsam fir (*Abies balsamea* (L.) Mill.), red spruce (*Picea rubens* Sarg.), and eastern hemlock (*Tsuga canadensis* (L.) Carrière)– have exhibited lower growth and survival responses to N deposition over time, while two other species– scarlet oak (*Quercus coccinea* Münchh.) and American basswood (*Tilia americana* L.)–exhibited greater growth and survival over time (Clark et al. 2023). Differences in nutrient limitation by species are influenced by species traits and nutrient requirements and by the productivity of sites to which they are adapted (Chapin 1980; Chapin et al. 1986). Community co-limitation in aboveground growth can therefore be possible even if individual species are found to be limited by a single nutrient if different species are limited by different nutrients (Güsewell 2004; Bracken et al. 2015).

Nutrient limitation can be described at the community level through the use of community-weighted means. Community-weighted means are the sums of mean values of traits, such as nutrient concentrations, for each species in the community, weighted by the relative abundance of those species in the community. These values thereby capture both within-species differences in trait values, which can be due to genetics or phenotypic plasticity in response to environmental change, and species composition, which can be a product of competitive interactions among species (Chapin 2003). These values have been shown to correlate well with other ecosystem properties, which can make them a good overall “marker” for ecosystem function (Garnier et al. 2004). For example, community-weighted SLA, LDMC, and foliar N were found to correlate well with net primary productivity, litter decomposition rate, and total soil carbon and N in a Mediterranean vineyard in France (Garnier et al. 2004). Many studies have since confirmed these observations more broadly, finding that traits expressed at a community-weighted level could provide broader insights into the relationship between the aboveground community and soil and nutrient cycling in that ecosystem. In a recent global analysis, community-weighted trait values were strongly correlated with local, edaphic factors (Bruelheide et al. 2018). Community-weighted LDMC and soil fertility have been shown to be correlated (Laughlin et al. 2015; Daou et al. 2021), as have community-weighted litter nutrient concentrations and soil fertility (Richardson et al. 2005; Laughlin et al. 2015). Changes in community-weighted foliar and litter N and P across a soil fertility gradient can be due both to changes in species composition as well as changes within species (Richardson et al. 2005). Community-weighted foliar N of overstory trees has also been found to correlate with ratios of fungi to bacteria ratios in soils (Wan et al. 2022). In these ways, community-weighted traits can correlate with ecosystem productivity and reflect environmental conditions, thereby serving as a strong candidate for study of limitation at the community level.

Nutrient-Addition Experiments to Study Nutrient Limitation

One of the best ways to investigate nutrient limitation is through nutrient-addition experiments, whereby the balance of nutrients in an ecosystem shifts and potentially alleviates nutrient limitations. These studies can provide more direct evidence for and insights on nutrient limitation than observational studies along environmental gradients. Nutrient-addition experiments have been considered our “best” information on nutrient limitation in the sense that an increase in net primary productivity in these studies clearly demonstrates the existence of a nutrient limitation (Vitousek and Howarth 1991). These studies can be difficult to interpret, however, in that it may take several years for the effects of treatment to actualize, species composition could change following nutrient addition, and soil processes could change following the addition of one nutrient in ways that could increase another nutrient as well (Güsewell 2004). That said, these studies still serve as a convenient way to study nutrient limitation, and long-term nutrient-addition studies are necessary to better understand the effects of plant species on nutrient cycling (Hobbie 2015). In temperate locations, such as northeastern North America, many nutrient-addition studies exist that can shed light on limitation by Ca, N, and P (Vadeboncoeur 2010).

Many nutrient-addition studies that observe the effects of Ca addition on ecosystems use either calcitic ($CaCO_3$) or dolomitic ($CaMg(CO_3)_2$) limestone. Many of these treatments are intended to increase pH, rather than increase Ca per se, and they consist of one slow-weathering application of Ca that is monitored over time. As shown by Moore et al. (2012), examining the 15-year response of sugar maple forests to dolomitic lime addition, it may take a decade for soil nutrition to change following this addition. Several of these one-time nutrient-addition studies have been monitored for over 20, even 30, years. Following a one-time application of dolomitic limestone in northern Pennsylvania, effects of Ca on foliar chemistry and increased growth of sugar maple persisted for 30 years, coupled with no change in American beech and decreased growth of black cherry, suggesting that species were limited by Ca to different extents, leading to changes in species composition (Long et al. 2022). Similarly long-lasting effects were documented 25 years following a single calcitic lime addition in European beech (*F. sylvatica*) stands in France, showing long-term changes in foliar chemistry and resorption and in litter decomposition rates but not morphological leaf traits (SLA, LDMC, stomatal density; Forey et al. 2015). Both of these studies originated during the 1980s, at a time when acidic deposition was substantially influencing forests and depleting forests of calcium (Likens et al. 1996; Schaberg et al. 2001).

Base cation depletion due to acidic deposition also prompted the watershed-scale addition of wollastonite (i.e., calcium silicate, $CaSiO_3$) to Watershed 1 at Hubbard Brook Experimental Forest in New Hampshire, USA, which has since demonstrated an increase in tree growth, particularly of sugar maple, since this one-time application in 1999 (Battles et al. 2014). In studies that apply wollastonite, however, it is important to also consider the effects of adding silica, as silica can improve water-use efficiency through decreasing stomatal conductance (Gao et al. 2006; Nascimento-Silva et al. 2023).

While N addition studies have been implemented in northeastern North America as a way to experimentally investigate the effects of anthropogenic N deposition on forests, such as the Chronic N Amendment Study at Harvard Forest (Magill et al. 2004), few fully factorial N x P studies exist in temperate forest ecosystems. Most of the forest studies included in Elser et al. (2007)'s meta-analysis on N and P co-limitation were located in tropical forests. In northeastern North America, where limitation by N, P, and Ca were evident from a meta-analysis of N, P, and Ca fertilization studies, few of these studies were factorial N x P experiments (Vadeboncoeur 2010). Previous factorial N x P experiments that involved a one-time application of nutrients have been conducted in New York (Lea et al. 1979) and Wisconsin (Stone 1980); these studies showed more of an increase with growth with N addition than other nutrients, suggesting N limitation. Three other N x P factorial experiments have involved continual application of N and P, with one six-year study in Ontario (Leech and Kim 1990), one two-year study in Connecticut (Finzi 2009), and one 10-year study in Maine (Safford and Czapowskyj 1986). These three experiments exhibited primarily N limitation, though the study in Maine showed Ca co-limitation of bigtooth aspen (*Populus grandidentata* Michx.), quaking aspen (*Populus tremuloides* Michx.), and white birch (Safford and Czapowskyj 1986). Other studies in temperate forests have been installed in the southeastern United States (Corbin et al. 2003), the United Kingdom (Newton and Pigott 1991), Germany (Fetzer et al. 2022), China (Zhang et al. 2018b), and Australia (O'Connell and

Mendham 2004). None of these factorial N x P studies has lasted as long as the Multiple Element Limitation in Northern Hardwood Ecosystems study in New Hampshire, which has been continually fertilized since 2011.

Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE)

Nutrient-addition experiments can be used to test theoretical models of nutrient limitation. Few of these studies exist in northern temperate forests, and MELNHE marks the longest running fully factorial N x P study. By adding N, P, and both in combination to elucidate nutrient limitations during forest development following clearcutting, MELNHE can test the MEL model and determine the extent to which predictions of multiple element limitation following clearcutting in northern hardwood forests are correct. The long duration of this study and its use of relatively modest nutrient-addition allows researchers to test trajectories of co-limitation that are not possible to observe in shorter studies with higher nutrient-addition rates, such as whether co-limitation occurs as a result of serial, single-nutrient limitation of N and P or simultaneous co-limitation by N and P (Davidson and Howarth 2007; Harpole et al. 2011).

MELNHE consists of 13 northern hardwood forest stands in central New Hampshire. Each stand has at least four plots: one is a control, one has been fertilized with N, one with P, and one with both N and P. Nutrient addition in these plots began in 2011. Nine of the MELNHE forest stands are at Bartlett Experimental Forest in Bartlett, NH. Six of these stands were third-growth and “mid-successional” (harvested 1975-90), and three are second-growth and “mature” (harvested 1883 and ~1890). Two of the MELNHE stands are at Hubbard Brook Experimental Forest in North Woodstock, NH, and the remaining two are at Jeffers Brook near Benton, NH. These sites each have one mid-successional stand (harvested ~1970-75) and one mature stand (harvested ~1900).

Established near the N, P, N+P, and control plots in a subset of stands are Ca-addition plots. In each of these plots, calcium in the form of wollastonite ($CaSiO_3$) was added once at a rate of 1150 kg ha^{-1}. Calcium-addition plots are located in stands C1, C6, and C8 at Bartlett Experimental Forest, in the mid-aged and mature stands at Jeffers Brook (JBM and JBO, respectively), in the mid-aged stand at Hubbard Brook Experimental Forest (HBM), and in a mature forest stand separate from the mature stand in the MELNHE study at Hubbard Brook, a different stand labeled HBCa.

Unlike the majority of N x P factorial studies that have been conducted in temperate deciduous and mixedwood forests, which have demonstrated N limitation, the MELNHE study has demonstrated P limitation, and more recently N x P co-limitation. In 2015, the first tree-inventory results showed a greater diameter-growth response of trees greater than 10 cm in diameter to P than to N (Goswami et al. 2018), though this response was driven by smaller trees; larger trees, including those used for foliar analysis, showed a greater response to N (Hong et al. 2022). Previous papers reporting foliar nutrient and resorption analyses from this time (2014 through 2016) also suggest primarily P limitation (Gonzales and Yanai 2019; Hong et al. 2022; Gonzales et al. 2023) The latest tree inventory, conducted in 2019, shows a greater response to N than in 2015 and now shows a greater response to N and P together than to N or P alone (Blumenthal in prep.). My dissertation addresses whether the most recent foliar nutrient and resorption analysis also indicates co-limitation by N.

To date, 29 graduate student theses or dissertations and over 45 manuscripts have been written in the context of the MELNHE study. My dissertation contributes to this growing body of knowledge on long-term nutrient addition, contributing insights on the effects of over a decade of low-dose annual addition of N and P on foliar traits, foliar and litter chemistry, and resorption efficiency, and long-term effects of a one-time addition of Ca on stomatal traits and carbon isotope composition. In particular, my dissertation investigates the following questions:

1. How do five foliar traits vary across N, P, and N+P addition treatments in six dominant tree species and at the community level, and are these responses consistent with responses of relative basal area increment to nutrient addition?
2. How do stomatal density, stomatal length, and carbon isotope composition vary with N, P, and $CaSiO_3$ addition in sugar maple and yellow birch trees, and how is variance partitioned in stomatal density and length measurements?
3. How do foliar and litter concentrations of N, P, K, Ca, and Mg and resorption efficiency of N and P change over time with N, P, and N+P addition at the community level?

These questions are explored through my dissertation, culminating in an increased understanding of the effects of nutrient availability and limitation on foliage and more insights into how changes to this ecosystem component influence nutrient forest nutrient cycling.

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CHAPTER 2: TREATMENT EFFECTS OF NITROGEN AND PHOSPHORUS ADDITION ON FOLIAR TRAITS IN SIX NORTHERN HARDWOOD TREE SPECIES

Abstract

Foliar traits can reflect plant strategies and influence plant fitness in response to environmental changes. Species may respond to changes in nutrient availability differently due to variation in trait plasticity. Trait values and community composition together can influence forest nutrient cycling. We compared five traits—foliar N, foliar P, specific leaf area (SLA), leaf dry matter content (LDMC), and carbon isotope composition ($\delta^{13}C$)—in six northern hardwood tree species (*Acer rubrum*, *Acer saccharum*, *Betula alleghaniensis*, *Betula papyrifera*, *Fagus grandifolia*, and *Prunus pensylvanica*) in a nitrogen (N) and phosphorus (P) fertilization study across 10 forest stands in New Hampshire, USA and considered how responses of these traits to N and P addition relate to nutrient limitation. Relationships among traits matched those observed in the leaf economics spectrum. Nutrient addition led to a shift in trait values towards the "acquisitive" side of the spectrum for all traits except $\delta^{13}C$, reflecting a tradeoff between water-use efficiency and nutrient-use efficiency. Treatment responses over time in annual relative basal area increment revealed that the *Betula* species were N-limited, but traits of all species responded to either or both N and P addition in ways that together could suggest N and P co-limitation in these communities. Two of the six species displayed lower foliar P under N addition, and three species displayed lower foliar N under P addition, which could indicate some degree of co-limitation. Community-weighted foliar N and P concentrations changed in ways that also could indicate co-limitation, whereas community-weighted carbon isotope composition responded to N addition. Specific leaf area (SLA), leaf dry matter content (LDMC), and carbon isotope composition differed with stand age within several species. Examining trait responses of tree species and communities to nutrient availability increases our understanding of biological mechanisms underlying the complex effects of nutrient availability on forests.

Introduction

Foliar traits, or measurable characteristics of leaves, can provide insights into plant strategies (Violle et al. 2007; Reich 2014). Certain foliar traits occupy a spectrum from values associated with more "acquisitive", fast-growing plants with short-lived leaves (higher specific leaf area (SLA), higher foliar nitrogen (N) and phosphorus (P) concentrations, greater photosynthetic capacity) to more "conservative", slow-growing plants with long-lived leaves (lower SLA, lower foliar N and P concentrations, lower photosynthetic capacity; Diaz et al. 2004). These relationships are referred to as the "leaf economics spectrum" (Wright et al. 2004). The traits originally included in this spectrum were SLA, foliar N, foliar P, leaf lifespan, leaf dark respiration, and photosynthetic capacity, and subsequent studies found that leaf dry matter content (LDMC) falls along this spectrum as well (e.g., Smart et al. 2017). This spectrum captures the idea that trait values tend to co-vary in ways that reflect tradeoffs: devoting resources into acquiring nutrients and growing quickly versus conserving nutrients and growing more slowly.

While these traits are largely determined by genetics and differ by species, considerable intraspecific variation also can occur (Albert et al. 2010). This variation within species can be influenced by genetics or environment (Bradshaw, 1965). Traits can be influenced by a variety of factors, such as the availability of light, water, and nutrients (Reich, 2014). In the context of fertilization studies, which are commonly used to investigate nutrient limitation, traits themselves cannot indicate whether a particular nutrient is limiting but can provide insight into how plants physically and physiologically respond to nutrient availability and limitation. For example, increases in nutrient concentrations with fertilizer addition may also lead to increases in SLA and LDMC, reflecting a shift in plant trait values that signifies a more "acquisitive" strategy in response to a change in nutrient availability (Iturrate-Garcia et al. 2020).

Foliar traits that relate to gas exchange and water use, such as carbon isotope composition ($\delta^{13}C$) can additionally provide insight on how metabolic processes such as photosynthesis and respiration change in response to nutrient availability. Carbon isotope composition is influenced by stomatal conductance; leaves that have lower stomatal densities and stomata that open less often tend to have higher $\delta^{13}C$, meaning that the cells discriminate less between ^{13}C and ^{12}C when fixing CO_2 (Farquhar et al. 1982). It is also positively correlated with intrinsic water-use efficiency, or iWUE, which represents the ratio of photosynthesis to stomatal conductance (Pérez-Harguindeguy et al. 2013).

While the leaf economics spectrum was developed to explain plant strategies in response to acquiring and conserving carbon and nutrients, more recent studies have shown that traits related to water use can also fall along this same spectrum (Reich 2014; Prieto et al. 2018). Since high iWUE reflects a conservative resource strategy, we would expect high δ^{13}C to fall along the more conservative end of the leaf economics spectrum (Prieto 2018). Associations of $\delta\,^{13}$C with the leaf economics spectrum, however, may conflict with other observations of tradeoffs in water-use efficiency and nutrient-use efficiency, potentially driven by the role of mass flow in nutrient uptake (Cramer et al. 2009). While we would expect most leaf economics traits to shift to the more 'acquisitive' side of the spectrum under nutrient addition, increasing nutrient availability could increase water-use efficiency, leading to an enrichment in ^{13}C under nutrient addition. Indeed, under nutrient addition, δ^{13}C nearly always either increases, suggesting ^{13}C enrichment (Raven et al. 2004). Differences in responses to changes in nutrient availability among traits may therefore complicate the interpretation of these traits falling along a single axis that represents plant strategy in the context of carbon, nutrient, and water use.

Species can differ in their growth and trait responses to nutrient availability. Most of this research in temperate forests has investigated effects of N addition. Recent N deposition studies have shown that northern hardwood species differ in response to N addition, with some species such as *Acer saccharum* Marsh. (sugar maple) and *Prunus serotina* Ehrh. (black cherry) increasing in growth rate with N addition and others, such as *Betula alleghaniensis* Britton (yellow birch) and *Fagus grandifolia* Ehrh. (American beech), decreasing (Clark et al. 2023; Pardo et al. 2019). Species can also differ in the magnitude to which their growth and trait values respond to environmental changes. For example, *Prunus pensylvanica* L.f. (pin cherry), a pioneer species, exhibited stronger growth and trait responses to complete, balanced nutrient addition than other species, such as *Betula papyrifera* (white birch; Fahey et al. 1998). Many other studies in this region have examined effects of N on tree growth and traits to better understand effects of anthropogenic N deposition. Much less is known about the effects of P availability on tree growth and traits in this region.

Differences in species-level responses to nutrient addition influence the community-level expression of these traits. Community-weighted means (CWM), which are calculated by weighting mean trait values with relative abundance of species, relate to environmental conditions. For example, CWM LDMC has been shown to decrease and CWM SLA to increase with increasing soil fertility (Daou et al. 2021), and local-scale factors such as soil conditions (e.g., nutrient availability) can significantly influence trait combinations and consequently trait CWMs

(Siefert et al. 2014; Bruelheide et al. 2018). Investigating how traits relate to each other, how they differ among species, how the response of these traits varies among species and at the community level, and how these responses relate to alleviation of nutrient limitations provides a more holistic perspective of how forests respond to changes in nutrient availability.

The purpose of this study is to quantify differences in foliar traits in response to long-term experimental nutrient addition treatments among six northern hardwood species and at the community level in mid-successional and mature forest stands. Specific objectives of this study were: 1) to explore relationships among five traits—four leaf economics spectrum traits (SLA, LDMC, foliar N, foliar P) and one gas-exchange trait ($\delta^{13}C$) —under nutrient addition to further investigate the role of gas-exchange and water use in the leaf economics spectrum 2) to determine how species- and community-level foliar trait values vary with experimental nutrient addition and between mid-successional and mature stands, and 3) determine whether N or P is more limiting to tree growth in these forests and whether changes in foliar traits with nutrient addition reflect these limitations, or co-limitation. The Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) experiment in the White Mountains of New Hampshire serves as an ideal location for these investigations. MELNHE is among the longest running N x P factorial fertilization studies in temperate deciduous forests; low doses of N and P fertilizer have been added to these forest stands since 2011.

We expected to see trait relationships consistent with the leaf economics spectrum. We also expected that high values of $\delta^{13}C$, representing iWUE, would represent a conservative strategy along the spectrum, being more strongly associated with low specific leaf area, high leaf dry matter content, and low foliar nutrient concentrations, but acknowledged that it might not change with nutrient addition in the same direction as the other four leaf-economics traits, given tradeoffs between water-use and nutrient-use efficiency. We hypothesized that these traits would exhibit changes in response to nutrient addition (Table 2-1), but that species would differ in the magnitude of their response, reflecting differences in strategy. We also hypothesized that foliar traits at community-weighted mean level in the mature stands would be more consistent with a conservative resource strategy than acquisitive. We expected that these trees might be N-limited, consistent with previous measurements of the same trees from this study in response to N and P addition and consistent with the prevailing hypotheses (Vitousek and Howarth 1991; Hong et al. 2022), but we also recognized that N and P co-limitation could be possible, given the most recent MELNHE tree diameter-growth results (Blumenthal et al. in prep.)

Table 2-1. Foliar traits measured in this study, what they represent, whether they are influenced by nutrients, light, and water, expected changes with N and P addition, and relevant references.

		Influenced by Availability of:				
Trait	**Represents**	**Nutrients**	**Light**	**Water**	**Expected Changes**	**References**
Foliar N	Photosynthetic capacity, community productivity, competitive ability	X	X	X	Increase with N addition, decrease with P addition if P is limiting	Bracken et al. 2015; Bruelheide et al. 2018; Wright et al. 2004; Young et al. 2023
Foliar P	Nucleic acids, lipid membranes, bioenergetic molecules (e.g., ATP)	X	X		Increase with P addition, decrease with N addition if N is limiting	Bracken et al. 2015; Wright et al. 2004; Young et al. 2023
Leaf dry matter content (LDMC)	Ratio of oven-dried to fresh mass. Negatively related to growth rate and decomposability, positively related to leaf lifespan	X		X	Decrease with nutrient addition	Daou et al. 2021; Siefert & Ritchie, 2016; Tatarko & Knops, 2018
Specific leaf area (SLA)	Area invested per unit mass of leaf. Relates positively to growth rate and photosynthetic capacity, negatively to leaf lifespan	X	X	X	Increase with nutrient addition	Iturrate-Garcia et al. 2020; Mo et al. 2020; Wright et al. 2004
$\delta^{13}C$	Photosynthetic capacity, intrinsic water use efficiency	X	X	X	Increase with nutrient addition	Cernusak et al. 2013; Raven et al. 2004

Methods

Site Description

Foliage was sampled in the MELNHE study in New Hampshire from 10 forest stands (Table 2-2). Six stands (C1, C2, C4, C6, C8, C9) were located at Bartlett Experimental Forest (BEF) in Bartlett, NH (44°03′N, 71°17′W), two stands (HBM, HBO) were located at Hubbard Brook Experimental Forest in Thornton, NH (43°56′N, 71°44′W), and two stands (JBM, JBO) were located at Jeffers Brook in Benton, NH (44°02′N, 71°53′W). These three sites differ in bedrock and parent material, with Jeffers Brook underlain by amphibolite, Hubbard Brook by granodiorite and schist, and Bartlett by granite; we expected differences in parent material to influence site fertility with Bartlett being the least fertile (e.g., lowest base saturation), Jeffers Brook most fertile, and Hubbard Brook intermediate Six of these stands were mid-successional, third-growth forests, which were last cut between 1970 and 1990, and the remaining four stands (C8, C9, HBO, JBO) were mature, second-growth forests, last harvested between 1883 and 1915 (Yanai et al. 2022). Mean annual precipitation at Hubbard Brook is around 1,400 mm (Campbell et al. 2010), mean air temperature in January is -9 °C and mean air temperature in July is 18 °C (USDA Forest Service 2022).

Forests were dominated by typical northern hardwood species following patterns related to secondary succession (Leak, 1991); mature forests were dominated by *Fagus grandifolia* Ehrh. (American beech), *Acer saccharum* Marsh. (sugar maple), and *Betula alleghaniensis* Ehrh. (yellow birch), while the youngest stands (C1 and C2) had more *Prunus pensylvanica* L.f. (pin cherry), *Betula papyrifera* Marsh. (white birch), and *Acer rubrum* L. (red maple). C4, C6, HBM, and JBM were dominated by a mix of these species. Soils were predominantly well drained or moderately well drained Spodosols developed in glacial till (Vadeboncoeur et al. 2014).

Each stand had four plots, which consisted of a 30 x 30-m inner measurement area with a 10-m buffer on each side (except for HBM and JBM plots, which had a 20 x 20-m measurement area with a 5-m buffer). Plots were treated annually with N (as NH_4NO_3, 30 kg ha^{-1} yr^{-1}), P (as NaH_2PO_4, 10 kg ha^{-1} yr^{-1}), both N and P, or neither. The 3:1 ratio of N to P added to these plots is much lower than N:P ratios typically seen in foliage (15:1 or 20:1), indicating that more P was added to the plots relative to plant demand, compared to N. This rate was chosen to account for occlusion of P in iron- and aluminum-containing minerals, primarily in soil B horizons, which makes P unavailable to plants (Wood et al. 1984; Weil and Brady 2017).

Foliar Sampling

Foliage samples were collected from Jeffers Brook (JBM and JBO) on July 29 and 30, 2021 and from Hubbard Brook (HBO and HBM) on August 2 and 3, 2021. Samples were collected from Bartlett (C1, C2, C4, C6, C8, and C9) on July 22, 28, 29 and August 1 and 2, 2022. We targeted three trees from the most abundant species in each stand, though occasionally only one or two trees of a species were present in a plot. We sampled the following tree species: *F. grandifolia, A. saccharum, A. rubrum, B. alleghaniensis, B. papyrifera,* and *P. pensylvanica* (Table 2-2). *P. pensylvanica* was sampled only in C1 and C2, as nearly all *P. pensylvanica* trees had died in the other stands by 2021. This effort captured at least 80% of the species composition by basal area for all stands except C4; 80% is recommended for species with relatively similar trait values(Pakeman and Quested 2007), which is true of northern hardwood overstory species. Stand C4, in which the sampled species represent 62% of basal area, has a large presence of quaking aspen (*Populus tremuloides* Michx.) and big-tooth aspen (*Populus grandidentata* Michx.), which were not sampled. One *A. rubrum* tree, the only one in stand HBO, was unintentionally sampled in the control plot; the foliage sampled from this tree was used to inform the community-weighted means in this plot but was not used in *A. rubrum* analyses.

Foliage samples were collected using a shotgun with steel shot from at least two sun-exposed portions of the canopy, and leaves were handled with gloves. Leaves were placed in gallon-sized plastic bags in the field with paper towels sprayed with distilled water to keep the samples hydrated until they were returned to the lab and refrigerated (Pérez-Harguindeguy et al. 2013). In total, 156 trees were sampled in 2021 and 270 trees were sampled in 2022.

Trait Measurements

Leaves that had little to no physical damage (from herbivory or steel shot) or disease were selected for trait measurements. On average, 9 leaves were selected per tree. We took photographs of each set of chosen leaves and took notes on the damage to aid interpretations of possible outliers. For a subset of 20 trees (at least three trees of each species sampled), we processed both a “damaged” and “undamaged” sample to compare damage effects on N, P, and leaf dry matter content (LDMC). Consistent effects of damage were not found, suggesting that including foliage with minor damage in the sample would yield small effects, if any, on these traits, and that there was little bias in choosing “undamaged” samples (Appendix A). If necessary to select damaged leaves, leaves with minor herbivory damage and intact margins were preferred over leaves with skeletonization or significant disease presence. No leaves with insect galls were selected for analysis.

Fresh leaves were weighed and scanned using a flatbed scanner to produce binary images at 300 DPI, which were used to calculate leaf area in ImageJ (https://imagej.net/ij/index.html). Leaves were then oven-dried at 60°C until constant weight to determine the dry mass. LDMC was calculated by dividing the dry mass by the fresh mass (Pérez-Harguindeguy et al. 2013). Specific leaf area (SLA) was calculated by dividing the leaf area (excluding holes and including petioles) by the dry mass (Pérez-Harguindeguy et al. 2013). These dried leaves were then ground using a Wiley mill with a 40-mesh screen, or a mortar and pestle if the dry mass was less than 1.0 g to minimize sample loss during processing. Subsamples of ground foliage (3.5-4.5 mg) were analyzed for δ^{13}C and N analysis using an Isoprime isotope-ratio mass spectrometer coupled with a Pyrocube combustion analyzer. Thirty-three samples were run in triplicate. Due to equipment malfunction, 110 of 426 samples could not be run for δ^{13}C, primarily from stands C2 and C6. Data from these two stands were consequently omitted from δ^{13}C analyses. Other subsamples of ground foliage (0.25 g) were microwave digested in 10 mL concentrated, trace metal-grade nitric acid using a MARS 6 microwave digestion system (CEM), diluted to 20% with deionized water, and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Optima 5300 DV, Perkin-Elmer) to obtain P concentrations. Duplicate samples were included with every acid digestion batch, and apple leaves (NIST 1515) were run as a tissue standard for N and P analyses.

For the NIST 1515 tissue standards, recovery of N was within 2.4% of the certified value on average, within 10.2% for all 29 NIST 1515 samples run. Replicates of N were within 9.1% of each other on average, with 32 of 33 replicates within 25% (maximum = 31%). Recovery of P was within 4% of the certified value on average, within 10% for all 36 NIST 1515 samples. Duplicates of P were within 3% of each other on average with a maximum of 13% difference; 16 of 19 duplicates were less than 5% different. NIST 1515 tissue standard δ^{13}C values were within 0.09 ‰ of the certified value on average, within 0.25 ‰ for all 15 NIST 1515 samples run, and replicates were within 0.12 ‰ on average, 0.25 ‰ at most.

Tree Growth Measurements

All trees ≥10 cm in diameter at breast height (DBH) were inventoried in the MELNHE study in late-spring 2011 (just after fertilization), 2015, and 2019 (Goswami et al. 2018; Blumenthal et al. in prep). To evaluate the annual relative basal area increment (RBAI) of the trees sampled for foliar traits in 2021 and 2022we used the DBH measurements in 2011 and 2019. Of the 426 trees sampled in this study, DBH measurements in 2011 and 2019 were available for 340 trees. This reduction is in part because 29 of the trees we sampled were in the plot buffer, which is not part of the tree inventory protocol. Also, only seven of the 24 *P. pensylvanica* trees had DBH values in 2011 and 2019 because many of the *P. pensylvanica* trees sampled in 2022 were less than 10 cm in diameter in 2011. Ten of the 426 trees did not have corresponding records in the tree inventory for unknown reasons.

RBAI was calculated for each tree as follows: $\mathrm{RBAI} = (1+ (\mathrm{BA}_{2019} - \mathrm{BA}_{2011})/\mathrm{BA}_{2011}))^{1/n})-1$, where BA_{2019} is the basal area of the stem in 2019, BA_{2011} is the basal area of the stem in 2011, and n is the number of years between measurements.

Data Analysis

To visualize relationships among traits, we performed a principal components analysis (PCA) using all species and all traits. This PCA was run in R (R Core Team 2022) using the ‘prcomp’ function and visualized using the ‘fviz_pca_biplot’ function of the ‘factoextra’ package (Kassambara and Mundt 2016). Species and treatments were visualized in separate PCA biplots to show how species compared and how suites of traits changed with nutrient addition treatment.

To determine whether the growth of each tree species was limited more by N or P, we ran separate linear mixed-effects models for RBAI for all species except for *P. pensylvanica*, which did not have enough trees. Fixed effects were addition of N, addition of P, the interaction of N and P, stand age, and site. Stand and plot within stand were included as random effects. Random effects of plot were zero for *F. grandifolia, A. rubrum, A. saccharum*, and *B. papyrifera*; therefore significant effects were interpreted with caution for these species (Appendix B). Analyses were performed in R version 4.2.2 (R Core Team 2022) with the ‘lme4’ and ‘lmerTest’ packages (Bates et al. 2015; Kuznetsova et al. 2017) using the ‘lmer’ function and Type III sums of squares and Satterthwaite degrees of freedom.

To further explore effects of N and P fertilization on foliar traits, we ran a linear mixed-effects model for each trait (five traits) for each species (six species): 30 models. Trait values at the tree level were used as response variables. Fixed effects were N addition, P addition, the interaction of N and P, and stand age (mid-successional and mature) and site. Site in these models served primarily as a blocking factor, as sites were sampled in different years and thereby the effects of sampling year and site were confounded. However, site was considered a fixed effect, rather than a random effect, because sites were deliberately selected based on differences in bedrock and, presumably, site productivity (see site description). Random effects were stand and plot within stand. Response variables were log transformed if necessary to meet assumptions of normality in the residuals (Gotelli and Ellison 2013). If assumptions could not be met (i.e., due to outliers), the models were run both with and without outliers; both sets of results are presented (Appendix C). Analyses were performed in R with the ‘lme4’ and ‘lmerTest’ packages (Bates et al. 2015; Kuznetsova et al. 2017) using the ‘lmer’ function and Type III sums of squares and Satterthwaite degrees of freedom. Site was removed from overfit models when doing so removed the singularity or enabled convergence; such models included foliar N for *A. rubrum* and *B. alleghaniensis*, LDMC for *B. papyrifera*, SLA for *A. saccharum* and *B. alleghaniensis*, and δ^{13}C for *A. rubrum* and *B. papyrifera* (Appendix C). Significant differences were characterized by calculating the differences in least-squares means with the ‘difflsmeans()’ function in ‘lmerTest’, using Satterthwaite degrees of freedom. The one *A. rubrum* tree in HBO was not included in *A. rubrum* because it was the only *A. rubrum* tree in the stand.

For six of the 30 trait models, stand effects were zero, which resulted in a singularity. This functionally resulted in the testing of site and stand age at the plot level, rather than at the stand level, which is a form of pseudoreplication. A concern with pseudoreplication is the reporting of factors as significant when they are not (Hurlbert 1984), but in only one of these models (δ^{13}C for *A. saccharum*) was site or stand age significant; this model should be interpreted with caution. Similarly, plot effects were zero in six of 30 models, which constitutes pseudoreplication for tests of N, P, and N x P, testing significance at the tree level rather than plot level. These models include foliar LDMC and SLA for *A. rubrum*, LDMC for *B. papyrifera*, SLA for *P. pensylvanica*, δ^{13}C for *B. alleghaniensis*, and foliar N for *A. saccharum*; these results were interpreted with caution. Removing an outlier remedied this issue for SLA in *B. papyrifera* and foliar N in *A. saccharum*, which in the case of *B. papyrifera* also eliminated the significance of a P-addition effect.

Linear mixed-effects models with the same fixed and random effects described above were also run for each trait at the community level using community-weighted means of each trait. CWMs for each plot ($\text{CWM}_{\text{Total}}$) were calculated using:

$$CWM = \sum_1^n p_{ij} x_{ij}$$

where *n* is the number of species, *p* is the proportion of basal that species *i* occupies in “community” (plot) *j*, and *x* is the mean trait value of species *i* in plot *j*. Fixed effects of these models were N, P, N x P, stand age, and site. Stand was a random effect. CWM SLA was logarithm-transformed to meet assumptions of normality. Due to missing data, CWM $\delta^{13}C$ was not calculated in stands C2 and C6. The one *A. rubrum* tree was included in CWM calculations for the control plot in HBO to improve accuracy.

To explore whether differences in CWMs with nutrient addition and stand age were due to species composition or within-species variability, these models were run using interspecific CWM values (CWM_{Inter}) and the contribution of intraspecific variability to CWM_{Total} (CWM_{Intra}; Lepš et al. 2011). CWM_{Inter} was calculated the same way as CWM_{Total} except that a study-wide mean trait value for each species was used instead of a plot-specific mean. This produced one mean for each species, with differences among CWM_{Inter} attributed to differences in species abundance across plots. CWM_{Intra} was calculated by subtracting CWM_{Inter} from CWM_{Total} (Lepš et al. 2011).

Results from all linear mixed-effects models run in this study were considered statistically significant if $p < \alpha = 0.05$ and were considered marginally statistically significant if $p < \alpha = 0.10$. We interpreted both significant and marginally significant results.

Table 2-2. Characteristics of MELNHE stands included in this study (Yanai et al. 2022). Species symbols are taken from the U.S. Department of Agriculture PLANTS database (Soil Conservation Service, 1982): ACRU = *Acer rubrum* (red maple), ACSA3 = *Acer saccharum* (sugar maple), BEAL2 = *Betula alleghaniensis* (yellow birch), BEPA = *Betula papyrifera* (white birch), FAGR = *Fagus grandifolia* (American beech), PRPE2 = *Prunus pensylvanica* (pin cherry). Species are listed in order from most to least abundant within stands.

Site	**Stand**	**Stand Age**	**Year Cut**	**Elevation (m)**	**Aspect**	**Slope (%)**	**Sampled Species**
BEF	C1	Mid-successional	1990	570	SE	5-20	BEPA, PRPE2, FAGR, BEAL2
	C2	Mid-successional	1988	340	NE	15-30	ACRU, FAGR, BEPA, PRPE2, BEAL2
	C4	Mid-successional	1979	410	NE	20-25	BEPA, ACRU, FAGR, BEAL2
	C6	Mid-successional	1975	460	NNW	13-20	ACRU, BEPA, BEAL2, FAGR
	C8	Mature	1883	330	NE	5-35	FAGR, ACSA3, BEAL2
	C9	Mature	1890	440	NE	10-35	ACSA3, FAGR, BEAL2
HB	HBM	Mid-successional	1970	500	S	10-25	BEAL2, BEPA, ACSA3, ACRU, FAGR
	HBO	Mature	1911	500	S	25-35	BEAL2, FAGR, ACSA3
JB	JBM	Mid-successional	~1975	730	WNW	25-35	BEAL2, BEPA, ACSA3
	JBO	Mature	1915	730	WNW	30-40	ACSA3, BEAL2, FAGR

Results

Relationships Among Foliar Traits

Foliar N, foliar P, SLA, and LDMC strongly co-varied with each other, all contributing to the first principal component axis (PC1), which explained 36% of variance in the data. In particular, foliar N and P were highly positively correlated with each other, and foliar N, P, and SLA were negatively correlated with LDMC (Figure 2-1). The second principal component axis (PC2) explained 27% of the variance and was most strongly explained by SLA and $\delta^{13}C$, which were negatively correlated with each other (Figure 2-1). Carbon isotope composition was not strongly correlated with PC1, but was slightly, positively correlated with LDMC and negatively correlated with foliar N and P (Figure 2-1). *Acer saccharum, A. rubrum,* and *F. grandifolia* clustered together in the PCA, indicating similarity in trait space; the centroids of these species clustered closer to high LDMC values and lower foliar N and P values, meaning that these three species all tended to have relatively high LDMC and low foliar N and P. *Prunus pensylvanica* was located the farthest from the other species, in a trait space indicating high values of foliar N and P and low values of LDMC (Figure 2-1A). *Betula papyrifera* and *B. alleghaniensis* were located between *P. pensylvanica* and the other species (Figure 2-1A).

Treatment centroids were very similar to each other, but the control centroid was located closer to the high LDMC end of PC1 than the P and N+P treatments, suggesting that the control trees were more likely to have high LDMC than P-addition treatments. The N and N+P addition treatments were located closer to the higher $\delta^{13}C$ end of PC2 (Figure 2-1B).

Tree Growth Response to N and P Addition and Stand Age

The mean annual relative basal area increment (RBAI, expressed in % per year) was 1.1 ± 0.3 higher with N addition for *B. alleghaniensis* ($F = 5.31$, $p = 0.03$) and 0.7 ± 0.3 higher with N addition for *B. papyrifera* ($F = 4.76$, $p = 0.03$; Figure 2-2, Appendix B), though *B. papyrifera* results should be interpreted cautiously due to pseudoreplication. RBAI was higher on average in mid-successional stands than mature stands for *A. saccharum* (2.0 ± 0.5 higher, 2.4 ± 0.06 higher without outlier; $F = 15.81$, $F = 15.14$ without outlier; $p < 0.01$, $p = 0.06$ without outlier), *F. grandifolia* (3.7 ± 1.4 higher; $F = 7.39$, $p = 0.04$), and *B. alleghaniensis* (2.3 ± 0.4 higher; $F = 7.83$, $p = 0.05$, Figure 2-2, Appendix B).

Trait Response to N and P Addition

As expected, foliar N was higher with N addition and foliar P was higher with P addition for every species ($p \leq 0.02$; Figures 2-3 and 2-4, Appendix C); the only exception was *A. rubrum*, for which we did not detect an increase of N with N addition ($p = 0.44$; Appendix C). For *F. grandifolia*, *P. pensylvanica*, *A. rubrum*, and *B. alleghaniensis*, foliar N was lower with P addition ($p = 0.09$, 0.07, 0.05, and < 0.01, respectively; Appendix C); foliar N did not consistently vary with P addition for *A. saccharum* or *B. papyrifera* ($p \geq 0.20$; Figure 2-3, Appendix C). For *F. grandifolia*, *P. pensylvanica*, and *B. alleghaniensis*, foliar P was lower with N addition ($p \leq 0.05$; Figure 2-5, Appendix C). Foliar P did not consistently vary with N addition for *A. rubrum* or *B. papyrifera* and was actually slightly higher with N addition for *A. saccharum* ($p = 0.02$; Figure 2-4, Appendix C). The interaction between N and P was significant for foliar P in *A. saccharum* and *B. alleghaniensis* ($p = 0.02$ and 0.05, respectively; Appendix C) because foliar P was lower with N+P addition than with P addition alone, and foliar P was even lower for the control and N-addition treatments, but the control and N-addition treatments did not differ from each other (Figure 2-4, Appendix C). We did not observe consistent effects of stand age or site on either foliar N or P. Repeating these species-level analyses with only the trees for which we had RBAI measurements produced slightly different results for several species. In particular, foliar N did not differ with P addition in *F. grandifolia* in the model with only trees for which we had RBAI measurements ($p \geq 0.35$, Appendix D), whereas it decreased with P addition in the full analysis. Likewise, foliar P did not differ with N addition in *F. grandifolia* in the RBAI measurement-only model ($p \geq 0.16$, Appendix D), whereas it decreased with N addition in the full analysis. Foliar N increased with P addition for *A. saccharum* in the model with only trees for which we had RBAI measurements ($F = 4.39$, $p = 0.04$), and foliar P decreased with N addition for *B. papyrifera* in the model with only trees for which we had RBAI measurements ($F = 5.17$, $p = 0.04$).

Community-weighted foliar N increased by 2.7 ± 0.5 mg g^{-1} with N addition ($F = 24.8$, $p < 0.01$) and decreased by 1.1 ± 0.5 mg g^{-1} with P addition ($F = 4.38$, $p = 0.05$; Figure 2-5, Appendix C). Community-weighted foliar P was 0.73 ± 0.05 mg g^{-1} higher with P addition than without ($F = 221.0$, $p < 0.01$), and was 0.31 ± 0.07 mg g^{-1} lower with N+P addition than P addition alone ($t = 4.42$, $p < 0.01$), but did not differ between the control and N addition treatments (difference in means = 0.01 ± 0.07 mg g^{-1}, $t = 0.17$, $p = 0.87$). These patterns were also detected for CWM_{Intra} ($p \leq 0.02$) but not CWM_{Inter} ($p \geq 0.23$; Appendix C).

LDMC was influenced by nutrient addition at the species level, but SLA was not (Figure 2-6, Figure 2-7). LDMC was 29.0 ± 11.2 mg g^{-1} lower on average with P addition for *A. saccharum* ($F = 6.75$, $p = 0.02$), and 27.8 ± 7.4 mg g^{-1} lower for *P. pensylvanica* after removing an outlier ($F = 14.18$, $p = 0.03$; Figure 2-6, Appendix C). Neither community-weighted LDMC nor SLA differed with N or P addition ($p \geq 0.47$, Appendix C).

Enrichment of ^{13}C increased with N addition for *B. papyrifera* and *A. saccharum*, indicated by an increase in $\delta^{13}C$ by 0.57 ± 0.28 ‰ for *B. papyrifera* ($F = 4.18$, $p = 0.07$) and by 0.39 ± 0.22 ‰ for *A. saccharum* ($F = 3.30$, $p = 0.09$; Figure 2-8, Appendix C). Community-weighted $\delta^{13}C$ was 0.30 ± 0.15 ‰ higher with N addition ($F = 3.73$, $p = 0.07$; Figure 2-5, Appendix C), which was driven by differences within species (CWM_{Intra} $\delta^{13}C$ $F = 4.22$, $p = 0.05$), but did not differ with P addition ($p = 0.41$). CWM_{Inter} $\delta^{13}C$ was 0.09 ± 0.04 ‰ greater on average with P addition ($F = 5.86$, $p = 0.03$), but CWM_{Intra} was not ($p = 0.75$; Appendix C), suggesting that differences in $\delta^{13}C$ were due to differences in species abundance, rather than within-species variability.

Effects of Stand Age and Site

Physical traits of foliage (SLA and LDMC) differed by stand age, with 27.5 ± 11.7 mg g^{-1} higher LDMC on average in mature than mid-successional stands for *F. grandifolia* ($F = 5.55$, $p = 0.06$) and 28.9 ± 9.7 mg g^{-1} higher LDMC on average in mature stands for *B. alleghaniensis* ($F = 8.87$, $p = 0.03$; Figure 2-6, Appendix C). LDMC was also higher at Hubbard Brook and Jeffers Brook than at low fertility Bartlett for *F. grandifolia* and *B. alleghaniensis* ($p = 0.09$ and 0.07 for *F. grandifolia* and *B. alleghaniensis*; Figure 2-6, Appendix C). SLA was 3.78 ± 1.28 m^2 kg^{-1} lower in mature than mid-successional stands for *A. saccharum* ($F = 8.69$, $p = 0.047$) and was 2.62 ± 1.02 m^2 kg^{-1} lower in mature stands *B. alleghaniensis* ($F = 6.65$, $p = 0.03$), but did not differ with stand age for *F. grandifolia* ($F = 0.42$, $p = 0.54$; Figure 2-7, Appendix C).

Community-weighted LDMC in mature stands was 50.2 ± 9.3 mg g^{-1} higher on average than in mid-successional stands ($F = 28.9$, $p = 0.05$), but SLA did not differ with stand age ($p = 0.89$, Figure 2-5, Appendix C). Stand age was significant for both CWM_{Inter} and CWM_{Intra} LDMC, meaning that the higher LDMC in mature stands was due both to species composition differences between mature and mid-successional stands and within-species variability ($p \leq 0.01$; Appendix C). CWM_{Intra} LDMC was higher at Hubbard Brook than Jeffers Brook and Bartlett Experimental Forest ($F = 4.71$, $p = 0.02$; Figure 5, Appendix C). CWM_{Inter} SLA was 1.47 ± 0.40 m^2 kg^{-1} higher in mature stands for SLA, reflecting species differences ($F = 13.336$, $p = 0.06$; Appendix C).

Enrichment of ^{13}C was greater in mature stands than mid-successional for *A.* saccharum, indicated by a mean $\delta^{13}C$ value that was 0.84 ± 0.26 ‰ higher in mature stands ($F = 10.22$, $p < 0.01$; Figure 2-8, Appendix C), but did not differ with stand age in any other species. While CWM_{Total} $\delta^{13}C$ did not differ with stand age, CWM_{Inter} $\delta^{13}C$ was 0.57 ± 0.20‰ lower in mature stands than in mid-successional stands ($F = 7.97$, $p = 0.05$), but CWM_{Intra} $\delta^{13}C$ was 0.59 ± 0.23‰ higher in mature stands ($F = 6.71$, $p = 0.06$; Appendix C).

Community-weighted foliar N was 1.9 ± 0.9 mg g^{-1} higher in mid-successional stands than in mature stands ($F = 5.02$, $p = 0.07$, Figure 2-5, Appendix C). Foliar N did not differ by age in *F. grandifolia*, *A. saccharum*, and *B. alleghaniensis*, the three species found in both stand age classes ($p \geq 0.22$, Figure 2-3, Appendix C).

Community-weighted foliar P differed by site ($F = 5.57$, $p = 0.04$; Figure 2-5, Appendix C), being 0.25 ± 0.08 mg g^{-1} higher at Hubbard Brook than at Bartlett Experimental Forest and 0.30 ± 0.10 mg g^{-1} higher at Hubbard Brook than at Jeffers Brook. No other traits differed by site.

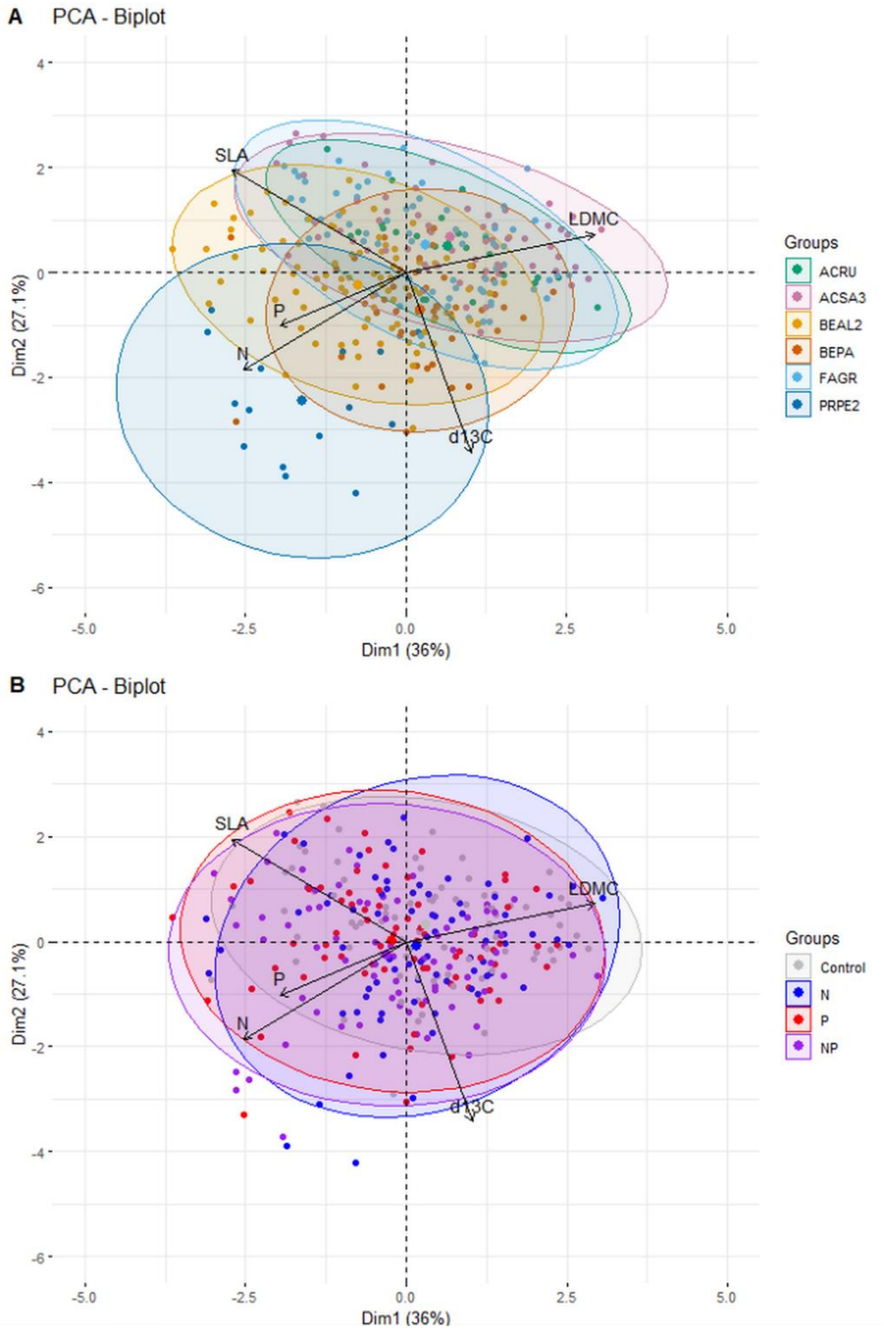

Figure 2-1. Principal components analysis using five foliar traits in 426 trees from six northern hardwood species located across 10 stands in a long-term N x P fertilization experiment, distinguished by species (A) and treatment (B). Centroids for each species or treatment group correspond in color and are slightly larger than individual tree points. Dimension 1 (PC1) explained 37% of the variability in trait values and Dimension 2 (PC2) explained 27%.

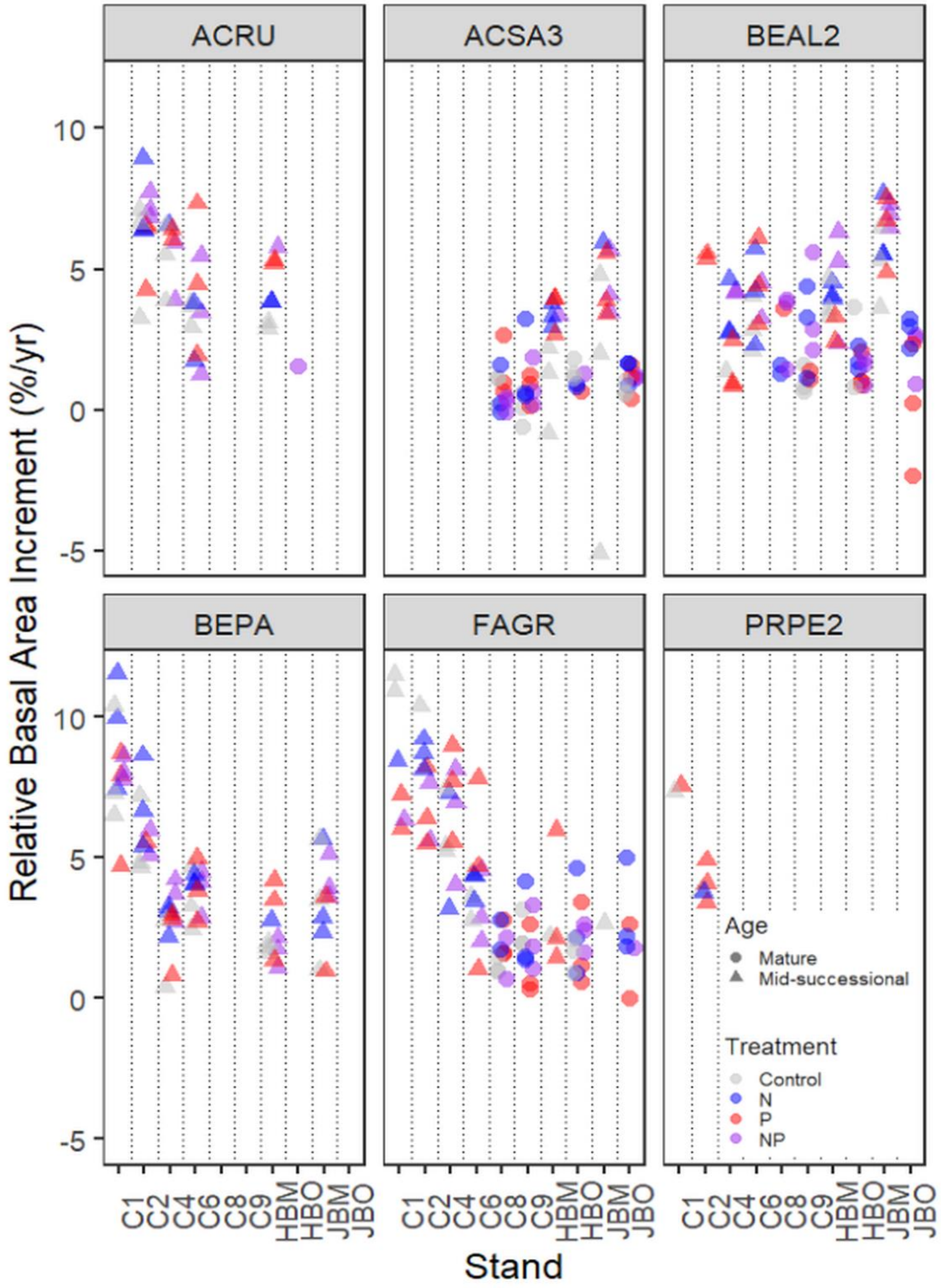

Figure 2-2. Relative basal area increment (% per year) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-aged and mature forest stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

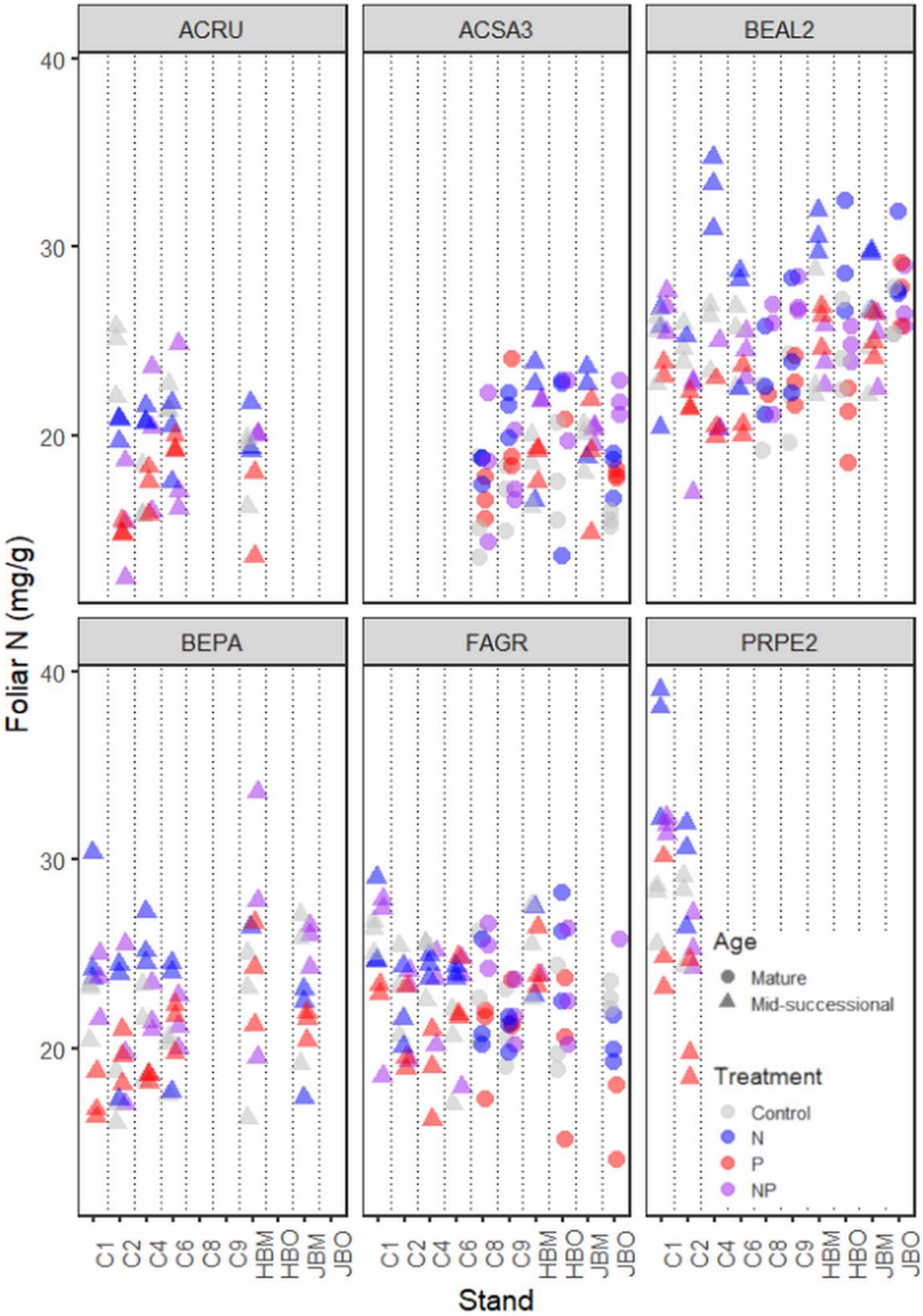

Figure 2-3. Foliar N concentrations (mg g^{-1}) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-successional and mature stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

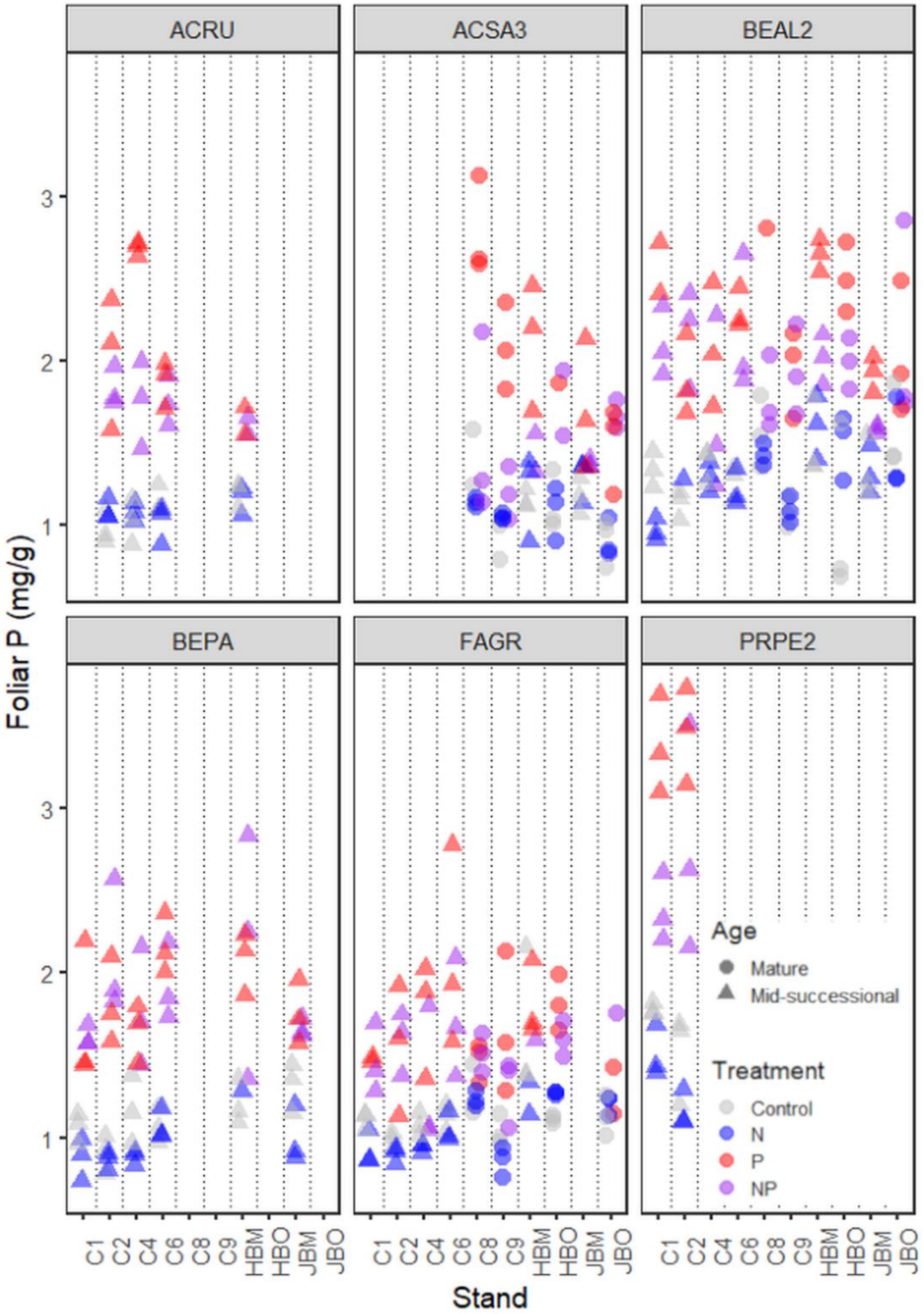

Figure 2-4. Foliar P concentrations (mg g^{-1}) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-successional and mature forest stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

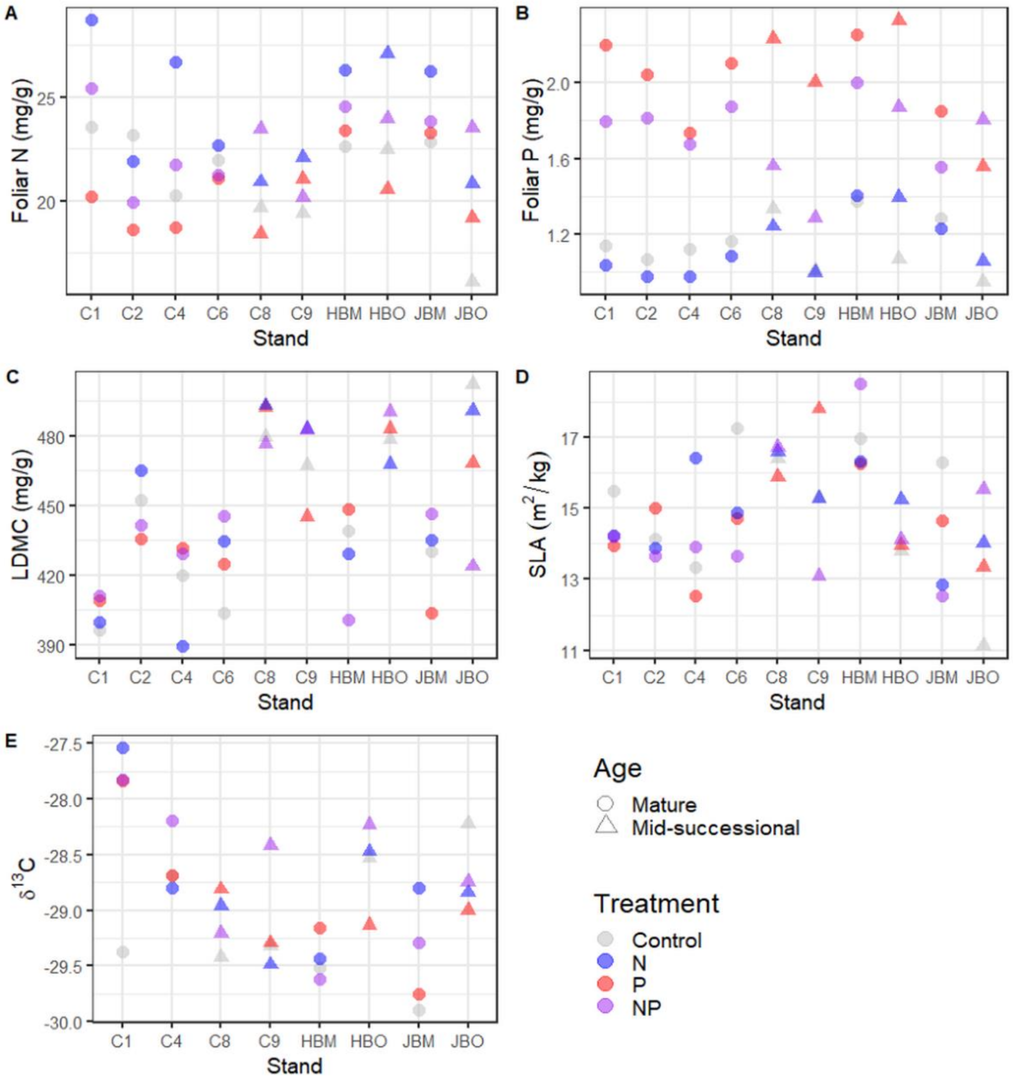

Figure 2-5. Community-weighted means of foliar N (A), P, (B), leaf dry matter content (LDMC, C), specific leaf area (SLA, D), and carbon-isotope composition (δ^{13}C, E) in forest plots receiving N addition (blue), P addition (red), N and P addition (purple), or no nutrients (control, gray).

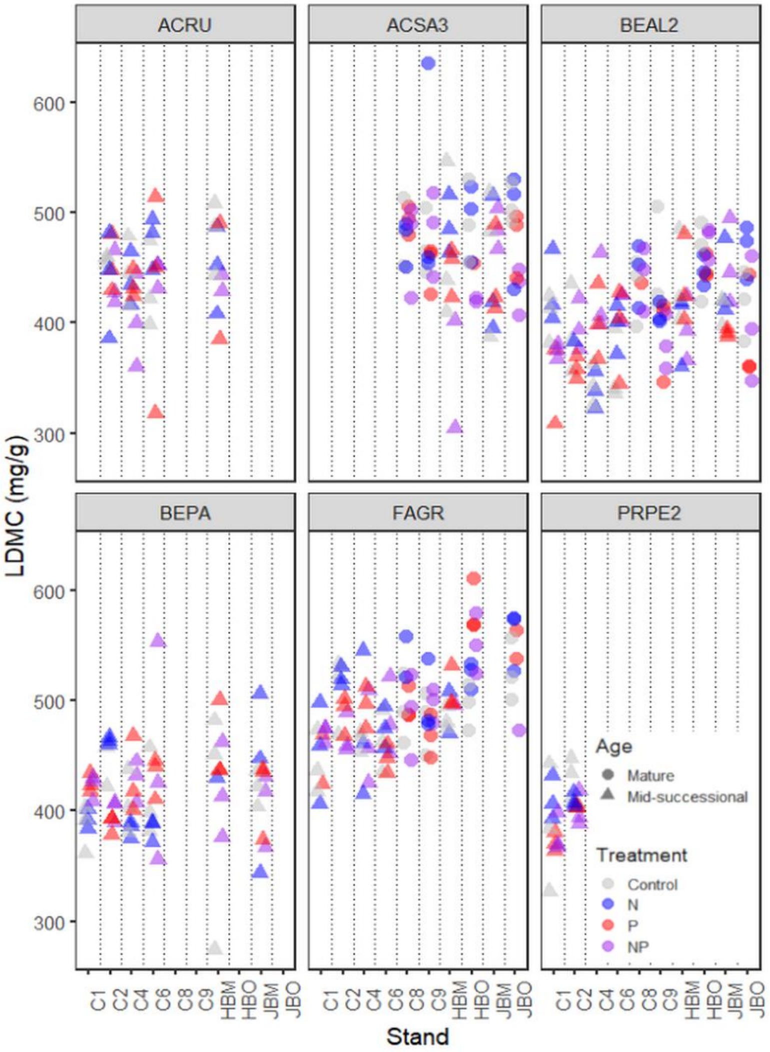

Figure 2-6. Foliar leaf dry matter content (LDMC, mg dry weight per g fresh weight) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-successional and mature forest stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

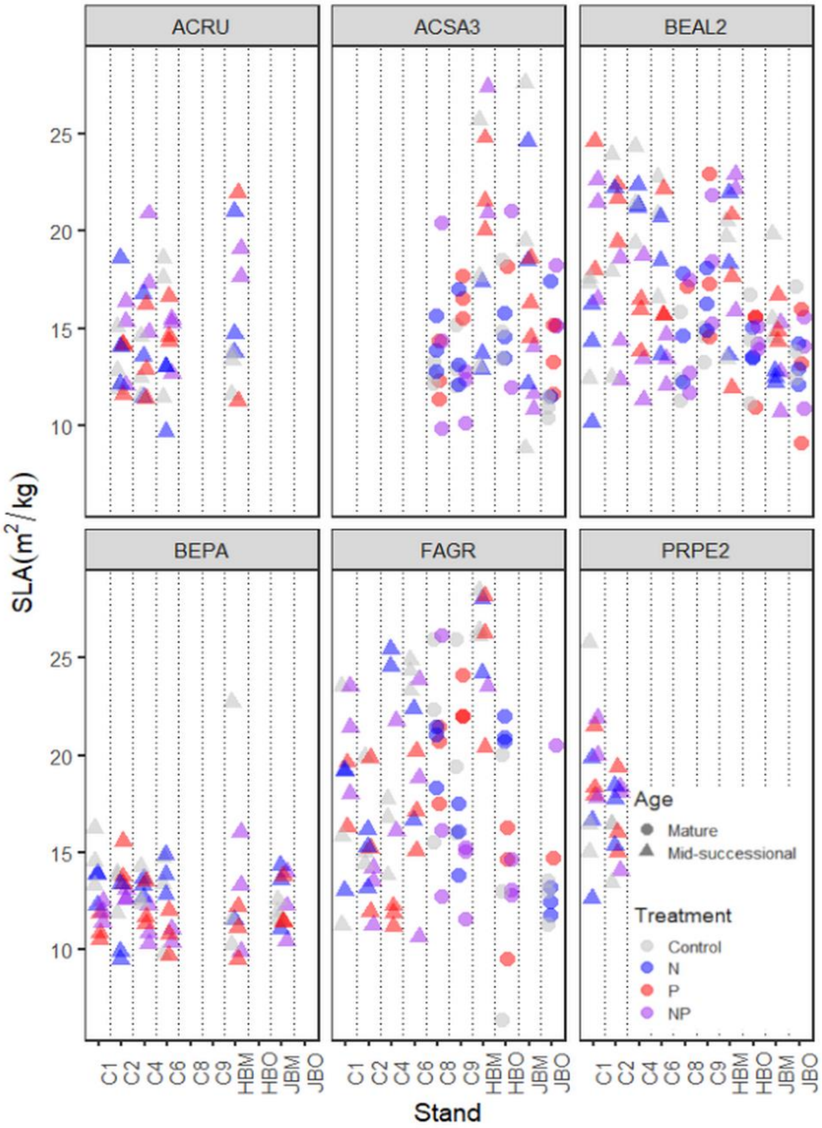

Figure 2-7. Foliar specific leaf area (SLA, m^2 per kg dry weight) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-successional and mature forest stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

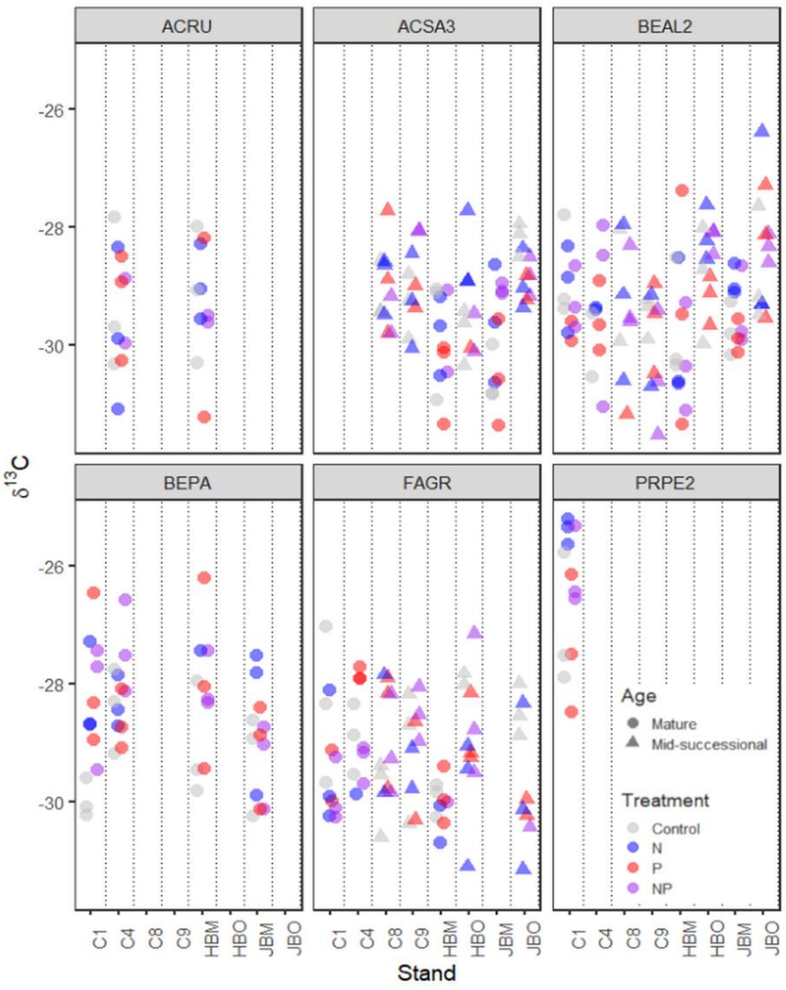

Figure 2-8. Foliar carbon isotope composition ($\delta^{13}C$, ‰) in individual trees of six species in plots that have received N (blue), P (red), N and P (purple), or neither N and P (gray) in mid-successional and mature forest stands. Tree species include *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), and *Prunus pensylvanica* (PRPE2).

Discussion

We sought to evaluate the response of foliar traits in six northern hardwood tree species to low-level, long-term N and P addition in a full-factorial experiment in order to improve our understanding of responses to changes in nutrient availability and limitation. The trait responses indicated an adherence to the leaf economics spectrum for four of the traits, with $\delta^{13}C$ displaying a slight association with a conservative plant strategy but a weak correlation with this spectrum overall. Four of these traits showed a shift in a more acquisitive resource strategy, though $\delta^{13}C$ changed in a way that indicated a more conservative strategy, suggesting a potential tradeoff between nutrient-use efficiency and water-use efficiency (e.g., Cramer et al. 2009). Our results also indicated differential and synergistic responses to N and P addition as well as some evidence of N and P co-limitation, at the species and the community level. By evaluating response of individual species and the community as a whole and separating out the intraspecific variation from interspecific variation in community-weighted means, we can better understand what drives the differences we see in response to N and P addition at the community level.

Relationships among foliar N, P, SLA, and LDMC were consistent with those predicted by the leaf economics spectrum, with high N, P, and SLA on one end and high LDMC on the other. In this way, the first PCA axis represents the leaf economics spectrum, which has commonly been observed in other trait-based studies (Wright et al. 2004; Zukswert and Prescott 2017; Hecking et al. 2022). Carbon isotope composition ($\delta^{13}C$) did not strongly correlate with this axis, but slightly fell on the same side of the leaf economics spectrum axis as LDMC, suggesting that high $\delta^{13}C$, indicating ^{13}C enrichment, was associated with a more conservative strategy; this is reasonable, considering that high $\delta^{13}C$ is associated with high intrinsic water-use efficiency (iWUE), and is consistent with past studies associating $\delta^{13}C$ with other traits in the leaf economics spectrum (e.g., Prieto et al. 2018; De La Riva et al. 2019). $\delta^{13}C$ was less strongly correlated with this axis than with the second axis, which suggested a negative relationship between $\delta^{13}C$ and SLA. A negative association between $\delta^{13}C$ and SLA has previously been reported in *Eucalyptus* species in Australia (Schulze et al. 2006). This negative association is also consistent with the spectrum of conservative to acquisitive traits in the leaf economics spectrum, as high $\delta^{13}C$ (i.e., higher iWUE) and low SLA are associated with conserving water and nutrients (Reich 2014; Prieto et al. 2018).

The low correlation of $\delta^{13}C$ with the PC1 axis could represent the fact that, unlike the other traits, nutrient addition does not shift $\delta^{13}C$ towards the acquisitive end of the leaf economics spectrum, but rather towards the conservative end. This response of $\delta^{13}C$ to nutrient addition has been widely reported and is more in line with a tradeoff between nutrient-use efficiency and water-use efficiency (Cramer et al. 2009), and it complicates the interpretation of a single axis of trait values that represent plant strategies in response to carbon, nutrient, and water use and acquisition (e.g., Reich 2014). The distribution of centroids associated with treatments suggested that the control treatment had the highest LDMC value, and that the nutrient addition treatments tended to have higher N and P, though the magnitude of these differences was small (Figure 2-1B). This result suggests a shift in trait values from conservative to more acquisitive following nutrient addition, particularly for traits that have associations with carbon and nutrient acquisition and use, which is consistent with observations of shifts in trait values following fertilizer addition in Arctic tundra shrubs (Iturrate-Garcia et al. 2020).

The PCA illustrated how species differed from each other in terms of their traits, suggesting that, based on their positions along the PC1 axis, *A. saccharum*, *F. grandifolia*, and *A. rubrum* were the most conservative species, followed by *B. alleghaniensis* and *B. papyrifera*, with *P. pensylvanica* being the most acquisitive (Figure 2-1A). This spectrum from conservative to acquisitive species aligns with the relative shade tolerance of these species, with *F. grandifolia and A. saccharum* being highly shade tolerant while *P. pensylvanica* is extremely shade intolerant, and *B. alleghaniensis, B. papyrifera*, and *A. rubrum* are intermediate species (Burns and Honkala 1990). This ordering of species along the PC1 axis provides more support for the association between the leaf economics spectrum and shade tolerance and is consistent with a previous fertilization study in this region suggesting that *P. pensylvanica* is particularly acquisitive (Fahey et al. 1998; Hallik et al. 2009). *Acer rubrum* appears to be a possible exception, as it clustered near the conservative end of the spectrum but tends to be intermediate in tolerance (Burns and Honkala 1990).

For *B. alleghaniensis* and *B. papyrifera*, we detected a response of RBAI to N addition, suggesting N limitation. Results for *B. papyrifera,* however, may be overstated due to pseudoreplication; while the effects of N and P should be tested at the plot level, they were tested at the tree level in *B. papyrifera*, which increases the probability of obtaining a false positive result. Nitrogen limitation is consistent with an earlier study in MELNHE by Hong et al. (2022), who also found in their analysis of RBAI from 2011 to 2015 that trees in these stands responded to N but not P. Many of the trees analyzed by Hong et al. (2022) were also included in this current study. N limitation is common in this region and is common in temperate forests in general (Vitousek and Howarth 1991; Finzi, 2009; Vadeboncoeur, 2010). Despite decades of N deposition in this area, foliar and soil N availability at Hubbard Brook, among other areas of the northeastern United States, have been decreasing over time perhaps as a result of increased carbon flux from the atmosphere to soils, promoting N limitation (Groffman et al. 2018; Mason et al. 2022). Initial RBAI analyses in MELNHE using all inventoried trees found primarily P limitation (Goswami et al. 2018), which contrasts with the result of Hong et al. (2022) for a subset of those trees due to a stronger response of small trees to P. More recent data from the full tree inventory in MELNHE, however, showed a greater response to N and P addition together than to either alone, suggesting N and P co-limitation (Blumenthal et al. in prep). Our current analyses provide some evidence of N limitation, but also potential co-limitation of N and P, in these trees. It is important to remember that our RBAI models do not include all the trees sampled in this study due to lack of pre-treatment diameter data for many of the sampled trees, particularly *P. pensylvanica*, because they were too small (< 10 cm in diameter at breast height) to be measured in 2011.

Foliar N and P concentrations in our stands demonstrated evidence of N and P co-limitation at the species level and the community level. Co-limitation in communities and populations is evident when the concentration of one nutrient in autotrophic tissues (e.g., foliage) decreases in the presence of the other, and vice versa; this suggests that different species or individuals within species are limited by different nutrients (Bracken et al. 2015). Either no change or an increase in one nutrient with the addition of the other can indicate biochemically dependent co-limitation (Saito et al. 2008; Bracken et al. 2015), which occurs when the addition of one nutrient synergistically increases the availability of the other.

At the species level, half of the species (*B. alleghaniensis*, *F. grandifolia*, and *P. pensylvanica*) exhibited a decrease in foliar N following P addition and vice versa, which suggests co-limitation. Among the other species, *Betula papyrifera* did not exhibit any measurable decreases in foliar N or P with the addition of the other nutrient, which could be evidence of biochemically dependent co-limitation, or evidence that *B. papyrifera* is more strongly limited by another resource. However, when only the trees for which we have RBAI measurements were analyzed foliar P exhibited a decline with N addition, which is consistent with the observation that N addition increased RBAI in *B. papyrifera* (Appendix D).

Both *Acer* species provided evidence of possible biochemically dependent co-limitation (Bracken et al. 2015). *Acer rubrum* exhibited a decrease in foliar N with P addition but no change in foliar P with N addition, which could suggest P limitation, but could also suggest biochemically dependent co-limitation, if N addition led to a consequent increase in P availability that offset a predicted decrease with N addition. This may be especially true for *A. saccharum*, for which foliar N did not change with P addition but foliar P measurably increased with N addition, consistent with observed increases in foliar P with increasing N deposition (Crowley et al. 2012). It is possible that N addition increased the production of phosphatase by roots and mycorrhizae, which would increase P availability, offsetting the expected decrease in foliar P with N addition and resulting in no net change in foliar P (Marklein & Houlton, 2012). Indeed, past research in these same forest stands prior to fertilization demonstrated a positive relationship between soil N availability and phosphatase production (Ratliff and Fisk 2016). In this way, both *Acer* species could be co-limited by N and P, and perhaps more so than for the other species, i.e., N addition might have stimulated a greater production of phosphatases by their roots and mycorrhizae. *Acer* trees exclusively form relationships with arbuscular mycorrhizae (AM; Brundrett and Tedersoo 2020), and AM trees have been associated with increased acid phosphatase activity relative to trees that form symbiosis with ectomycorrhizal species (Ma et al. 2021), which lends further support for this hypothesis.

These diverse foliar responses to N and P at the species level, which indicate P limitation or co-limitation, suggest community-level co-limitation. Community-weighted foliar N decreased with P addition, and foliar P was lower with N+P addition than with P alone, but there was no difference between the control and the N addition treatment. We observed the same relationships in $\mathrm{CWM_{Intra}}$ (but not $\mathrm{CWM_{Inter}}$) for both nutrients, verifying that these response to N and P addition are due primarily to within-species differences among plots, rather than pre-existing or developing differences in species composition among plots. In one sense, this could suggest P limitation, in that P addition suppressed concentrations of N, but N alone did not suppress foliar P. The interaction between N and P, however, more likely indicates co-limitation, in that N addition may have suppressed foliar P concentrations when both N and P were added due to a dilution effect (Jarrell and Beverly 1981) resulting from greater tree growth with both N and P than with P alone (Ostertag and DiManno 2016). The lack of difference between the control and N treatments could be a sign of increased P availability with N addition, through phosphatase production (Marklein and Houlton 2012) or increased fine root production and turnover (Ma et al. 2021). Notably, in three mature stands of the MELNHE study, N addition caused increased root growth especially in mineral soil, presumably promoting increased access to soil P (Shan et al. 2022). These responses could indicate a coupling of N and P and potential co-limitation; this interpretation is corroborated by evidence for both N and P limitation among species. More research is needed to further elucidate mechanisms for an N-induced increase in P availability.

We observed decreases in LDMC with P addition (but not N addition) for *P. pensylvanica* and *A. saccharum*. We had initially predicted that we would see a decrease in LDMC with the addition of a limiting nutrient (Table 2-1). These results would thereby suggest a limiting role for P in at least *P. pensylvanica* and *A. saccharum*, in that greater P leads to a reduction in LDMC, which tends to be associated with a more acquisitive strategy and greater photosynthetic rate. Our RBAI results, however, did not enable us to evaluate which nutrients limit *P. pensylvanica* growth, and did not produce evidence of either N or P limitation of *A. saccharum*. In observational studies, plants with lower LDMC have been observed in areas with high soil P and vice versa (Cui et al. 2022). In fertilization studies, LDMC has been observed to decrease with P addition in some cases, such as an alpine plant community in Tibet (Liu et al. 2017). LDMC has been observed to decrease with increasing foliar P as well (Wu et al. 2020). These relationships could be due to the relationship between leaf water content and P, as P accumulates in cells as inorganic P solutes, which could lead to the increase of free and bound water in leaves, thereby lowering LDMC (Singh et al. 2006). The majority of N in leaves, however, is bound into proteins, with an estimated 50 to 80% of N in leaves in chloroplasts, incorporated into chlorophyll and proteins such as RuBisCO (Makino and Osmond 1991; Estiarte et al. 2022). The observed change in LDMC with P addition and not N, therefore, could be due to differences in N and P biochemistry within leaves, as an increase in N may not have the same osmotic effect as an increase in P.

We observed an increase in $\delta^{13}C$ with N addition in *B. papyrifera* and *A. saccharum* as well as at the community level, which together provide evidence for N limitation. *Betula papyrifera* also demonstrated an increase in RBAI with N addition, suggesting that $\delta^{13}C$ might assist in detecting nutrient limitations of tree growth. An increase in $\delta^{13}C$ with N addition could reflect an increase in photosynthetic capacity, which would increase the concentrations of CO_2 fixed during photosynthesis for the same stomatal conductance (Raven et al. 2004). $\delta^{13}C$ has been shown to increase with tree height, reflecting an increase in resistance of hydraulics; an increase in tree growth and in $\delta^{13}C$ with N addition could reflect an increase in tree height, though the differences we are seeing in $\delta^{13}C$ are too small to be explained by only changes in height (Vadeboncoeur et al. 2020). Higher CWM_{Inter} $\delta^{13}C$ with P addition, but lack of detectable change in CWM_{Intra} $\delta^{13}C$ or CWM_{Total} $\delta^{13}C$ with P addition, suggests that this P-addition result may be due to differences in species composition among plots, rather than within-species differences in $\delta^{13}C$.

Physical traits, foliar N, and $\delta^{13}C$ exhibited differences due to stand age. These effects were most clear and consistent for LDMC. The mature stands are dominated more by *F. grandifolia* and *A. saccharum*, relatively conservative species, and do not have any *P. pensylvanica* or *B. papyrifera*, the most acquisitive species in our study. This difference in species composition explains the higher $\mathrm{CWM_{Intra}}$ LDMC, higher SLA, and lower foliar N in mature stands, as SLA was lower in *B. papyrifera* and *P. pensylvanica* than the other species and foliar N was higher in *P. pensylvanica* than several of the species found in mature stands (Figure 2-3, Figure 2-7). Community-weighted $\delta^{13}C$ did not differ with stand age, but $\mathrm{CWM_{Inter}}$ of $\delta^{13}C$ was lower in mature stands while $\mathrm{CWM_{Intra}}$ was higher. The lower $\mathrm{CWM_{Inter}}$ of $\delta^{13}C$ in mature stands is likely due to the absence of *P. pensylvanica,* which had the highest $\delta^{13}C$ of all species studied (Figure 2-8). At the species level, lower SLA in mature stands for *B. alleghaniensis* and *A. saccharum*, and higher $\delta^{13}C$ in mature stands for *A. saccharum* are all consistent with past studies demonstrating changes in traits with tree height (Falster et al. 2018; Vadeboncoeur et al. 2020). If trees in the mature stands are taller, on average, than those in the mid-successional stands, then differences in tree height could in part explain differences in traits with stand age.

These results support hypotheses that either or both N and P are limiting within these northern hardwood forest communities, although they provide slightly more direct evidence for N limitation in tree growth, at least in *Betula* species. Traits differed in their responses to N and P, and these responses differed among species, but in general, changes in trait values with N and P addition were consistent with changes towards a more acquisitive resource strategy: higher foliar nutrient values, lower LDMC, and higher SLA. These results suggest that it may be important to remember that changes in N and P can differ in their effects on traits due to their different and multiple roles in plant biology, complicating the comparison between the two nutrients. This study provides hypotheses to further test relating to individual species' differences in response to nutrient addition and how these differences contribute to community-level changes.

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CHAPTER 3: RESPONSES OF STOMATAL DENSITY AND CARBON ISOTOPE COMPOSITION OF SUGAR MAPLE AND YELLOW BIRCH FOLIAGE TO N, P, AND $CASIO_3$ FERTILZATION

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Abstract

Stomatal density, stomatal length, and carbon isotope composition can all provide insights into environmental controls on photosynthesis and transpiration. Stomatal measurements can be time-consuming; it is therefore wise to consider efficient sampling schemes. Knowing the variance partitioning at different measurement levels (i.e., among stands, plots, trees, leaves, within leaves) can aid in making informed decisions around where to focus sampling effort. In this study, we explored the effects of nitrogen (N), phosphorus (P), and calcium silicate ($CaSiO_3$) addition on stomatal density, length, and carbon isotope composition ($\delta^{13}C$) of sugar maple (*Acer saccharum* Marsh.) and yellow birch (*Betula alleghaniensis* Britton). We observed a positive but small (8%) increase in stomatal density with P addition and an increase in $\delta^{13}C$ with N and $CaSiO_3$ addition in sugar maple, but we did not observe effects of nutrient addition on these characteristics in yellow birch. Variability was highest within leaves and among trees for stomatal density and highest among stomata for stomatal length. To reduce variability and increase chances of detecting treatment differences in stomatal density and length, future protocols should consider pre-treatment and repeated measurements of trees over time or measure more trees per plot, increase the number of leaf impressions or standardize their locations, measure more stomata per image, and ensure consistent light availability.

Introduction

Stomata are crucial for photosynthesis, respiration, and transpiration. The density and size of stomata together influence the capacity for photosynthesis and transpiration (Sack et al. 2003; Wang et al. 2015). Along with regulation of stomatal conductance, these characteristics can influence water-use efficiency (Franks et al. 2015). Changes in stomatal density and length could scale up to influence water-use efficiency (Mastrotheodoros et al. 2017) and net primary production (Wang et al. 2015) at the ecosystem scale. Therefore, in the context of increased CO_2 and increasingly variable precipitation expected with global climate change (Campbell et al. 2009), it is important to understand influences on stomatal characteristics to understand how individual plants, species, and ecosystems will respond.

Stomatal characteristics have a recognized genetic basis (Shimada et al. 2011), varying strongly with phylogeny (Liu et al. 2018), and are influenced by environmental factors such as light availability (Gay and Hurd 1975), atmospheric CO_2 concentrations (Woodward and Kelly 1995), soil moisture (Sun et al. 2014), and temperature (Beerling and Chaloner, 1993; Hill et al. 2014). Stomatal density has also been found to vary with tree height, though the direction of this effect is inconsistent (Woodruff et al. 2010; Kenzo et al. 2012), perhaps due to confounding effects of canopy position. This plasticity is just one component of plants' overall water-use strategy (Deans et al. 2020; Xu et al. 2021); other biological influences on plant water use, in addition to stomatal density and length, include regulation of stomatal conductance (Lawson and Vialet-Chabrand 2019), leaf area and sapwood area (Wullschleger et al. 1998), wood anatomy (Meinzer et al. 2013), and rooting architecture (Weigelt et al. 2021).

While effects of some environmental factors on stomatal characteristics are well known, studies on the effects of soil nutrient availability have yielded inconsistent results. In response to nitrogen (N) addition, stomatal density has increased in hybrid poplar (*Populus* x *euramericana* (Dode) Guiner var Dorskamp) and salmonberry (*Rubus spectabilis* Pursh; Siegwolf et al. 2001; van den Top et al. 2018) but decreased in *Arabidopsis thaliana* (L.) Heynh. (Cai et al. 2017). In response to the addition of phosphorus (P), stomatal density has increased in cowpea (*Vigna sinensis* L.) and chickpeas (*Cicer arietinum* L.; Sekiya and Yano 2008; Chtouki et al. 2022) or remained unchanged in *A. thaliana* and in six woody savanna species in Brazil (Cai et al. 2017; Costa et al. 2021). Effects of P addition on stomatal density were found to be positive in chickpea leaves when soil moisture was adequate (Chtouki et al. 2022) and positive in in cowpea leaves when atmospheric CO_2 concentrations were high (Sekiya and Yano 2008). In response to calcium carbonate addition, no change in stomatal density in mature European beech trees

(*Fagus sylvatica* L.) was detected (Forey et al. 2015). Other studies have observed a decrease in stomatal conductance with silicon fertilization (Gao et al. 2006; Vandegeer et al. 2021). Little research has been conducted on the effects of nutrient addition on stomatal characteristics in mature trees, and no research has been conducted on stomatal characteristics in a fully factorial N x P addition experiment.

Stomatal density and length can influence photosynthetic capacity and transpiration, but the actual rate of either process also depends on stomatal conductance. One way to observe the cumulative outcome of stomatal conductance over the lifetime of a leaf is to measure the stable carbon (C) isotope ratio ($\delta^{13}C$), which is related to the discrimination between ^{12}C and ^{13}C as leaves convert atmospheric carbon dioxide to photosynthate (Farquhar et al. 1982). $\delta^{13}C$ can thus provide insight on how nutrient addition might influence physiological function. $\delta^{13}C$ relates to intrinsic water-use efficiency (iWUE): the ratio of photosynthesis (A) to stomatal conductance (g_s), or of micromoles of CO_2 consumed in photosynthesis to moles of water lost due to transpiration (Pérez-Harguindeguy et al. 2013). iWUE has been shown to increase with added N and P when adding N or P increases photosynthetic capacity (Raven et al. 2004), so we would expect $\delta^{13}C$ to also increase with N or P addition, particularly if N or P limits tree growth. Increases in transpiration with calcium (Ca) or silicate (SiO_3) addition (Green et al. 2013) could result in decreased iWUE and thereby a decrease in $\delta^{13}C$, though iWUE could increase if photosynthetic capacity were to increase more than transpiration or if total leaf area were to increase, both of which are possible given increases in forest production following $CaSiO_3$ addition (Battles et al. 2014; Fahey et al. 2022). Other environmental factors that can influence $\delta^{13}C$ include climate (Rao et al. 2017), tree height, and light availability, though comparisons of $\delta^{13}C$ in shaded vs. open-canopy trees suggest that light availability and canopy position are most influential than tree height in influencing these characteristics (Vadeboncoeur et al. 2020).

Stomatal characteristics are easy and inexpensive to measure, but measuring a sufficient number to detect treatment effects in a large study can be time consuming. Decisions must be made at a variety of levels; for example, researchers must decide how many trees to sample (per species, plot, treatment, etc.), how many leaves of each tree to sample, how many impressions to make, the area of the leaf blade to study, and how many stomata for which to measure length. It is wise to consider the sampling intensity at each level to enhance the ability to detect treatment effects. Variance partitioning analysis, in which a linear mixed-effects model is run on data with no fixed effects and with nested random effects (Messier et al. 2010), is often used in trait studies to quantify variability at different taxonomic or otherwise hierarchical levels (Messier et al. 2010; Albert et al. 2010; Burton et al. 2017; Hecking et al.

2022). By showing at which level(s) variance is highest, researchers can optimize sampling intensity across multiple levels of organization.

Here, we measure the effects of N, P, and $CaSiO_3$ addition on stomatal density, stomatal length, and carbon isotope composition and determine how variance is partitioned across sites, stands, plots, trees, and leaves in our experimental design. Our research took place in a study of Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE), a long-term nutrient addition experiment in New Hampshire, USA that investigates the effects of long-term, low-dose applications of N (ammonium nitrate), P (monosodium phosphate), and $CaSiO_3$ (as the calcium silicate mineral wollastonite). We studied two of the most abundant tree species—yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum* Marsh)—for this analysis. We expected to see an increase in stomatal density with nutrient addition and an increase in $\delta^{13}C$ with N and P addition and a decrease in $\delta^{13}C$ with $CaSiO_3$ addition, consistent with observed trends in iWUE with nutrient addition (Raven et al. 2004; Green et al. 2013). In addition to characterizing the effects of nutrient addition on stomatal characteristics, we aimed to consider how our variance partitioning analyses could improve future sampling protocols.

Methods

Site Description

The 11 even-aged forest stands used in this study were located in three sites in the White Mountains of New Hampshire: Jeffers Brook (44°02′N, 71°53′W), Hubbard Brook Experimental Forest (43°56′N, 71°44′W), and Bartlett Experimental Forest (44°03′N, 71°17′W). Mean precipitation at Hubbard Brook Experimental Forest is ~1,400 mm per year (Campbell et al. 2010), and the mean monthly air temperature is -9 °C in January and 18 °C in July (USDA Forest Service, 2022). The climate at Bartlett Experimental Forest and Jeffers Brook is similar to that of Hubbard Brook (PRISM 2022). Six of these stands were located at Bartlett (C1, C2, C4, C6, C8, and C9), while three were located at Hubbard Brook (HBCa, HBM, HBO) and two at Jeffers Brook (JBM, JBO; Table 3-1). Six stands were mid-successional (clearcut between 1970 and 1990), and the remaining five were mature, harvested between 1883 and 1915 (Table 3-1). Forests were dominated by typical northern hardwood species, namely American beech (*Fagus grandifolia* Ehrh.), sugar maple, yellow birch, red maple (*Acer rubrum* L.), white birch (*Betula papyrifera* Marsh.), and pin cherry (*Prunus pensylvanica* L.f.). Mature forest stands were dominated by American beech, sugar maple, and yellow birch, while mid-successional stands had greater proportions of red maple, white birch, and pin cherry. Soils were acidic, sandy-loam Spodosols developed in glacial drift deposited 14,000 years ago (Vadeboncoeur et al. 2014).

Each stand had at least four plots, which were 50 x 50 m, consisting of a 30 x 30-m measurement area with a 10-m buffer (but HBM and JBM plots were 30 x 30 m, consisting of a 20 x 20-m inner plot with a 5-m buffer). Four plots in each stand were treated annually with N (as NH_4NO_3, 30 kg ha^{-1} yr^{-1}), P (as NaH_2PO_4, 10 kg ha^{-1} yr^{-1}), both N and P, or neither. These doses are relatively modest but have resulted in increased nutrient concentrations in foliage (Hong et al. 2022; Gonzales et al. 2023). Stands C1, C6, C8, HBM, JBM, and JBO have a fifth plot where calcium $CaSiO_3$ (wollastonite) was added once at 1150 kg ha^{-1}. This application rate matches that applied in Watershed 1 at Hubbard Brook (Battles et al. 2014). The HBCa stand at Hubbard Brook had only a control and $CaSiO_3$plot. Treatments began in 2011 in all stands except for HBM, in which $CaSiO_3$ was added in 2015.

Foliar Sampling and Laboratory Analysis

Sunlit foliage was sampled from two locations in canopies of dominant and codominant sugar maple and yellow birch trees (> 10 cm diameter at breast height, Table 3-1) using a shotgun. Sugar maple and yellow birch were selected due to their abundance in the study sites. Foliage was sampled in late July and early August in 2021 at Hubbard Brook and Jeffers Brook and in 2022 at Bartlett; foliage from 85 sugar maple trees and 137 yellow birch trees were sampled in total (Table 3-1). Most trees were sampled from the measurement area, though occasionally trees from the buffer were sampled if there were not enough suitable trees in the inner plot.

Three leaves from each tree were selected for stomatal density and length measurements. These leaves were air-dried and pressed in a plant press for at least 72 hours (van den Top et al. 2018). To help account for the effect of variability in light availability due to differences in canopy position (Keenan and Niinemets 2016; Young et al. 2023), additional foliage with complete petioles and little to no evidence of insect damage were used to calculate specific leaf area (SLA), which is the ratio of leaf area to dry mass. Area was calculated in ImageJ (https://imagej.net/ij/index.html) using 300 dpi computer scans and mass was measured on the same leaves dried at 60 °C. This additional foliage was subsequently ground using a Wiley mill with a 40-mesh sieve or a mortar and pestle if the dry mass was less than 1.0 g. Stable carbon isotope ratios were measured on ground, homogenized foliage subsamples (3.5-4.5 mg) using an Isoprime isotope-ratio mass spectrometer coupled with a Pyrocube combustion analyzer; there was one ground foliage sample per tree. Values are reported in delta notation (Coplen 2011). Thirteen of 222 samples were run in triplicate for quality control purposes. $\delta^{13}C$ values for NIST 1515 (apple leaf) tissue standards were within 0.25 ‰ of the certified value. Triplicates were within 0.25 ‰, averaging 0.11 ‰. Carbon isotope data were available for 81 of 85 sugar maple trees and 112 of 137 yellow birch trees due to equipment malfunction. Fortunately, these samples include trees from each species in each treatment across both stand ages in all three sites.

Stomatal Density and Length Measurements

Stomata were visualized using nail polish impressions (Figure 3-1; Sonti et al. 2021). Clear nail polish was applied in three approximately 1 x 1-cm squares on each leaf: one near the base of the leaf, one in the middle of the leaf, and one at the top of the leaf, adjacent to the midvein. Impressions were mounted on clear slides using transparent tape and viewed using light microscopy at 400× magnification. One photograph was taken of each impression. Care was taken to photograph an area on the impression in which stomata were as clear as possible and veins were minimally present; this biased the count relative to the whole leaf but minimized variability due to vein presence. Stomata were counted in each image using ImageJ software and converted to units of stomata per square millimeter. In total, 846 sugar maple images and 1,354 yellow birch images were analyzed; for 5 yellow birch trees, we did not make images for one of the three leaves due to pubescence on the underside of leaves, and for an additional two yellow birch trees and one sugar maple tree, poor impressions led to unusable images (i.e., impressions where in no place could we get a clear image in which we could clearly recognize stomata). Length of three stomata were measured in each image for 15 yellow birch trees in JBM and for 35 sugar maple trees across HBM, HBO, and JBM (405 stomata for yellow birch, 945 stomata for sugar maple). Stomata density and length measurements (Zukswert et al. 2023a) and microscope photos (Zukswert et al. 2023b) are published through the Environmental Data Initiative.

Data Analysis

Linear mixed-effects modeling was used to quantify the effect of nutrient addition on stomatal density and δ^{13}C. Analyses were performed in R (R Core Team 2022) with the 'lme4' and 'lmerTest' packages (Bates et al. 2015; Kuznetsova et al. 2017) using Type III sums of squares and Satterthwaite degrees of freedom. We ran two separate models to analyze the effects of N and P on stomatal density (NxP analysis): one for sugar maple and one for yellow birch. We ran two additional models to analyze effects of N and P on δ^{13}C in sugar maple and yellow birch. Data for these four models came from stands that had a control, N, P, and NP plot. Categorical fixed effects for each model included N addition, P addition, the interaction of N and P, stand age (mid-successional and mature), and site. The N and P variables represent a factorial ANOVA. Because sites were sampled in two different years, "site" effects are confounded with sampling year and primarily serve a blocking function. Random effects for stomatal density models included stand, plot within stand, tree within plot, and leaf within tree. Random effects for $\delta^{13}C$ models included site, stand within site, and plot within stand. Stomatal density was either logarithm or cube-root transformed to meet assumptions of normality and homoscedasticity of the residuals (Cox 2011; Gotelli and Ellison 2013). $\delta^{13}C$ was not transformed. Fixed-effect and random-effect adjusted R^2 approximations were calculated using the 'rsq' package (Zhang 2022). Main effects (i.e., mean differences between factor levels: P vs. no P, $CaSiO_3$ vs. no $CaSiO_3$) were calculated using the 'difflsmeans' function in the lmerTest package.

Stand-level random effects were zero for the sugar maple stomatal density analysis and $\delta^{13}C$ analysis for the NxP analysis, and plot-level random effects were zero for the yellow birch $\delta^{13}C$ analyses, resulting in singularities. For sugar maple, this meant that stand age and site were tested on the plot level as opposed to stand level, and for yellow birch, this meant that N, P, and N x P were tested on the tree level as opposed to plot level, which increases the danger of accepting a significant effect that is not truly significant. Fortunately, for most of these tests, the effects were not significant. However, the positive effect of stand age on $\delta^{13}C$ in the sugar maple NxP analysis should be interpreted with caution.

Four linear mixed-effects models were constructed to quantify the effects of $CaSiO_3$ addition on stomatal density and $\delta^{13}C$ ($CaSiO_3$ analysis) for sugar maple and yellow birch. Data for these models came from stands that had control and $CaSiO_3$ plots. Categorical fixed effects included $CaSiO_3$ addition, stand age class, and site. Random effects for stomatal density models included stand, plot within stand, tree within plot, and leaf within tree. Random effects for $\delta^{13}C$ models included stand and plot within stand. Stomatal density was logarithm transformed to meet assumptions of normality for yellow birch; $\delta^{13}C$ was not transformed. Fixed-effect and random-effect adjusted R^2 approximations were calculated using the 'rsq.lmm()' function in the 'rsq' package (Zhang 2022). Singularity errors resulted for sugar maple stomatal density (stand and plot-level effects were zero), yellow birch stomatal density (stand-level effects were zero), and yellow birch $\delta^{13}C$ (plot-level effects were zero), but in no cases did these instances of pseudoreplication produce statistically significant results.

To further evaluate sources of variability in stomatal density, we explored the relationship between SLA and stomatal density using a Spearman rho rank correlation test, calculated using the ‘HMisc’ package (Harrell and Dupont 2023). To further aid in interpreting results, we also ran a linear mixed-effects model for specific leaf area in yellow birch trees, with age as a fixed-effect factor and stand and plot within stand as random effects.

Nested random-effects models were used to perform a variance partitioning analysis to correspond with each of the four stomatal density models described above. Four nested random-effects models were also constructed for the four possible comparisons of stomatal length (sugar maple in NxP plots, sugar maple in $CaSiO_3$ plots, yellow birch in NxP plots, yellow birch in $CaSiO_3$ plots). Random effects for stomatal density models were site, stand within site, plot within stand, tree within plot, leaf within tree, and image within leaf, while stomatal length models also included a level for stomate within image. No fixed effects were included. Variance at each level was extracted from the model output and visualized graphically in R as the proportion of the total variance (R Core Team 2022). We assume in this analysis that any nutrient-addition treatment effect on variance would be present at the plot level and recognize that variance due to plot and treatment are indistinguishable given that there is only one plot of each treatment type in each stand. This analysis assumes that nutrient addition does not influence variance at other levels of measurement.

Upon performing a variance partitioning analysis on stomatal length and noting the extremely high variance within images and low variance at the plot level, we abandoned further stomatal length measurements and decided not to analyze these data statistically. These results are presented graphically.

Table 3-1. Characteristics of stands used in a study of nutrient addition effects on stomatal density in yellow birch (YB) and sugar maple (SM). Sites are Bartlett Experimental Forest (Bartlett), Hubbard Brook Experimental Forest (Hubbard Brook), and Jeffers Brook. The number of trees sampled (*n*) is listed for each species in each stand. Note that SM was not sampled in the four mid-successional stands at Bartlett.

Site	**Stand**	**Age**	**Year Cut**	**Plots**	**Species (*n*)**
Bartlett	C1	Mid-successional	1990	Control, N, P, NP, $CaSiO_3$	YB (12)
	C2	Mid-successional	1988	Control, N, P, NP	YB (10)
	C4	Mid-successional	1978	Control, N, P, NP	YB (12)
	C6	Mid-successional	1975	Control, N, P, NP, $CaSiO_3$	YB (15)
	C8	Mature	1883	Control, N, P, NP, $CaSiO_3$	SM (15), YB (15)
	C9	Mature	~1890	Control, N, P, NP	SM (12), YB (12)
Hubbard Brook	HBM	Mid-successional	1970	Control, N, P, NP, $CaSiO_3$	SM (14), YB (14)
	HBCa	Mature	~1910	Control, $CaSiO_3$	SM (5), YB (6)
	HBO	Mature	~1900	Control, N, P NP	SM (9), YB (12)
Jeffers Brook	JBM	Mid-successional	~1975	Control, N, P, NP, $CaSiO_3$	SM (14), YB (15)
	JBO	Mature	~1900	Control, N, P, NP, $CaSiO_3$	SM (15), YB (15)

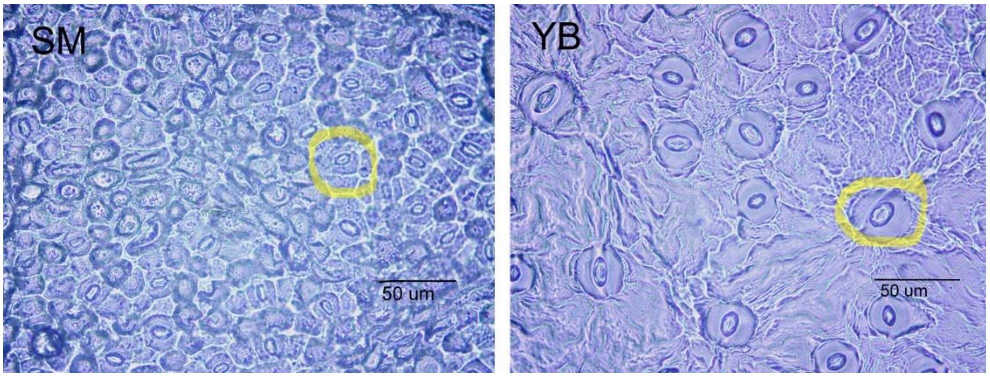

Figure 3-1. Examples of images used to measure stomatal density in sugar maple (SM, left) and yellow birch (YB, right). These leaves are from trees located in Stand C8 Plot 1, which is a P-addition plot at Bartlett Experimental Forest. Images were taken of clear nail polish films using a light microscope at 400× magnification. One stomate is circled in yellow in each image. All images used in this study are published through the Environmental Data Initiative (Zukswert et al. 2023b).

Results

For sugar maple, stomatal density was 35 ± 19 stomata per mm^2 (8%) higher, on average, with P addition (P-addition effect for ln-transformed stomatal density $F = 4.10$, $p = 0.06$; Table 3-2), though stomatal density was not higher with P addition than the control in two of the six forest stands (Figure 3-2). Stomatal density was not consistently affected by N addition (N-addition effect $F = 0.02$, $p = 0.88$; Table 3-2), and the NxP interaction was not significant ($F = 0.01$, $p = 0.91$, Table 3-2). Overall, fixed effects in the sugar maple stomatal density NxP model explained 3.3% of variance and random effects explained 45.4% (Appendix E). $\delta^{13}C$ in sugar maple was 0.51 ± 0.23 ‰ higher with N addition ($F = 4.05$, $p = 0.06$, Table 3-3, Figure 3-3), but not affected by P addition ($F = 0.08$ $p = 0.78$; Table 3-3) nor the NxP interaction ($F = 0.14$, $p = 0.71$, Table 3-3). Stomatal density was not influenced by $CaSiO_3$ addition ($F = 0.02$, $p = 0.88$; Table 3-2), but $\delta^{13}C$ was 0.76 ± 0.38 ‰ higher with $CaSiO_3$ addition ($F = 4.95$, $p = 0.08$; Table 3-3). $\delta^{13}C$ and stomatal density were not significantly correlated in sugar maple (Spearman $\rho = -0.04$, $p = 0.29$). $\delta^{13}C$ was higher in mature stands than younger forests for sugar maple trees, but this effect was only significant in the NxP analysis ($F = 9.57$, $p < 0.01$).

For yellow birch, though striking differences in stomatal density among nutrient treatments seem evident within several stands (Figure 3-2), stomatal density was not consistently affected by N or P addition across all the stands (N-addition *F* = 0.81, *p* = 0.38*;* P-addition *F* = 0.42, *p* = 0.52; Table 3-2). No patterns in $\delta^{13}C$ were evident with either N or P addition (N-addition *F* = 1.18, *p* = 0.28; P-addition *F* = 0.84, *p* = 0.36; Table 3-3). The interaction between N and P was not significant for either stomatal density or $\delta^{13}C$ ($p \geq 0.58$; Tables 3-2 and 3-3). Neither stomatal density nor length differed consistently due to $CaSiO_3$ addition in yellow birch (Table 3-2, Figures 3-2 and 3-4).

No consistent differences in stomatal length were observed among N or P addition treatments for either species (Figure 3-4). $\delta^{13}C$ was weakly positively correlated with stomatal density in yellow birch (Spearman ρ = 0.05, *p* = 0.09). In yellow birch, stomatal density was higher in thicker leaves with lower SLA values, more so for yellow birch (Spearman ρ = -0.34, $p < 0.01$) than for sugar maple (Spearman ρ = -0.08, *p* = 0.03, Figure 3-5). SLA in yellow birch was lower in mature stands than in mid-successional stands (*F* = 8.367 *p* = 0.02, Figure 3-6, Appendix F).

Most variance in stomatal density was found among images (i.e., within leaves) and among trees for both sugar maple and yellow birch in both the NxP analysis and the $CaSiO_3$ analysis (Figure 3-7). More than 50% of the variance was among images for sugar maple, with the next largest source of variance being among trees. For yellow birch, variance among trees was highest, but nearly equivalent to variance among images in both analyses (Figure 3-7).

By far, most of the variance in stomatal length was measured among stomata within the same image: over 60% (Figure 3-7). In yellow birch, the next largest source of variance was among trees, while in sugar maple, variance was next highest among images in the $CaSiO_3$ analysis and among stands for the NxP analysis (Figure 3-7).

Table 3-2. Effects of nutrient addition and stand age on stomatal density in sugar maple and yellow birch leaves based on four linear mixed-effects models. Each model is listed in italics. Analyses examined effects of N and P addition (SM *n* = 588 images, YB *n* = 1,000 images) and effects of $CaSiO_3$ addition (SM *n* = 258 images, YB *n* = 354) in mid-successional and mature stands. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. Stomatal density was log-transformed for sugar maple in the NxP analysis and yellow birch in the Ca analysis, and cube-root transformed for yellow birch in the NxP analysis, to meet normality assumptions for residuals (Shapiro-Wilk test $p \geq 0.05$). Random effects included stand, plot within stand, tree within plot, and leaf within tree.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Sugar Maple: N x P (ln)					
N	0.001	1	16	0.023	0.882
P	0.102	1	16	4.100	0.060
Age	0.013	1	16	0.538	0.474
Site	0.006	2	16	0.118	0.889
N x P	<0.001	1	16	0.014	0.908
Sugar Maple: $CaSiO_3$					
$CaSiO_3$	142.7	1	24	0.024	0.877
Age	376.2	1	24	0.065	0.802
Site	3558.4	2	24	0.306	0.740
Yellow Birch: N x P (cube root)					
N	0.079	1	27	0.807	0.377
P	0.041	1	27	0.417	0.524
Age	0.112	1	6	1.135	0.329
Site	0.137	2	6	0.696	0.537
N x P	0.031	1	27	0.311	0.582
Yellow Birch: $CaSiO_3$ (ln)					
$CaSiO_3$	0.018	1	9	0.947	0.357
Age	0.058	1	9	3.121	0.113
Site	0.030	2	9	0.793	0.483

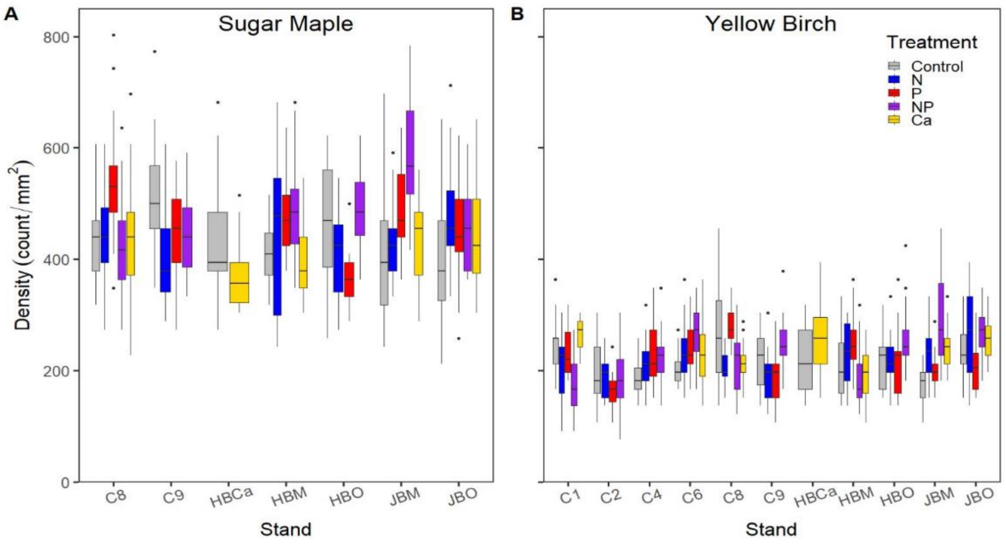

Figure 3-2. Stomatal density (count per mm^2) of sugar maple leaves (SM, left) and yellow birch leaves (YB, right). Note that yellow birch occurs in all 11 study stands but sugar maple only occurs in seven.

Table 3-3. Effects of nutrient addition and stand age on $\delta^{13}C$ in sugar maple and yellow birch leaves based on four linear mixed-effects models. Each model is listed in italics. Analyses examined effects of N and P addition (SM n = 67 trees, YB n = 92 trees) and effects of $CaSiO_3$ addition (SM n = 35 trees, YB n = 41 trees) in mid-successional and mature stands. Sums of squares (SS), and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. Random effects included stand and plot within stand.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Sugar Maple: N x P					
N	1.707	1	18	4.052	0.059
P	0.034	1	19	0.081	0.779
Age	4.030	1	18	9.568	0.006
Site	1.167	2	18	1.385	0.276
N x P	0.061	1	18	0.144	0.709
Sugar Maple: $CaSiO_3$					
$CaSiO_3$	1.991	1	5	4.952	0.078
Age	1.467	1	3	3.650	0.164
Site	0.060	2	3	0.075	0.929
Yellow Birch: N x P					
N	0.952	1	82	1.180	0.281
P	0.676	1	82	0.838	0.363
Age	0.772	1	5	0.957	0.373
Site	1.004	2	5	0.622	0.573
N x P	0.022	1	82	0.027	0.869
Yellow Birch: $CaSiO_3$					
$CaSiO_3$	1.514	1	36	2.660	0.112
Age	1.385	1	4	2.432	0.197
Site	0.007	2	4	0.007	0.994

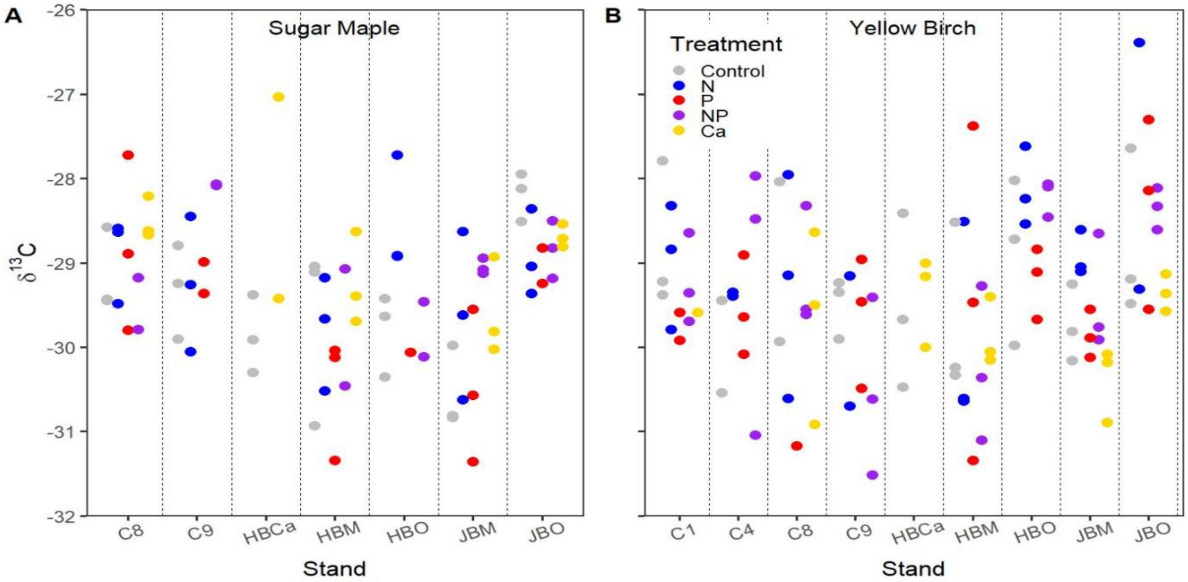

Figure 3-3. δ^{13}C in sugar maple (SM) trees (A) and yellow birch (YB) leaves (B).

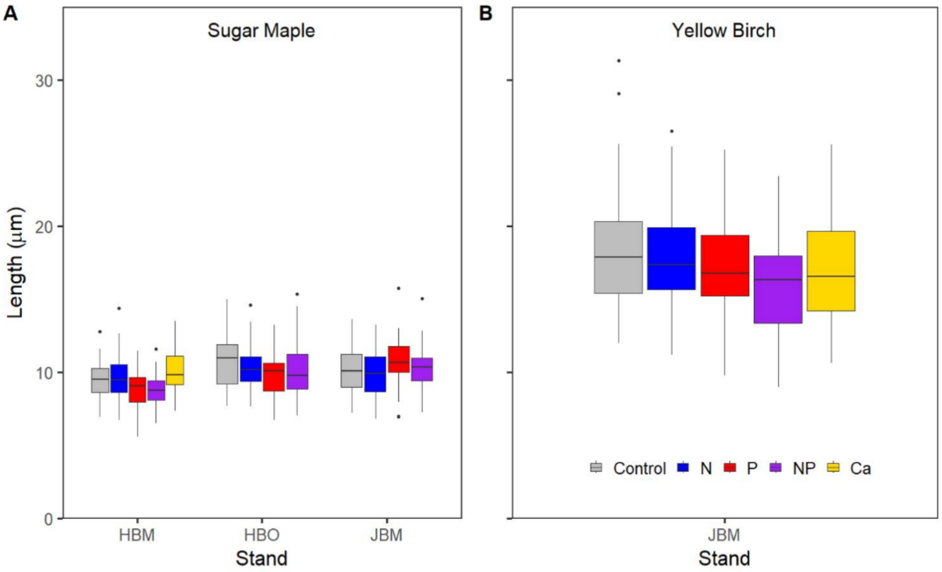

Figure 3-4. Stomatal length (in micrometers) in leaves of sugar maple (SM) trees (A) and yellow birch (YB) leaves (B).

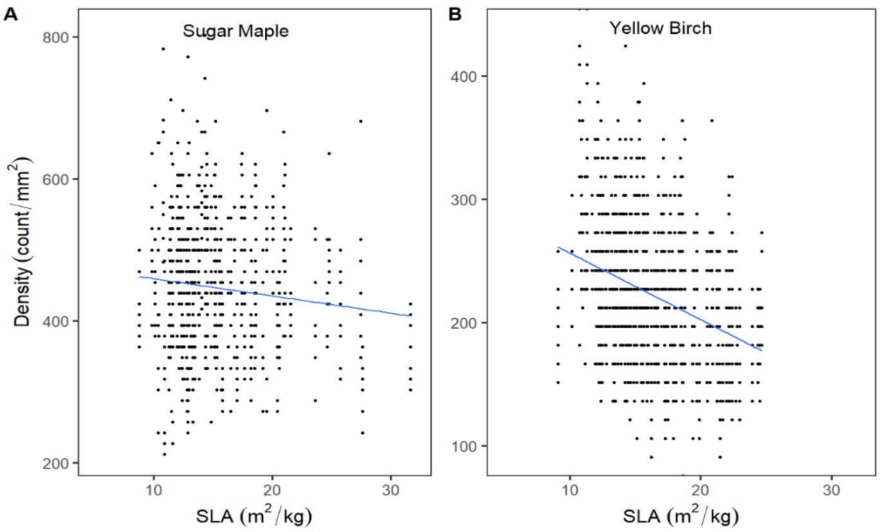

Figure 3-5. Relationship between specific leaf area (SLA, m^2 kg^{-1}) and stomatal density in sugar maple (A) and yellow birch (B) trees across stands included in both NxP and $CaSiO_3$ analyses. The blue line represents a regression line and gray area represents the 95% confidence interval.

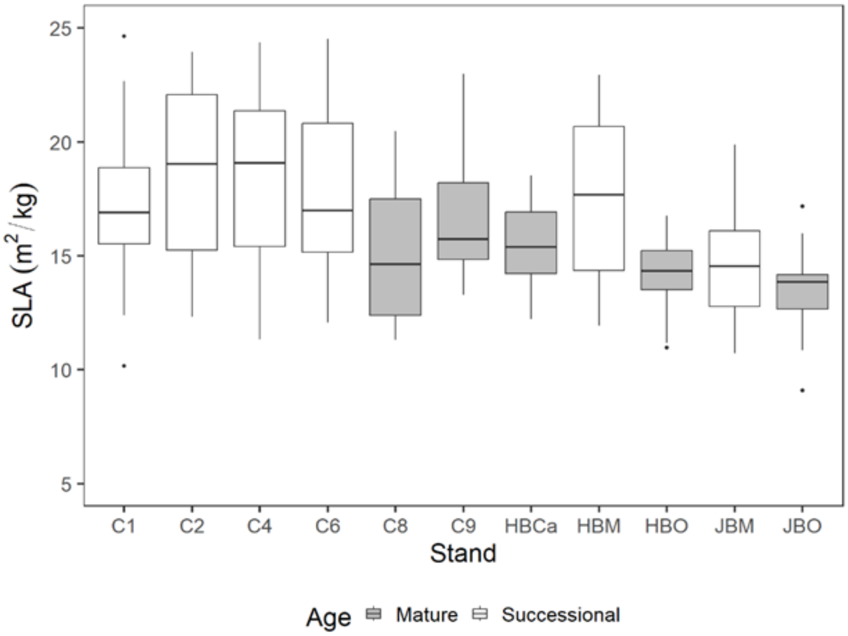

Figure 3-6. Relationship between specific leaf area (SLA, m^2 kg^{-1}) and stand age in yellow birch (*Betula alleghaniensis* Britt.) trees. Mid-successional stands (white) were harvested 32-52 years ago, and mature stands (gray) were harvested 112-139 years ago.

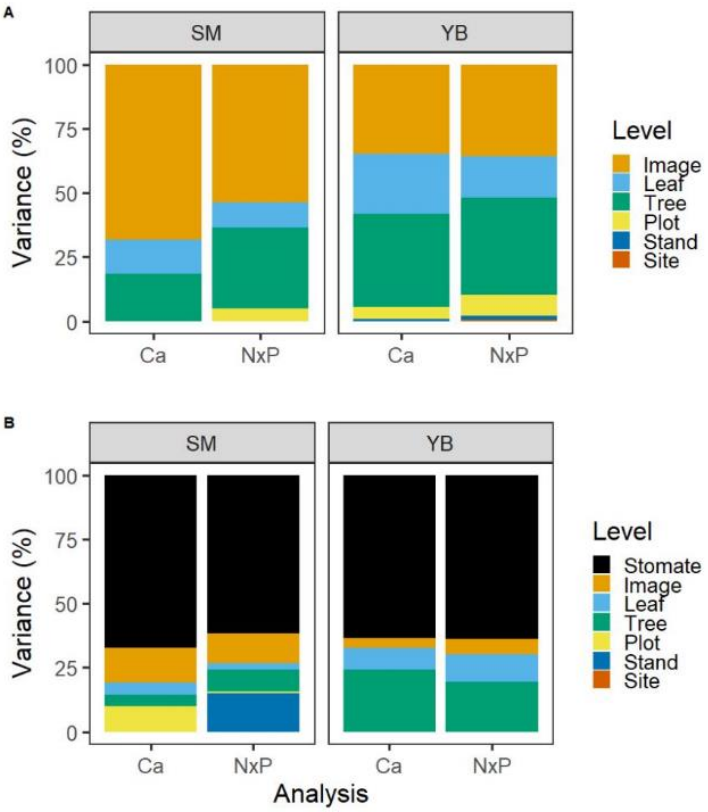

Figure 3-7. Percent of variance attributed to each level of measurement for stomatal density (A) and stomatal length (B) in sugar maple (SM) and yellow birch (YB) following N, P, or $CaSiO_3$ addition.

Discussion

Increased stomatal density in sugar maple with P addition (Table 3-2) is consistent with two previous studies of P addition and stomatal density, namely a laboratory experiment involving cowpea (*Vigna sinensis* L.; Sekiya and Yano 2008) and a long-term (20-year) N x P fertilization field study of several woody plant species in a Brazilian savanna, though these increases in stomatal density were not statistically significant in the latter study (Costa et al. 2021). Our study is the first report of a possible effect of P availability on stomatal density in a temperate forest tree species. Other studies on the effects of P availability on stomatal density in herbaceous plants have not detected an effect of P addition, just as we did not observe an effect of P addition on yellow birch stomatal density. For example, Cai et al. (2017), who also compared effects of both N and P, did not find significant effects of P addition on stomatal density in *Arabidopsis thaliana* (L.) Heynh. The studies that do report a significant effect of P addition on stomatal density report significant interactions with soil moisture and atmospheric CO_2, suggesting a greater effect of P on stomatal density in sufficiently wet conditions (Sekiya and Yano 2008; Chtouki et al. 2022) and with increasing atmospheric CO_2 (Sekiya and Yano 2008).

Stomatal density and length can influence maximum stomatal conductance, though actual conductance is also influenced by pore area within stomates and the rate at which stomata open and close (Fanourakis et al. 2015). An increase in stomatal density with P addition can thereby increase the maximum stomatal conductance, which has implications for both photosynthesis and transpiration. Stomatal density and length are often negatively correlated (Bertolino et al. 2019), and small stomata are believed to open and close more quickly. As a result, leaf surfaces with higher densities of smaller stomata can increase maximum conductance (and thereby increase photosynthetic capacity) in adequate environmental conditions while providing more control and faster rates of stomatal opening and closure in inadequate conditions, such as during drought or periods of high atmospheric water demand (Drake et al. 2013). We did not see a difference in stomatal length with P addition in our study, though we measured this variable on only a subset of trees, and variability in length among stomata within the same image was remarkably high (Figure 3-7).

While P addition was associated with increased stomatal density in sugar maple, it was not associated with differences in $\delta^{13}C$. $\delta^{13}C$ increased with both N and $CaSiO_3$ addition in sugar maple. Under constant light and atmospheric conditions, an increase in $\delta^{13}C$ indicates lower internal CO_2 concentrations and therefore reduced stomatal conductance relative to photosynthesis, i.e., increased iWUE (Dawson et al. 2002); thus, iWUE increased with N and $CaSiO_3$ addition in these trees. Increases in iWUE with N addition are often attributed to an increase in photosynthesis, due to increases in RuBisCO and chlorophyll (Raven et al. 2004). Indeed, N addition, but not P addition, has been shown to increase chlorophyll concentrations in sugar maple trees in the MELNHE study (Young et al. 2023). While $CaSiO_3$ addition has previously been associated with increased transpiration, silicon in general has been observed to decrease stomatal conductance by reducing the flexibility of stomatal guard cell walls, which can increase iWUE (Gao et al. 2006; Nascimento-Silva et al. 2023).

The observation of greater $\delta^{13}C$ in sugar maple in mature stands is not surprising, as $\delta^{13}C$ and thereby iWUE are known to increase with tree height (Vadeboncoeur et al. 2020). Variability among trees in stand age effects on δ13C, and thereby iWUE, has been attributed to both tree- and stand-scale differences in tree height, as well as to canopy structure differences that affect sub-canopy vapor pressure deficit difference, and light availability, with iWUE increasing as these factors increase (Farquhar et al. 1989; Duquesnay et al. 1998; Vadeboncoeur et al.

2020). Stomatal characteristics sampled at the top of the canopy did not differ with stand age in our study, which is consistent with past findings of no relationship with stand age and inconsistent relationships with tree height (Woodruff et al. 2010; Kenzo et al. 2012; Li et al. 2021).

Our variance partitioning analysis addressed the variability at all the spatial scales involved in our sampling design and suggested that stomatal density measurements were most variable among trees and within leaves (Figure 3-7). The consistency in stomatal density within individual trees may reflect a genetic component of stomatal density (Shimada et al. 2011; Zhang et al. 2012) or differences in environmental conditions among trees. To improve the detectability of treatment effects on stomatal density, it may therefore be helpful to measure stomatal density over time in the same trees. Measuring edaphic indicators, such as pre-dawn water potential at a dry time of year, or monitoring soil variables, such as soil moisture, at the tree level may also help future studies explain variability among trees or plots due to environmental conditions among trees and improve the detectability of a treatment effect.

The second highest level of variance in stomatal density occurred within leaves (i.e., among images), which could be attributed both to natural variability and to measurement error. The variability of stomatal density across leaf surfaces can vary naturally, and stomatal density measured near the edge of the blade has been shown to be lower than it is for the rest of the leaf in European beech (Forey et al. 2015). While we attempted to standardize the placement of the three impressions, we acknowledge that the impressions near the top and bottom of the leaf in particular could at times be influenced by proximity to the edge of the leaf. Future protocols could minimize this variability by taking more impressions, mostly in the middle of the leaf, or measuring more than three leaves and taking only one impression per leaf in a central location. Variability was relatively higher within leaves for sugar maple than for yellow birch, which could be due in part to observer error. Sugar maple stomata tended to be very small and generally left less distinct impressions that were more difficult to recognize in comparison to yellow birch (Figure 3-1). Sugar maple leaves tended to have more small veins, which were difficult to exclude from images.

Variance in stomatal length was highest by far among stomata within the same images, ranging from 62 to 67% of variance. Only three stomata per image were measured in this protocol, as has been done by others (e.g., Sonti et al. 2021, though they also took three images on different spots within each impression, instead of one). Measuring more than three stomata per image, and thereby increasing the sampling intensity at this measurement level, would best reduce the uncertainty associated with stomatal length. Increasing the number of stomata per image, however, would substantially increase time and effort. Making all stomatal density and length measurements for one tree (e.g., 3 leaves) took 75 minutes on average, with 35 minutes devoted to stomatal length measurements. It is therefore important to account for the tradeoff between increasing sampling effort and resource constraints.

One way to substantially decrease time and effort for stomatal density measurements is to automate the counting process. Several programs using neural networks exist, such as Stomatal Counter (Fetter et al. 2019). We elected to count stomata manually to eliminate error due to automation, which we anticipated could be high especially for sugar maple or images of poor quality. In future studies, using a neural network system could save time and thereby potentially support a greater sample size.

Light availability could cause additional variation in stomatal density and length, as stomatal density, like many other leaf traits (Pérez-Harguindeguy et al. 2013; Coble and Cavaleri 2015), has been shown to change with light availability, especially early in leaf development (Gay and Hurd 1975). While we strove to sample only fully sunlit leaves in our study, it is possible that some of our leaves were not as fully sunlit as others, which may be more common than previously thought of ‘sunlit leaves’ (Keenan and Niinemets 2016). In yellow birch in particular, SLA was negatively correlated with stomatal density, and SLA was lower in the older stands (Figure 3-6). This could be due to differences in the size of these trees, as SLA has been shown to decrease with increasing tree height, likely because an increased SLA no longer increases growth rate in older trees, which tend to be taller (Liu et al. 2010; Falster et al. 2018). It could alternatively mean that these leaves were more fully sunlit than the leaves in the mid-successional stands in the NxP analysis, where the dense forest canopies impair visibility during shotgun sampling. Alternative methods such as tree climbing (Young et al. 2023) and unmanned aerial vehicles (UAV) offer more control in selecting fully sunlit leaves for analysis (Schweiger et al. 2020), which could reduce variability due to light availability and thereby improve the detectability of treatment effects on stomatal characteristics.

Our study suggests that soil P availability might influence stomatal density in certain tree species, such as sugar maple. These increases in stomatal density could then have cascading effects on photosynthetic capacity and transpiration, increasing the capacity for these trees to take in atmospheric CO_2 but also increasing their capacity to transpire. More research is needed, perhaps in more controlled conditions and with attention to variance at different measurement levels, to confirm these hypotheses. While N and $CaSiO_3$ addition did not seem to influence stomatal characteristics in either species, N and $CaSiO_3$ addition did positively correlate with $\delta^{13}C$ in sugar maple, suggesting a possible influence of N on photosynthetic capacity and $CaSiO_3$ on conductance. Future studies should consider measuring stomatal characteristics in more trees or in the same tree over time, ideally considering pre-treatment conditions where applicable, standardizing impression location or measuring more impressions per leaf, measuring more stomata per impression, and controlling for canopy position.

Data and Material Availability

Stomatal density and length measurements and the corresponding microscope images are published in the Environmental Data Initiative.

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CHAPTER 4: CHANGES IN FOLIAR CHEMISTRY AND NUTRIENT RESORPTION IN NORTHERN HARDWOOD FORESTS IN RESPONSE TO LONG-TERM EXPERIMENTAL NITROGEN AND PHOSPHORUS ADDITION

Abstract

Long assumed to be nitrogen-limited, hardwood forests in the northeastern United States have also increased in production in response to phosphorus and calcium additions, indicating that these forests may be co-limited by multiple elements. Foliar and litter chemistry measured over time in fertilization experiments can provide insight on physiological responses to altered nutrient availability, nutrient limitation and ways in which nutrient cycles are interrelated. We compared community-level foliar and litter chemistry and resorption efficiency in a long-term nitrogen (N) and phosphorus (P) fertilization study across 10 forest stands distributed across three sites in New Hampshire. We measured N, P, calcium (Ca), magnesium (Mg), and potassium (K) in foliage from sunlit portions of dominant and codominant trees and in fresh litter collected during peak litterfall in 2008-2010 (pretreatment) and again in 2014-16 and 2021-22. Foliar N and P concentrations indicated co-limitation in 2014-16 by lower concentrations of one nutrient following the addition of the other, suggesting a dilution effect, and in 2012-22 by an interactive effect of N and P addition: foliar P concentrations were lower under N+P addition, consistent with a dilution effect following a greater response of tree growth to N+P addition, which was observed in these sites in 2019. Changes in litter N and P concentrations with N and P addition mirrored those in foliar N and P. Resorption efficiency of N and P decreased with addition of these respective nutrients and P resorption efficiency was higher in the N+P treatment than the P treatment. Foliar Ca and litter Ca and K decreased with N addition but increased with P addition. Results indicated N and P co-limitation and revealed interactions among N, and P, and base cations.

Introduction

Plant production in temperate forests has long been assumed to be primarily nitrogen (N) limited (Vitousek and Howarth 1991). In the late 20th and early 21st century, however, more evidence emerged suggesting that temperate forests could be limited by phosphorus (P) due to the composition of the parent material, loss of P through leaching and erosion, and anthropogenic causes such as N deposition (Vitousek et al. 2010; Peñuelas et al. 2013). Co-limitation by N, P, and Ca has also been observed in temperate forests in the northeastern USA (Vadeboncoeur 2010), perhaps in part due to a legacy of N deposition. These observations reflect more recent ecological theory suggesting that plants should tend towards limitation by multiple elements simultaneously and allocate relatively more resources towards acquiring nutrients that are in greater demand, thereby optimizing resource allocation and uptake (Bloom et al. 1985; Rastetter and Shaver 1992). Nutrient limitation of plant production in temperate forests, therefore, is more complex than once assumed.

A principal mechanism of nutrient conservation in forests is foliar resorption, in which a high proportion (60-70%; Brant and Chen 2015) of foliar N and P content is translocated from leaves to perennial tissues prior to abscission. While energetically costly (Killingbeck 2004), resorption reduces the requirement for root uptake of N and P from soil; therefore, resorption rates tend to be higher in environments with low soil fertility (Brant and Chen 2015). Two measures of resorption are often used: resorption efficiency, which is the proportion of leaf nutrient content resorbed, and resorption proficiency, which is the nutrient concentration of senesced leaves following resorption (Killingbeck 1996). Both measures of resorption are effective, indirect indicators of plant nutrient limitation (Killingbeck 1996; Han et al. 2013; Ostertag and DiManno 2016; Hong et al. 2022), and addition of a limiting nutrient usually results in increased foliar concentrations and reduced resorption of that nutrient (Yuan and Chen 2015), and decreased foliar concentrations of non-limiting nutrients due to a dilution effect (Jarrell and Beverly 1981; Bracken et al. 2015). A dilution effect can occur when the addition of a growth-limiting nutrient increases the rate of dry matter accumulation more than it increases the accumulation rate of other nutrients (Jarrell and Beverly 1981). Thus, both foliar nutrient concentrations and foliar resorption can be used to infer plant nutrient limitation.

Demonstrating nutrient co-limitation of plant production in natural tree communities on the basis of foliar nutrients and resorption presents further complexities. In particular, when measuring changes in tissue chemistry following nutrient addition in autotrophic communities, a decrease in the concentration of one nutrient with addition of the other and vice versa could indicate “community co-limitation”; community co-limitation occurs when different species or individuals within a community are limited by different nutrients, which is expressed as dilution of foliar nutrient concentrations in certain species or individuals when different nutrients are added (Bracken et al. 2015). Alternatively, an increase or no change in the concentration of one nutrient with the addition of the other could indicate “biochemically dependent co-limitation” (Saito et al. 2008; Bracken et al. 2015), which occurs when individuals are limited by the same two nutrients if the addition of one nutrient enhances the other. For example, the addition of N can stimulate the production of phosphatases, which makes more P available, increasing the concentration of foliar P as well as foliar N (Marklein and Houlton 2012).

Similarly, nutrient resorption is expected to increase for one nutrient with the addition of another, limiting nutrient (Yuan and Chen 2015). For example, if N is limiting, P concentrations in litter (resorption proficiency) should decrease with N addition. Resorption efficiency is also expected to decrease with addition of the nutrient in question but increase if another, more limiting nutrient is added (Han et al. 2013; Yuan and Chen 2015). Resorption proficiency has been found to be more strongly influenced by nutrient availability than is resorption efficiency, perhaps because the nutrient concentrations in litter are traits that can be more directly acted upon by natural selection than the proportion of nutrients resorbed (Killingbeck 1996; Wright and Westoby 2003).

The uptake and concentrations of calcium (Ca), potassium (K), and magnesium (Mg) may be influenced by changes in relative availability resulting from N or P addition. Decreases in foliar Ca, Mg, and K concentrations with N or P addition could arise in part from a dilution effect (Jarrell and Beverly 1981). In this case, the addition of a limiting nutrient would decrease the concentration of non-limiting nutrients. Decreases in Ca, Mg, and K concentrations could also arise from nutrient addition-induced changes in the availability of other nutrients in the environment, such as a decrease in soil base cations (e.g., Ca, Mg, K) as a result of soil acidification from ammonium nitrate addition (Lucas et al. 2011; Tian and Niu 2015). These nutrient cycles could also be coupled, meaning that changing the availability of one nutrient changes the availability of the other (Fiorentino et al. 2003; Finzi et al. 2011; Marklein and Houlton 2012).

The Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) study, the longest running N x P factorial fertilization study in a temperate forest ecosystem, was developed to investigate N and P co-limitation in northern hardwood forests. This study in New Hampshire, USA, in which fertilization began in 2011, provides a unique opportunity to confirm whether changes in foliar and litter chemistry and in resorption efficiency over time are consistent with measurements of plant production and changes in nutrient limitation. Average growth of trees ≥ 10 cm in diameter at breast height in the first four years of this study was primarily limited by P (Goswami et al. 2018), but the larger trees were limited by N (Hong et al. 2022), consistent with other observations that smaller trees tend to be more P-limited than larger trees, which tend to be more N-limited (Li et al. 2018). In the subsequent four-year period, however, tree-growth was co-limited by both N and P in many of the stands (Blumenthal in prep.).

The objective of this study was to quantify the effects of long-term, low-level N and P additions on foliar chemistry and nutrient resorption in northern hardwood forests. Foliage and freshly fallen leaf litter were collected by species before treatment from 2008-10, after treatment from 2014-16, and most recently in 2021-22. Community-weighted means of foliar and litter nutrient concentrations, and resorption efficiency of N and P, were calculated and used to assess community-level responses to changes in nutrient availability; this approach integrates species' responses to changes in nutrient availability and is driven by changes in nutrient concentrations and relative abundance of species (Lepš et al. 2011).

Given the latest tree-growth results, we expected foliar and litter chemistry to indicate co-limitation by N and P. That is, if N and P are indeed co-limiting, we would expect to observe a decrease in concentrations of foliar P with N addition and a decrease in concentrations of foliar N with P addition. If there is biochemically dependent co-limitation, we would expect to see either no change or an increase in concentrations of one nutrient with the addition of the other. Decreases in Ca, Mg, and K with N or P addition due to a dilution effect might also be expected. We would also expect to see a decrease in litter P concentrations with N addition and decrease in litter N concentrations with P addition, indicating an increase in resorption proficiency with the addition of other nutrients. Similarly, resorption efficiency of P should increase with N addition and resorption efficiency of N should increase with P addition in the case of N and P co-limitation.

Methods

Site Description

Green foliage and freshly senescent leaf litter were collected in 10 even-aged northern hardwood forest stands in the MELNHE study, established across three sites in New Hampshire. Six stands were located at the Bartlett Experimental Forest in Bartlett, NH (44°03′N, 71°17′W): stands C1, C2, C4, and C6 were mid-successional, third-growth forests (most recently harvested between 1975 and 1990) and stands C8 and C9 were mature, second-growth forests (harvested between 1883 and 1890). Two stands were located at Hubbard Brook Experimental Forest in Woodstock, NH (44°02′N, 71°53′W): stand HBM was mid-successional (last harvested in 1970) while HBO was mature (harvested in 1911). Two stands were located at Jeffers Brook in Benton, NH (44°02′N, 71°53′W): stand JBM was mid-successional (last harvested in ~1975), while JBO was mature (harvested in 1915, Table 4-1). Mean annual precipitation at Hubbard Brook was around 1,400 mm (Campbell et al. 2010), mean air temperature in January was -9 °C, and mean air temperature in July was 18 °C (USDA Forest Service 2022).

Dominant tree species in the mature stands included *Fagus grandifolia* Ehrh. (American beech), *Acer saccharum* Marsh. (sugar maple), and *Betula alleghaniensis* Britt. (yellow birch). The youngest stands (C1 and C2) were dominated by *Betula papyrifera* Marsh. (white birch), *Prunus pensylvanica* L.f. (pin cherry), and *Acer rubrum* L. (red maple); other mid-successional stands consisted of a mix of all species except *P. pensylvanica* (Table 4-1, Figure 4-1). Soils were primarily well drained or moderately well drained Spodosols on glacial till (Vadeboncoeur et al. 2014). Bedrock differed across sites, with Jeffers Brook underlain by amphibolite, Hubbard Brook by granodiorite and schist, and Bartlett by granite; due to differences in bedrock these sites were expected to represent a broad range of soil fertility across the region, with Jeffers Brook having higher soil base saturation than Hubbard Brook and Bartlett.

In each stand in the MELNHE study we established four 50 x 50 m treatment plots, consisting of a 30 x 30-m inner measurement area with a 10-m buffer (except HBM and JBM, which had a 20 x 20-m measurement area with a 5-m buffer in order to fit treatment plots in areas of similar species composition and uniform management history). Plots have been treated annually since 2011 with N (30 kg N ha^{-1} year^{-1} as NH_4NO_3), P (10 kg P ha^{-1} year^{-1} as NaH_2PO_4), both N and P, or neither, as an untreated control. The 3:1 ratio of N to P added to these plots is lower than N:P ratios typically seen in foliage, indicating that more P was added to the plots relative to plant demand compared to N; this higher rate of P addition was chosen to account for the occlusion of P in iron- and aluminum-containing minerals, making some P unavailable to plants (Wood et al. 1984; Weil and Brady 2017).

Foliar Sampling and Processing

Foliage was collected between July 22 and August 16 from 40 plots in each of 10 stands in the MELNHE study using a shotgun (Youngentob et al. 2016) within three distinct sampling intervals between 2008 and 2022: 2008-10 (pre-treatment), 2014-16, and 2021-22. Leaves were sampled from at least two sunlit portions of the canopies of dominant or codominant trees. All dominant species were sampled from all stands in 2008, 2009, and 2010. In 2014, all dominant species in C2 were sampled. In 2015, only *F. grandifolia*, *A. saccharum*, and *A. rubrum* were sampled across all stands, while in 2016, *P. pensylvanica*, *B. alleghaniensis*, and *B. papyrifera* were sampled across all stands. All six species were sampled in 2021 at Hubbard Brook and Jeffers Brook and in 2022 at Bartlett (Table 4-1). Leaves that had little to no damage or disease were selected for chemical analysis. If necessary to choose among damaged leaves, leaves with minor herbivory or intact margins were preferred over leaves with skeletonization or disease.

Foliar samples collected from 2008 to 2015 were frozen after collection, then oven-dried at 60 °C, ground through a 20 mesh screen, ashed in a muffle furnace and hot-plate digested with 5 mL of 6N nitric acid before being diluted to 20% with deionized water and analyzed for P, Ca, Mg, and K using inductively coupled plasma optical emission spectroscopy (ICP-OES; Optima 5300 DV, Perkin-Elmer; See et al 2015; Gonzales and Yanai 2019; Hong et al. 2022). Foliar samples collected in 2016 were frozen, oven-dried, ground through a 40 mesh screen, then 0.25 g subsamples were digested with 10 mL of concentrated nitric acid using a MARS 6 microwave digestion system (CEM), then diluted to 20% with deionized water and analyzed using ICP-OES. Standard reference material (NIST 1515) recovery for samples collected in 2014-16 was within 15% for Ca and K (mean = 3.5% and 4.4%, respectively), within 20% for P (mean = 3.5%), and within 25% for Mg (mean = 2.8%), and duplicates were within 23% (Hong et al. 2021). Nitrogen concentrations in samples from 2008 through 2016 were measured through combustion in a CN analyzer (FlashEA 1112 analyzer, Thermo Scientific); recovery of N in NIST 1515 samples was within 11% for all samples.

In 2021 and 2022, sample handling procedures were modified to accommodate measurements of physical leaf characteristics (see Chapter 2). Leaf samples were refrigerated after collection and processed within a week. Samples were oven-dried at 60°C and ground through a Wiley mill with a 40 mesh screen, then 0.25 g of oven-dried foliage from each sample was digested in 10 mL concentrated nitric acid using a MARS 6 microwave digestion system (CEM), diluted to 20% with deionized water, and analyzed using ICP-OES. Standard reference material (NIST 1515) replicates were within 10% for all elements of interest. All duplicates were within 14% for elements of interest. To measure N concentrations, subsamples of ground foliage (3.5-4.5 mg) were analyzed using a CN analyzer in 2008-16 (FlashEA 1112, Thermo Scientific) and a mass spectrometer in 2021-22 (Isoprime VisION, Elementar); recovery of N in NIST 1515 samples was within 11% for all samples and duplicates were within 31% (mean = 9.2%).

For a subset of 20 trees (at least three of each species sampled) in 2021, we processed both a “damaged” and “undamaged” sample to compare damage effects on N, P, Ca, K, and Mg. We detected a slight decrease in Ca and Mg concentrations in the damaged samples, but no detectable effects on N, P, or K (Appendix G).

Leaf Litter Sampling and Processing

In rain-free periods during peak litterfall in early to mid-October between 2009 and 2022, fresh leaf litter was collected from the same species and stands in which foliage had been sampled the previous summer. In 2009 and 2010, litter was collected from nets suspended ~1 m aboveground (12-mm polypropylene mesh). In 2014 through 2016 and 2021 through 2022, recently fallen litter was collected haphazardly from the ground. In 2021 and 2022, freshly senesced leaves free of damage and disease were selected using the same criteria used to select foliage in an attempt to match the foliage and litter samples used for resorption measurements (Van Heerwaarden et al. 2003). Between 9 and 30 leaves were collected per species per plot in 2021-22. Because we aimed select leaf litter that more closely matched the foliar samples from 2021 and 2022, which were primarily sunlit leaves sourced near the top of the trees, these litter samples are not representative of all leaf litter falling at the plot scale.

Litter samples were processed in the same way as the corresponding foliage samples (See et al. 2015; Gonzales and Yanai 2019). Recovery of P was within 15%, K was within 17%, Ca was within 13%, and Mg was within 15% of certified values for NIST 1515 samples from 2014 through 2016, and recovery for all elements was within 8% for NIST 1515 samples from 2021 and 2022. Concentrations of all elements in duplicates were within 9% of each other (Zukswert et al. 2022). In all years, subsamples of ground litter (3.5-4.5 mg) were analyzed using a CN analyzer (FlashEA 1112 analyzer, Thermo Scientific). Recovery of N was within 5% for NIST 1515 standards when running pre-treatment data, within 21% for samples collected between 2014-16 (mean = within 4%) and within 13% for samples collected between 2021-22 (mean = within 5%, Zukswert et al. 2022).

Data Analysis

To explore the effects of N and P fertilization on foliar and litter chemistry, we ran linear mixed-effects models for each of the five nutrients measured in foliage and litter and for resorption efficiency of N and P. Models were run in R (R Core Team 2022) using the ‘lme4’ (Bates et al. 2015) and ‘lmerTest’ packages (Kuznetsova et al. 2017). Species-level models of foliar and litter nutrient concentrations and resorption efficiency for *F. grandifolia*, *A. saccharum*, *B. alleghaniensis*, and *B. papyrifera* are available as supplementary material and were not interpreted in depth in this study. Models for *A. rubrum* and *P. pensylvanica* were not constructed due to small sample size, but differences in nutrient concentrations among plots were visualized graphically (Appendix H). Resorption efficiency was corrected for organic matter loss during resorption using a standard mass-loss correction factor for deciduous angiosperms (0.784; Vergutz et al. 2012)

We ran linear mixed-effects models at the community level using community-weighted means. We ran separate models for the data from 2014-16 and from 2021-22, meaning that we ran 20 models of community-weighted values in total. Community weighted means for each plot (CWM_{Total}) were calculated using:

$$CWM = \sum_{1}^{n} p_{ij} x_{ij}$$

where *n* is the number of species, *p* is the proportion of basal area of that species *i* occupies in the “community” (plot) *j*, and *x* is the mean trait value of species *i* in plot *j*. Fixed effects of these models were N, P, the interaction of N and P, stand age, site, and mean pre-treatment CWM_{Total} for that nutrient. Stand was a random effect. Site was used as a fixed effect because sites were initially selected for the experiment based on presumed differences in soil fertility (see Site Description). Site is confounded with sampling year, however, and thus serves primarily as a blocking factor. Site was removed from overfit models when doing so removed the singularity or enabled convergence.; these models only included CWM_{Total} litter Ca in 2014-16 and 2021-22 (Table 4). Post-hoc tests were conducted when interactions were present to compare differences in least squares means among treatments.

In the pre-treatment sampling period, a few species that were collected as foliage in the previous summer were not collected as litter because they did not fall into the nets. These observations were imputed. Pre-treatment concentrations of N in leaf litter were imputed for four plot-species combinations (out of 167; 2%) and pre-treatment concentrations of Ca, P, K, and Mg were imputed for 12 plot-species combinations (7%) using the ‘predictive mean matching’ method from the ‘mice’ (“Multiple Imputation by Chained Equations”) package in R (Buuren and Groothuis-Oudshoorn 2011). Similarly, concentrations of Ca, P, K, and Mg were imputed for four plot-species combinations (out of 168, 2%) in the 2016 litter.

To explore whether significant differences in community-weighted means were due to differences in species composition or within-species variation, we calculated interspecific CWM values ($\mathrm{CWM}_{\mathrm{Inter}}$). These values use the overall mean value of each species and convey the contribution of differences in species abundance to $\mathrm{CWM}_{\mathrm{Total}}$, and the contribution of intraspecific (within species) variation to $\mathrm{CWM}_{\mathrm{Total}}$ ($\mathrm{CWM}_{\mathrm{Intra}}$; Lepš et al. 2011). We ran linear mixed-effects models with the same fixed and random effects as the $\mathrm{CWM}_{\mathrm{Total}}$ models. $\mathrm{CWM}_{\mathrm{Inter}}$ was calculated the same way as $\mathrm{CWM}_{\mathrm{Total}}$ except that one study-wide species mean concentration was used instead of plot-specific means. Resulting differences among $\mathrm{CWM}_{\mathrm{Inter}}$ were attributed to differences in species abundances across plots. Pre-treatment $\mathrm{CWM}_{\mathrm{Inter}}$ was used as a covariate in these models. $\mathrm{CWM}_{\mathrm{Intra}}$ was calculated by subtracting $\mathrm{CWM}_{\mathrm{Inter}}$ from $\mathrm{CWM}_{\mathrm{Total}}$ (Lepš et al. 2011), and pre-treatment $\mathrm{CWM}_{\mathrm{Intra}}$ was also included as a covariate. Site was removed from overfit models when doing so removed the singularity or enabled convergence: these models included the following $\mathrm{CWM}_{\mathrm{Intra}}$ models: foliar N (2021-22), foliar P (2021-22), litter P (2014-16), litter Ca (2021-22), litter K (2021-22), and PRE (2014-16; Appendix I). Post-hoc tests were conducted when interactions were present, comparing differences in least squares means among treatments.

For six of the 2021-22 models, stand effects were zero, which resulted in a singularity, functionally resulting in the testing of site and age at the plot level, rather than stand level. This is a form of pseudoreplication (Hurlbert 1984) and could result in the reporting of factors as significant when they are not. Age was not significant ($p < 0.05$) in any of these six models, but site was significant in the foliar P $\mathrm{CWM}_{\mathrm{Total}}$ model; this result should be interpreted with caution. To test the effects of outliers, models for $\mathrm{CWM}_{\mathrm{Total}}$ and $\mathrm{CWM}_{\mathrm{Intra}}$ foliar K were run with and without stand HBO. To ensure that the residuals of the $\mathrm{CWM}_{\mathrm{Total}}$ model for foliar N were normally distributed, $\mathrm{CWM}_{\mathrm{Total}}$ was log transformed (Gotelli and Ellison 2013). No transformations improved the normality of residuals for the model of $\mathrm{CWM}_{\mathrm{Intra}}$ foliar N measured in 2021-22 or the models of $\mathrm{CWM}_{\mathrm{Inter}}$ for foliar N and P measured in 2014-16, so the results of these models must be interpreted with caution.

Resorption efficiency of N and P were calculated for each species in 2014-16 and in 2021-22 using concentrations of nutrients in foliage and litter using the following equation (Vergutz et al. 2012):

$$NuRE = \left(1 - \frac{Nu_{sen}}{Nu_{gr}} \times MLCF\right) \times 100$$

where NuRE is the resorption efficiency of a nutrient, $\mathrm{Nu}_{\mathrm{sen}}$ is the concentration of the given nutrient in senesced leaves (leaf litter), $\mathrm{Nu}_{\mathrm{gr}}$ is the concentration of the nutrient in green foliage, and the MLCF is the mass loss correction factor (0.784 for deciduous angiosperms; Vergutz et al. 2012). These species-level resorption efficiencies were then used to calculate the $\text{CWM}_{\text{Total}}$, $\text{CWM}_{\text{Inter}}$, and $\text{CWM}_{\text{Intra}}$ for N and P resorption efficiency. We ran linear mixed-effects models for each of these six CWM resorption efficiency variables with fixed effects including N, P, the interaction of N and P, site, stand age, and pre-treatment CWM, with stand as a random effect. Models for $\text{CWM}_{\text{Total}}$ and $\text{CWM}_{\text{Intra}}$ N resorption efficiency measured in 2021-22 were run with and without stand C9 to compare the results with and without outliers. Models for $\text{CWM}_{\text{Total}}$ and $\text{CWM}_{\text{Intra}}$ P resorption efficiency measured in 2021-22 were run with and without C2 to compare results with and without outliers. Stand-level random effects for these P resorption efficiency models run with and without C2 were zero; consequently, site and stand age were tested at the plot level instead of stand level and were interpreted with caution.

For all linear mixed-effects models run in this study, results were considered statistically significant if $p < 0.05$ and marginally statistically significant if $p < 0.10$. We interpreted both significant and marginally significant results.

Table 4-1. Characteristics of sampled MELNHE stands, including stand age, the year it was last cut, elevation, aspect, slope, and species sampled. Species sampled were ACRU (*Acer rubrum*), ACSA3 (*Acer saccharum*), BEAL2 (*Betula alleghaniensis*), BEPA (*Betula papyrifera*), FAGR (*Fagus grandifolia*), and PRPE2 (*Prunus pensylvanica*). Species are listed in order of proportion of total basal area, from most abundant to least in each stand.

Site	Stand	Stand Age	Year Cut	Elevation (m)	Aspect	Slope (%)	Sampled Species 2014-16	Sampled Species 2021-22
BEF	C1	Mid-successional	1990	570	SE	5-20%	BEPA, PRPE2, FAGR, ACRU	BEPA, PRPE2, FAGR, BEAL2
	C2	Mid-successional	1988	340	NE	15-30%	ACRU, FAGR, BEPA, PRPE2, BEAL2	ACRU, FAGR, BEPA, PRPE2, BEAL2
	C4	Mid-successional	1979	410	NE	20-25%	BEPA, ACRU, FAGR, BEAL2, PRPE2	BEPA, ACRU, FAGR, BEAL2
	C6	Mid-successional	1975	460	NNW	13-20%	ACRU, BEPA, BEAL2, FAGR, PRPE2	ACRU, BEPA, BEAL2, FAGR
	C8	Mature	1883	330	NE	5-35%	FAGR, ACSA3, BEAL2	FAGR, ACSA3, BEAL2
	C9	Mature	1890	440	NE	10-35%	ACSA3, FAGR, BEAL2	ACSA3, FAGR, BEAL2
HB	HBM	Mid-successional	1970	500	S	10-25%	BEAL2, BEPA, ACRU, ACSA3, FAGR	BEAL2, BEPA, ACRU, ACSA3, FAGR
	HBO	Mature	1911	500	S	25-35%	BEAL2, FAGR, ACSA3	BEAL2, FAGR, ACSA3
JB	JBM	Mid-successional	~1975	730	WNW	25-35%	BEAL2, BEPA, ACSA3, PRPE2	BEAL2, BEPA, ACSA3
	JBO	Mature	1915	730	WNW	30-40%	ACSA3, BEAL2, FAGR	ACSA3, BEAL2, FAGR

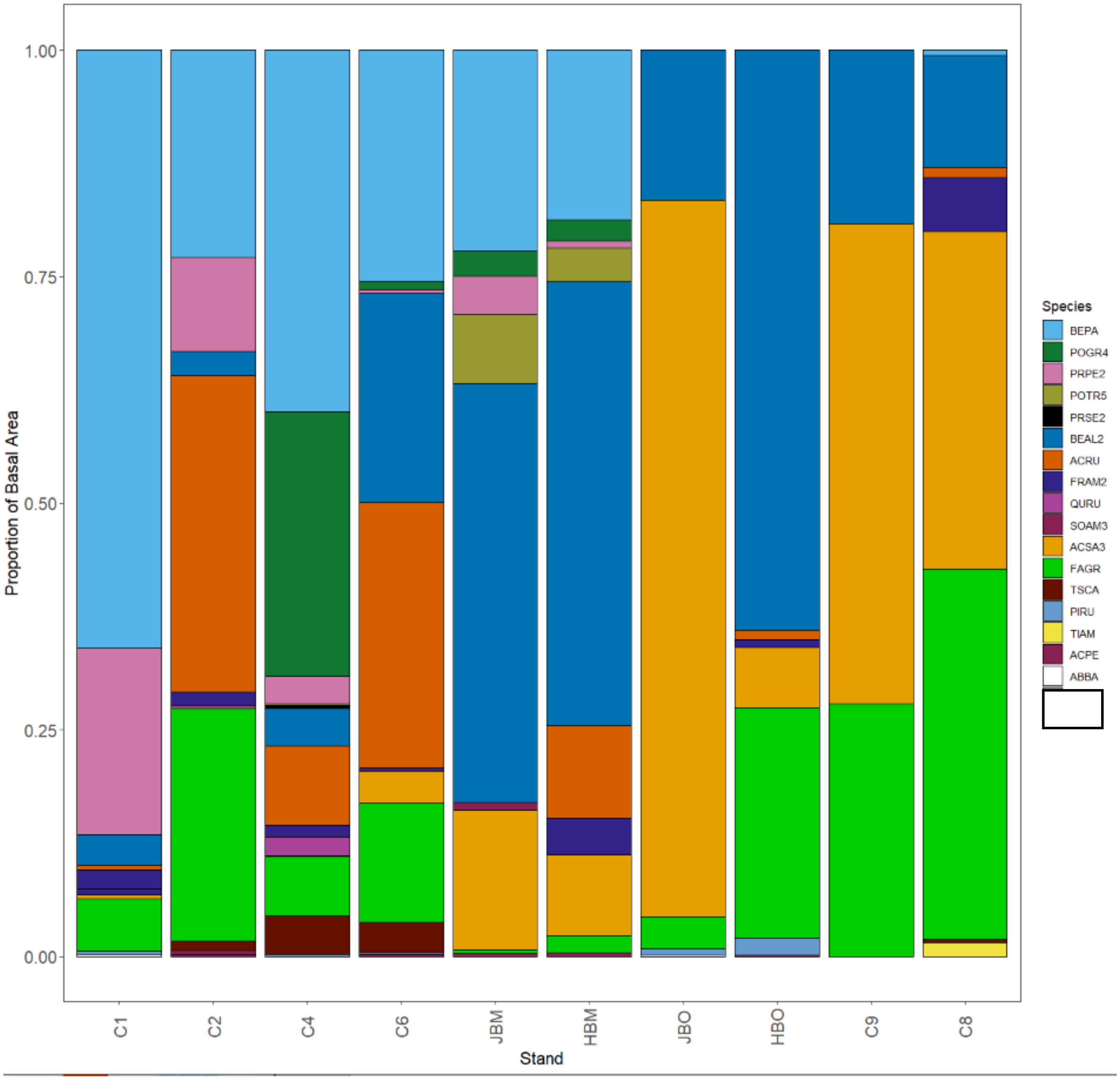

Figure 4-1. Proportion of basal area by species of trees greater than 10 cm in diameter in each stand in 2019. Species include *Abies balsamea* (ABBA), *Acer pensylvanicum* (ACPE), *Acer rubrum* (ACRU), *Acer saccharum* (ACSA3), *Betula alleghaniensis* (BEAL2), *Betula papyrifera* (BEPA), *Fagus grandifolia* (FAGR), *Fraxinus americana* (FRAM2), *Picea rubens* (PIRU), *Populus grandidentata* (POGR4), *Populus tremuloides* (POTR5), *Prunus pensylvanica* (PRPE2), *Quercus rubra* (QURU), *Sorbus americana* (SOAM3), *Tilia americana* (TIAM), and *Tsuga canadensis* (TSCA). Species are grouped in the legend by shade tolerance, with shade intolerant at the top (BEPA – PRSE2), intermediate shade tolerance in the middle (BEAL2 – SOAM3), and shade tolerant at the bottom (ACSA3 – ABBA). Within shade-tolerance groups, species are ordered from greatest to least basal area across the study sites. Stands are ordered from youngest to oldest from left to right.

Results

Foliar Nutrients

Community-weighted foliar N increased with N addition and decreased with P addition by a similar magnitude in both 2014-16 and 2021-22. Specifically, CWM_{Total} N was 2.77 ± 0.34 mg g^{-1} higher on average with N addition in 2014-16 ($F = 66.32$, $p < 0.01$, Table 4-2) and 2.61 ± 0.53 mg g^{-1} higher on average with N addition in 2021-22 ($F = 24.21$, $p < 0.01$; Table 4-3), and CWM_{Total} N was 0.87 ± 0.34 mg g^{-1} lower with P addition in 2014-16 ($F = 6.60$, $p = 0.02$; Table 4-2) and 1.2 ± 0.53 mg g^{-1} lower with P addition in 2021-22 ($F = 4.76$, $p = 0.04$; Table 4-3, Figure 4-2). These changes, and all other changes in foliar nutrient CWM_{Total} with N or P addition, were driven primarily by changes in CWM_{Intra} over time, indicating changes due to within-species variation or plasticity (Appendix I).

Community-weighted foliar P continued to increase with P addition over both sampling intervals, but not by as much in the N+P treatment. CWM_{Total} P was 0.46 ± 0.03 mg g^{-1} higher on average with P addition in 2014-16 ($F = 264.34$, $p < 0.01$; Table 4-2) and 0.10 ± 0.03 mg g^{-1} lower on average with N addition in 2014-16 ($F = 11.74$, $p < 0.01$; Table 4-2), with no interaction between N and P. In 2021-22, however, CWM_{Total} P was 0.88 ± 0.07 mg g^{-1} higher on average with P addition alone than with no nutrient addition ($t = -12.84$, $p < 0.01$), but was only 0.57 ± 0.07 mg g^{-1} higher with N+P addition than with no nutrient addition ($t = -8.30$ $p < 0.01$; Table 3, Figure 4-2). In the N addition, CWM_{Total} P was 0.04 ± 0.07 mg g^{-1} lower than in the control treatment, but this difference was not statistically significant ($t = 0.58$, $p = 0.56$). Foliar P was higher in Hubbard Brook than in Bartlett Experimental Forest and Jeffers Brook in 2014-16 ($F = 6.50$, $p = 0.04$; Table 4-2, Figure 4-2) and in 2021-22 ($F = 8.60$, $p < 0.01$; Table 4-3, Figure 4-2).

Community-weighted foliar Ca decreased with N addition and increased with P addition in both sampling periods. CWM_{Total} Ca was 0.63 ± 0.21 mg g^{-1} lower with N addition in 2014-16 ($F = 9.26$, $p < 0.01$; Table 4-2) and 0.87 ± 0.27 mg g^{-1} lower with N addition in 2021-22 ($F = 10.75$, $p < 0.01$; Table 4-3, Figure 4-2). CWM_{Total} Ca was 0.54 ± 0.21 mg g^{-1} higher with P addition in 2014-16 ($F = 6.87$, $p = 0.01$; Table 4-2) and 0.96 ± 0.27 mg g^{-1} higher with P addition in 2021-22 ($F = 12.89$, $p < 0.01$; Table 4-3, Figure 4-2).. CWM_{Total} Ca was greater at Jeffers Brook than in Hubbard Brook or Bartlett Experimental Forest in 2014-16 ($F = 10.05$, $p = 0.01$; Table 4-2); no site-level differences were detected in 2021-22 (Table 4-2, Table 4-3, Figure 4-2).

Community-weighted foliar K and Mg decreased with N addition in some sampling periods. CWM_{Total} K did not differ with nutrient addition in 2014-16 but decreased with N addition in 2021-22; it was 0.60 ± 0.26 mg g^{-1} lower on average (F =5.53, p = 0.03), and 0.72 ± 0.23 mg g^{-1} lower when excluding HBO (F = 9.65, p < 0.01; Table 4-3, Figure 4-2).. CWM_{Total} Mg, however, was slightly lower with N addition in 2014-16 (F = 6.00, p = 0.02; Table 4-2), but did not differ with nutrient addition in 2021-22 (p > 0.12; Table 4-3, Figure 4-2).

For all five nutrients in all CWM calculations, pre-treatment concentrations were positively correlated with the concentrations measured during the sampling period ($p \leq 0.02$; Table 4-2, Table 4-3, Appendix I). Nutrient concentrations did not differ with stand age (p > 0.17; Table 4-2, Table 4-3, Figure 4-2).

Litter Nutrients

Community-weighted litter N increased with N addition and decreased with P addition in both 2014-16 and 2021-22 sampling periods. Specifically, CWM_{Total} N was 1.32 ± 0.28 mg g^{-1} higher on average in the N-addition plots in 2014-16 (F = 21.76, p < 0.01; Table 4-4) and 1.55 ± 0.30 mg g^{-1} higher in 2021-22 (F = 26.78 p < 0.01; Table 4-5, Figure 4-3). Litter CWM_{Total} N was 0.63 ± 0.28 mg g^{-1} lower with P addition in 2014-16 (F = 5.07, p = 0.03; Table 4-4) and 0.62 ± 0.30 mg g^{-1} lower in 2021-22 (F = 4.27, p = 0.05; Table 4-5, Figure 4-3). These differences in litter N with nutrient addition, and all other differences in CWM_{Total} litter nutrients with N and P addition, were driven primarily by changes in within-species variation (Appendix I). Pre-treatment N was positively correlated with litter N in 2021-22 (F = 21.78, p < 0.01; Table 4-5) but not 2014-16 (F = 1.63, p = 0.21; Table 4-4, Figure 4-3). No differences due to stand age or site were detected.

Community-weighted litter P, like foliar P, increased in the P-addition treatments over time, but not by as much in the N+P as for P alone. CWM_{Total} P was 0.61 ± 0.09 mg g^{-1} higher in the P-addition plots than in the control plots in 2014-16 (t = -6.86, p < 0.01) and 1.95 ± 0.14 mg g^{-1} higher in 2021-22 (t = -14.11, p < 0.01), but only 0.19 ± 0.09 mg g^{-1} higher in the N+P plots than in the control plots in 2014-16 (t = -2.13, p = 0.04) and only 0.99 ± 0.14 mg g^{-1} higher in 2021-22 (t = -7.15, p < 0.01). Litter P did not differ between the control and N plots in 2014-16 (t = 0.12, p = 0.91) or in 2021-22 (t = 0.24, p =0.81; Figure 4-3). Pre-treatment P was not correlated with litter P, and no differences due to stand age or site were detected.

Community-weighted litter Ca was not detectably affected by N or P addition in 2014-16 ($p \geq 0.11$; Table 4-4), but in 2021-22, CWM_{Total} Ca decreased by 1.15 ± 0.39 mg g^{-1} with N addition (F = 8.64, p < 0.01) and increased by 1.20 ± 0.40 mg g^{-1} with P addition, on average ($F = 9.16$, $p < 0.01$; Table 4-5). Site differences were detected: community-weighted litter Ca was 2.41 ± 0.68 mg g^{-1} higher in Jeffers Brook than Bartlett and 2.06 ± 0.73 mg g^{-1} higher in Jeffers Brook than Hubbard Brook (Figure 4-3). In 2014-16, litter Ca was higher in mature stands than mid-successional stands ($F = 4.76$, $p = 0.07$; Table 4-4, Figure 4-3), but it did not differ with age in 2021-22 ($F = 0.37$, $p = 0.57$; Table 4-5, Figure 4-3). Pre-treatment litter Ca was correlated with litter Ca in both sampling periods (Table 4-4, Table 4-5, Figure 4-3).

Community-weighted litter K was not detectably affected by N or P addition in 2014-16, but in 2021-22, CWM_{Total} K was 0.81 ± 0.23 mg g^{-1} higher in the P-addition plots ($F = 12.72$, $p < 0.01$; Table 4-5, Figure 4-3). Litter K was highest in Bartlett Experimental Forest in 2014-16 ($F = 3.72$, $p = 0.09$; Table 4-4) and in 2021-22 ($F = 5.41$, $p = 0.05$; Table 4-5). Litter K did not differ with stand age ($p \geq 0.25$) and was positively correlated with pre-treatment K only for 2021-22 ($F = 22.64$, $p < 0.01$, Table 4-5).

Community-weighted litter Mg was 0.13± 0.07 mg g^{-1} lower on average with N addition in 2021-22 ($F = 4.00$, $p = 0.06$; Table 4-5, Figure 4-3), but did not differ with N or P addition in either 2014-16 or 2021-22 ($p > 0.11$; Table 4-4, Figure 4-3). Litter Mg differed by site in both sampling periods, but in different ways: Jeffers Brook had the lowest litter Mg in 2014-16 but the highest in 2021-22 (Table 4-4, Table 4-5, Figure 4-3). Litter Mg was not influenced by stand age ($p > 0.24$; Table 4-4, Table 4-5, Figure 4-3) and was positively correlated with pre-treatment litter Mg for both 2014-16 ($F = 52.74$ $p < 0.01$; Table 4-4) and 2021-22 ($F = 18.24$, $p < 0.01$; Table 4-5).

N and P Resorption Efficiency

Community-weighted N resorption efficiency (NRE) did not differ with nutrient addition in the 2014-16 sampling period but decreased with N addition in 2021-22. CWM_{Total} NRE (expressed in %) was 2.5 ± 1.2 lower on average with N addition ($F = 4.05$, $p = 0.04$), and 2.0 ± 1.0 when excluding stand C9 ($F = 4.82$, $p < 0.01$; Table 4-6, Figure 4-4). These changes in NRE were driven by within-species variation (Appendix I). NRE did not differ across sites or with stand age and was correlated with pre-treatment NRE only in 2021-22 ($p < 0.01$; Table 4-6, Table 4-7, Figure 4-4).

In both sampling intervals, community-weighted P resorption efficiency (PRE) was lower than controls in the P-addition plots and the N+P addition plots but did not differ from the control with N addition. CWM_{Total} PRE in 2014-16 (expressed in %) was 21.8 ± 3.6 lower in the P-addition plots than in the control plots ($t = 6.00$, $p < 0.01$), but did not differ among the control, N, and N+P plots (Figure 4-4). In 2021-22, PRE did not differ between the control and N plots ($t = -0.11$, $p = 0.92$; $t = -0.05$, $p = 0.96$ when C2 was removed), but PRE was 65.8 ± 5.2 lower in the P-addition plots than in the control plots ($t = 12.52$, $p < 0.01$), 62.72 ± 4.07 lower with C2 removed ($t = 15.40$, $p < 0.01$), and 38.6 ± 5.3 lower in the N+P plots than in the control plots ($t = 7.34$, $p < 0.01$), 33.5 ± 4.1 lower in the N+P plots than in the control plots with C2 removed ($t = 8.22$, $p < 0.01$; Table 4-6, Table 4-7, Figure 4-4). $\text{CWM}_{\text{Total}}$ PRE differed by site in 2014-16 but not in 2021-22; specifically, Jeffers Brook had a higher PRE than the other two stands ($F = 6.83$, $p = 0.03$; Table 4-6, Figure 4-4). These differences were driven by changes in within-species variation (Appendix I).

Table 4-2. The 3-to-5-year response of foliar nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed with linear mixed-effects models. The response variables are community-weighted means ($\text{CWM}_{\text{Total}}$). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar CWMs, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***P***
Nitrogen					
N	76.122	1	27	66.320	<0.001
P	7.575	1	27	6.600	0.016
Age	0.345	1	8	0.381	0.555
Pre-N CWM	19.105	1	27	16.645	<0.001
Site	0.421	2	7	0.1484	0.836
N x P	0.461	1	27	0.402	0.532
Phosphorus					
N	0.096	1	26	11.741	0.002
P	2.154	1	26	264.345	<0.001
Age	0.021	1	5	2.578	0.170
Pre-P CWM	0.110	1	20	13.488	0.001
Site	0.106	2	5	6.499	0.039
N x P	0.003	1	26	0.393	0.536
Calcium					
N	3.905	1	26	9.264	0.005
P	2.897	1	26	6.873	0.014
Age	0.029	1	5	0.069	0.803
Pre-Ca CWM	10.084	1	26	23.923	<0.001
Site	8.474	2	6	10.052	0.014
N x P	0.184	1	26	0.436	0.515
Potassium					
N	0.315	1	27	0.583	0.452
P	0.777	1	27	1.437	0.241
Age	0.182	1	7	0.336	0.580
Pre-K CWM	16.779	1	21	31.032	<0.001
Site	0.148	2	6	0.137	0.875
N x P	0.390	1	26	0.721	0.404
Magnesium					
N	0.154	1	27	6.004	0.021
P	0.004	1	27	0.138	0.713
Age	0.000	1	7	0.004	0.949
Pre-Mg CWM	1.005	1	13	39.215	<0.001
Site	0.105	2	6	2.042	0.212
N x P	0.013	1	27	0.494	0.488

Table 4-3. The 10-to-11-year response of foliar nitrogen, phosphorus, calcium, potassium, and magnesium to nutrient addition and stand age, analyzed using linear mixed-effects models. The response variables are community-weighted means ($\text{CWM}_{\text{Total}}$). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar CWMs, site, and the interaction of N and P. Stand was a random effect. The K model was re-run without HBO, to remove outliers; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	67.730	1	27	24.207	<0.001
P	13.322	1	27	4.761	0.038
Age	2.664	1	7	0.952	0.360
Pre-N CWM	18.313	1	15	6.545	0.022
Site	9.685	2	6	1.731	0.251
N x P	1.300	1	27	0.465	0.501
Phosphorus					
N	0.303	1	32	13.039	0.001
P	5.481	1	32	236.245	<0.001
Age	0.020	1	32	0.859	0.361
Pre-P CWM	0.155	1	32	6.661	0.015
Site	0.399	2	32	8.597	<0.001
N x P	0.178	1	32	7.690	0.009
Calcium					
N	7.576	1	32	10.746	0.003
P	9.088	1	32	12.891	<0.001
Age	0.882	1	32	1.251	0.272
Pre-Ca CWM	29.987	1	32	42.536	<0.001
Site	0.074	2	32	0.053	0.948
N x P	0.091	1	32	0.130	0.721
Potassium					
N	3.493 (4.588)	1	26 (23)	5.528 (9.646)	0.027 (0.005)
P	0.353 (0.057)	1	25 (23)	0.559 (0.120)	0.462 (0.732)
Age	0.644 (0.138)	1	5 (5)	1.019 (0.291)	0.356 (0.615)
Pre-K CWM	7.115 (5.862)	1	21 (23)	11.262 (12.322)	0.003 (0.002)
Site	0.472 (0.805)	2	5 (4)	0.374 (0.846)	0.707 (0.493)
N x P	0.111 (0.059)	1	25 (23)	0.176 (0.125)	0.679 (0.727)
Magnesium					
N	0.087	1	32	2.567	0.119
P	0.003	1	32	0.098	0.756
Age	0.001	1	32	0.041	0.840
Pre-Mg CWM	2.389	1	32	70.375	<0.001
Site	0.142	2	32	2.086	0.141
N x P	0.016	1	32	0.482	0.493

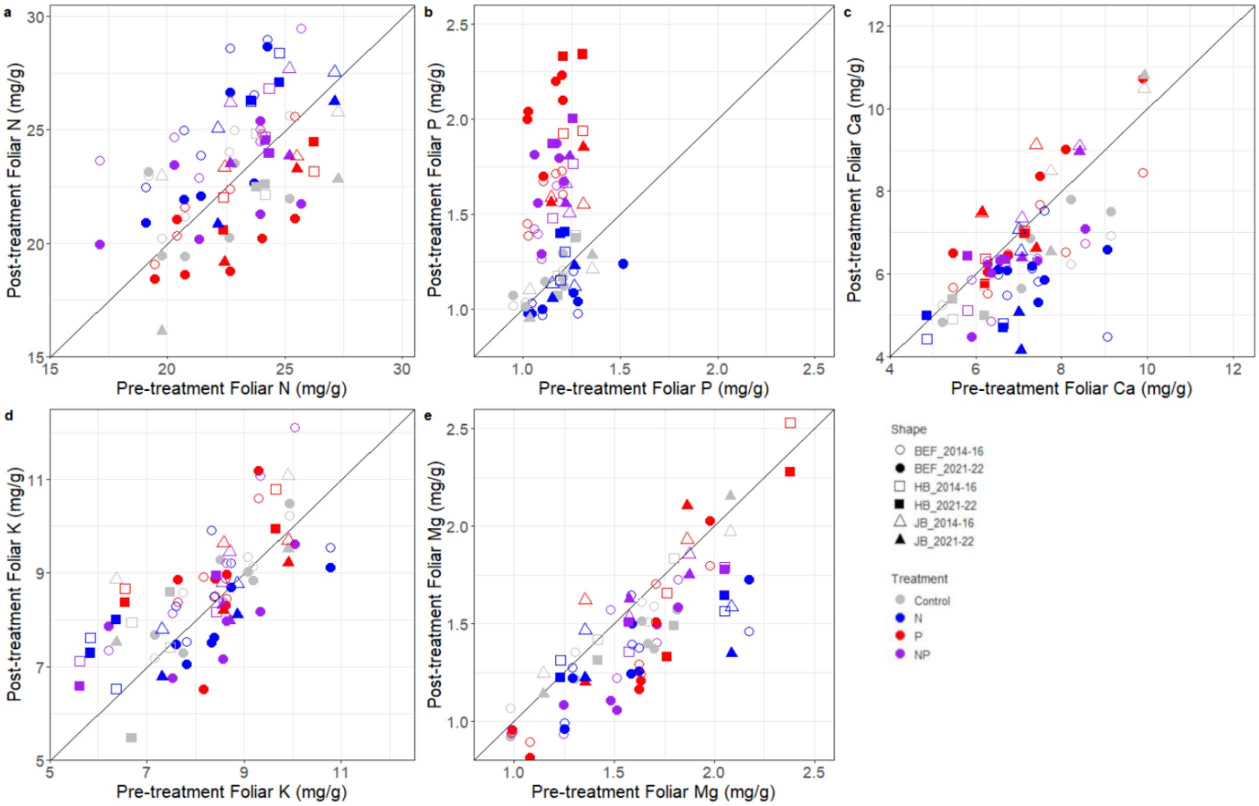

Figure 4-2. Foliar N (a), P (b), Ca (c), K (d), and Mg (e) concentrations measured before nutrient addition in 2008-10 ("pre-treatment") and after treatment in 2014-16 and 2021-21 ("post-treatment"). Each data point represents a plot, and values are community-weighted means.

Table 4-4. The 3-to-5-year response of litter nitrogen, phosphorus, calcium, potassium, and magnesium to nutrient addition, stand age, and site, analyzed using linear mixed-effects models. Response variables are community-weighted means (CWM_{Total}). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter CWMs, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***P***
Nitrogen					
N	17.066	1	27	21.762	<0.001
P	3.975	1	27	5.061	0.033
Age	0.763	1	8	0.973	0.353
Pre-N CWM	1.276	1	29	1.628	0.212
Site	0.717	2	7	0.457	0.651
N x P	1.657	1	27	2.113	0.158
Phosphorus					
N	0.455	1	26	11.632	0.002
P	1.586	1	26	40.513	<0.001
Age	0.001	1	7	0.016	0.903
Pre-P CWM	0.021	1	32	0.533	0.470
Site	Excluded				
N x P	0.406	1	26	10.359	0.003
Calcium					
N	4.111	1	27	2.878	0.101
P	2.108	1	28	1.476	0.235
Age	6.799	1	7	4.759	0.066
Pre-Ca CWM	4.541	1	14	3.179	0.100
Site	Excluded				
N x P	1.061	1	26	0.743	0.397
Potassium (ln)					
N	0.221	1	27	2.831	0.104
P	0.010	1	27	0.132	0.720
Age	0.123	1	8	1.573	0.247
Pre-K CWM	0.072	1	32	0.915	0.346
Site	0.582	2	6	3.718	0.085
N x P	0.015	1	26	0.192	0.665
Magnesium					
N	0.084	1	27	2.780	0.107
P	0.045	1	28	1.494	0.232
Age	0.051	1	6	1.687	0.244
Pre-Mg CWM	1.598	1	9	52.736	<0.001
Site	0.907	2	6	14.958	0.005
N x P	0.074	1	27	2.428	0.131

Table 4-5. The 10-to-11-year response of litter nitrogen, phosphorus, calcium, potassium, and magnesium to nutrient addition, stand age, and site, analyzed using linear mixed-effects models. Response variables are community-weighted means ($\text{CWM}_{\text{Total}}$). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N and P addition, stand age, pre-treatment litter CWMs, site, and the N x P interaction. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***P***
Nitrogen					
N	23.842	1	27	26.783	<0.001
P	3.803	1	27	4.272	0.049
Age	1.711	1	8	1.922	0.204
Pre-N CWM	19.391	1	17	21.784	<0.001
Site	1.351	2	7	0.759	0.505
N x P	1.632	1	27	1.834	0.187
Phosphorus					
N	2.440	1	27	25.693	<0.001
P	21.485	1	27	226.247	<0.001
Age	0.124	1	6	1.309	0.298
Pre-P CWM	0.045	1	23	0.475	0.498
Site	0.307	2	6	1.618	0.279
N x P	2.102	1	27	22.136	<0.001
Calcium					
N	12.929	1	26	8.642	0.007
P	13.701	1	27	9.158	0.005
Age	0.551	1	6	0.369	0.566
Pre-Ca CWM	51.112	1	16	34.163	<0.001
Site	Excluded				
N x P	0.045	1	25	0.030	0.864
Potassium					
N	0.377	1	27	0.734	0.399
P	6.529	1	26	12.723	0.001
Age	0.730	1	6	1.421	0.280
Pre-K CWM	11.634	1	9	22.635	0.001
Site	5.562	2	5	5.411	0.057
N x P	1.330	1	26	2.587	0.120
Magnesium					
N	0.182	1	27	4.002	0.056
P	0.010	1	29	0.211	0.650
Age	0.075	1	6	1.655	0.246
Pre-Mg CWM	0.830	1	15	18.243	<0.001
Site	0.564	2	6	6.200	0.034
N x P	0.000	1	27	0.000	0.998

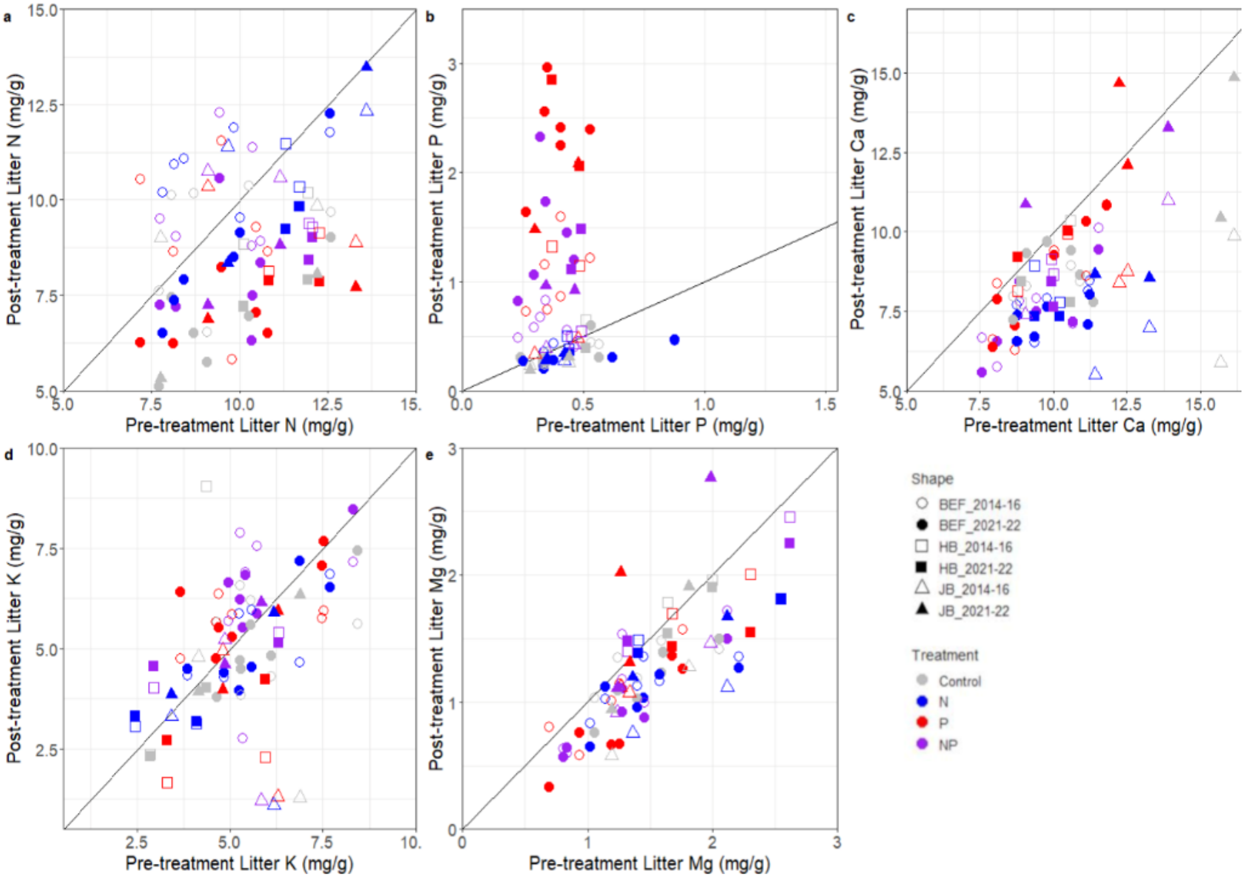

Figure 4-3. Litter N (a), P (b), Ca (c), K (d), and Mg (e) concentrations measured before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-21 ("post-treatment"). Each point is a plot, and values are community-weighted means.

Table 4-6. The 3-to-5-year response of N resorption efficiency (NRE) and P resorption efficiency (PRE) to nutrient addition, stand age, and site, analyzed using linear mixed-effects models. The response variables are community-weighted means (CWM_{Total}). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment (2008-10) resorption efficiency CWMs, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
NRE					
N	75.322	1	26	0.453	0.507
P	1.551	1	26	0.132	0.719
Age	26.311	1	7	2.237	0.181
Pre-NRE CWM	7.312	1	29	0.622	0.437
Site	11.755	2	6	0.500	0.628
N x P	27.283	1	26	2.320	0.140
PRE					
N	684.24	1	27	10.337	0.003
P	1589.70	1	27	24.015	<0.001
Age	2.66	1	6	0.040	0.847
Pre-PRE CWM	50.68	1	20	0.766	0.392
Site	904.37	2	6	6.831	0.029
N x P	809.71	1	27	12.232	0.002

Table 4-7. The 10-to-11-year response of N resorption efficiency (NRE) and P resorption efficiency (PRE) to nutrient addition, stand age, and site, analyzed using linear mixed-effects models. The response variables are community-weighted means (CWM_{Total}). Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment (2008-10) resorption efficiency CWMs, site, , the interaction of N and P. Stand was a random effect. The NRE model was re-run without C9, and the PRE model was re-rerun without C2, to remove outliers; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
NRE					
N	61.182 (35.763)	1	26 (23)	4.946 (4.822)	0.035 (0.019)
P	0.034 (8.448)	1	26 (23)	0.003 (1.139)	0.959 (0.297)
Age	28.444 (0.137)	1	7 (7)	2.299 (0.019)	0.173 (0.896)
Pre-NRE CWM	122.310 (57.655)	1	30 (27)	9.888 (7.773)	0.004 (0.010)
Site	27.289 (23.608)	2	6 (6)	1.103 (1.591)	0.388 (0.281)
N x P	10.092 (27.510)	1	26 (23)	0.816 (3.709)	0.375 (0.066)
PRE					
N	1895.1 (1940.2)	1	26 (28)	13.810 (26.023)	0.001 (<0.001)
P	26724.6 (20444.6)	1	26 (28)	194.751 (274.209)	<0.001 (<0.001)
Age	124.8 (36.1)	1	5 (28)	0.909 (0.485)	0.381 (0.492)
Pre-PRE CWM	25.9 (39.0)	1	19 (28)	0.188 (0.523)	0.669 (0.476)
Site	397.5 (130.5)	2	5 (28)	1.448 (1.750)	0.319 (0.192)
N x P	1721.1 (1868.6)	1	26 (28)	12.542 (25.063)	0.002 (<0.001)

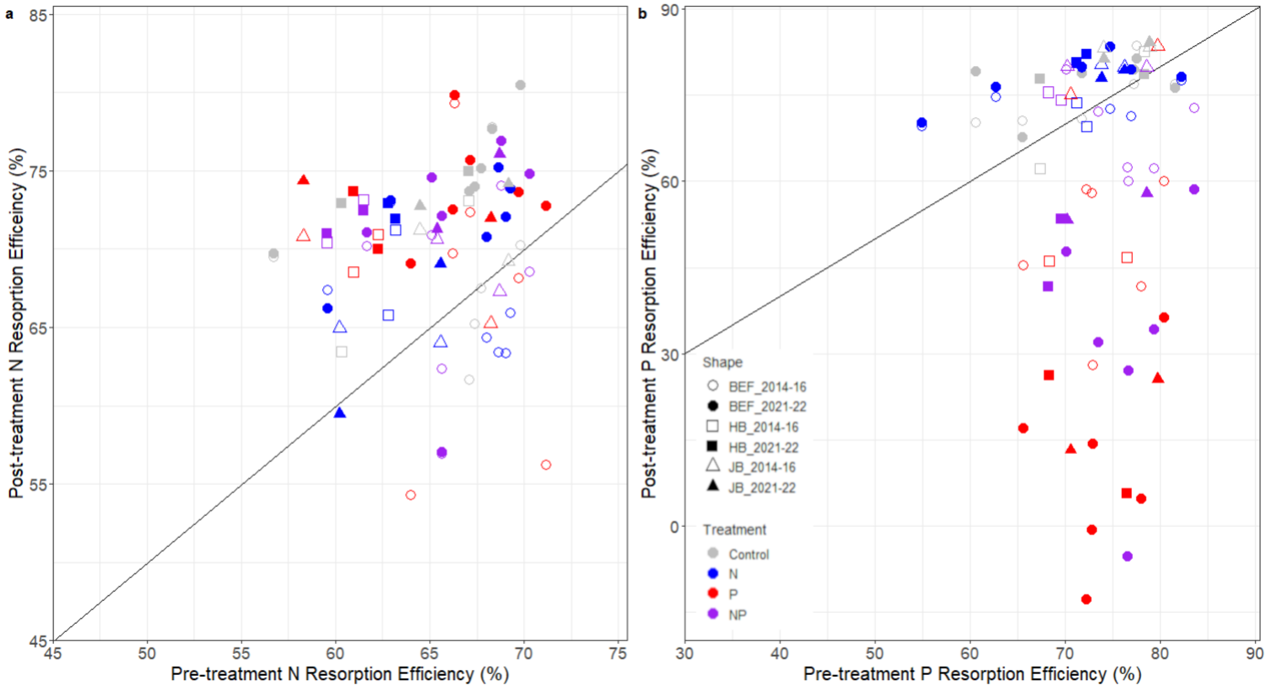

Figure 4-4. N resorption efficiency (a) and P resorption efficiency (b) measured before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-21 ("post-treatment"). Each point is a plot, and values are community-weighted means.

Discussion

Long-term responses of community-weighted foliar N and P to experimental additions of N and P indicated co-limitation more than N or P limitation alone. In 2014-16, changes in CWM_{Total} foliar N and P following nutrient addition were consistent with community co-limitation in that foliar N concentrations decreased with P addition and vice versa. In 2021-22, however, foliar N decreased with P addition, but foliar P did not decrease with N addition alone, which indicates P limitation. There was, however, an interaction between N and P, such that foliar P was lower under the addition of both N and P than P alone. This interaction is consistent in part with biochemically dependent co-limitation (Bracken et al. 2015). There are several mechanisms by which N addition could increase P uptake in trees; some of these mechanisms include increased fine root production and turnover (Ma et al. 2021) and increased phosphatase production (Marklein and Houlton 2012). Indeed, before fertilization, phosphatase activity was higher in the MELNHE stands with greater N availability (Ratliff and Fisk 2016). Moreover, in three mature MELNHE stands, an increase in fine root growth was observed in response to N addition (Shan et al. 2022).

Therefore, the increase in foliar P with N addition was probably associated with these mechanisms and could have offset decreases in foliar P due to dilution. When P was added in addition to N, the sufficiency of P in this treatment would presumably have reduced the value of these P-acquisition strategies, leading to a possible dilution effect as growth increased with the addition of both N and P. This result is consistent with results of a meta-analysis of responses of foliar N and P to nutrient addition (Ostertag and DiManno 2016) and with the latest tree inventory results, which indicated greater diameter growth in the N+P treatment than with N or P addition alone (Blumenthal in prep.). This result did not quite suggest biochemically dependent co-limitation in which both elements increase the availability of the other when added (Bracken et al. 2015); rather, these results suggest that N addition may have increased acquisition of P, but P addition did not seem to affect acquisition of N. These results were driven by within-species variation, indicating that these changes were due to changes in nutrient concentration in foliage with nutrient addition and not due to differences in species composition among plots, or to changes in relative species abundance in plots over time.

Both foliar N and P increased with the addition of these respective nutrients, but the magnitude of the difference in foliar N with and without N addition remained consistent from 2014-16 to 2021-22, despite the continued addition of N, whereas the magnitude of the effect of P addition on foliar P with and without P addition increased over time. One possible interpretation is that N addition led to an increase in biomass that could lead to increases in foliar N content but not concentration, whereas P concentrations may have continued to increase over time following P addition through “luxury consumption” (van den Driessche 1974; Van Wijk et al. 2003). Nitrogen addition has been shown to increase leaf area index (LAI), which could be due to increases in leaf abundance or area with added N (e.g., Cramer et al. 2000; Zhang et al. 2018). An increase in LAI with N addition could result in greater foliar N content in trees but not necessarily greater foliar N concentration. Measurements of LAI could confirm this explanation.

Other possible explanations for this lack of a reciprocal response in foliar nutrition could relate to the differences in N and P fertilizers and their application rates. Nitrate from the N fertilizer is easily leached, more so than phosphate from the P fertilizer (Weil and Brady 2017); it is possible that more applied N was lost through leaching compared to P. The different rates at which N and P were applied in this study could also help explain this discrepancy. We added more N than P to account for the greater demand for N relative to P in plants. For example, pre-treatment mass ratios of foliar N to foliar P concentrations in our study ranged between 8:1 and 33:1 with an average of 20:1, suggesting that these trees take up about 20 times more N than P, on average. In our study, the fertilizer N:P ratio was 3:1. While we have added more N relative to P, the ratio is narrower than in foliage. The reason to add more P than N relative to plant demand was the occlusion of P in iron- and aluminum-containing minerals, primarily in soil B horizons, which makes P unavailable to plants (Wood et al. 1984; Weil and Brady 2017). If we added much more P relative to demand than N, however, we might expect more luxury consumption of foliar P in the P-addition treatments than foliar N in the N-addition treatments.

Changes in community-weighted concentrations of N and P in leaf litter with nutrient addition resembled changes in foliar N and P. We expected that the resorption proficiency would decrease in the other nutrient following the addition of a limiting nutrient (Killingbeck 1996). A global meta-analysis of foliar and litter N and P concentrations in N and P-addition studies (Yuan and Chen 2015) revealed responses similar to ours: 1) an increase in litter N with N addition and no interaction between N and P, 2) a greater increase in litter P with P addition, and 3) smaller increase in litter P with P addition when N and P were added together. The decrease in CWM_{Total} litter N with P addition in both 2014-16 and 2021-22 could indicate P limitation, whereas the lack of a decrease in CWM_{Total} litter P with N addition could indicate increased P availability stimulated by N addition. The lower litter P concentrations in N+P compared to P plots could indicate possible N limitation. In this way, the litter N and P results complement the foliar N and P results, further suggesting co-limitation, and they could be explained by the same mechanisms.

Community-weighted NRE decreased over time with N addition, and community-weighted PRE decreased over time with P addition. The magnitude of the response of resorption efficiency to nutrient addition was much greater for P than for N, which has been attributed to the greater variability of foliar P and litter P concentrations in general and to the greater fraction of mobile P in cells than mobile N in previous studies (Han et al. 2013; Estiarte et al. 2022). We did not observe an increase in resorption efficiency in the other nutrient with the addition of either nutrient, as we had expected (Yan et al. 2018). A lack of an observed effect of nutrient addition on resorption of the other nutrient is consistent with results from a meta-analysis of N and P addition studies, which also showed a clearer response of nutrient addition to litter proficiency than to resorption efficiency (Yuan and Chen 2015). Phosphorus resorption efficiency decreased by less in the N+P addition treatment than with P addition alone, though, which could be consistent with a dilution effect following greater overall tree growth following the addition of both N and P addition than P addition alone.

The decrease in community-weighted foliar Ca and K with N addition could provide further evidence for N limitation in these forests, if these decreases are interpreted as a dilution effect. It is important to also consider the potential acidifying effect of ammonium nitrate, which has been associated with decreases in soil base cation concentrations (Lucas et al. 2011; Tian and Niu 2015; Moore and Houle 2023). However, a meta-analysis of N-addition studies showed significant acidifying effects of N addition when rates of N addition were greater than 50 kg ha^{-1} yr^{-1} (Tian and Niu 2015), which is greater than the 30 kg ha^{-1} yr^{-1} rate applied in the MELNHE plots. Soil chemistry was last measured in MELNHE plots in 2017; while mineral soil pH decreased by 0.2 following N addition between 2009 and 2017 (Fisk 2022), concurrent decreases in exchangeable soil Ca and K concentrations were not observed (Walsh 2022). There was also no evidence of lower tree growth with N addition (Goswami et al. 2018; Blumenthal in prep.), which might be expected if N were acidifying and impairing tree nutrition (Gradowski and Thomas 2006; Moore and Houle 2023). Therefore, while ammonium nitrate may have acidified the soil in 2021-22, there is little evidence for this in the soil data we have to date and there is evidence for N limitation, which would also be consistent with a decrease in Ca and K due to a dilution effect (Hong et al. 2022).

The increase in foliar and litter Ca with P addition was perplexing, particularly if P is also limiting and might be expected to have a diluting effect on Ca (Jarrell and Beverly 1981). Calcium addition has been shown to increase foliar P concentrations, as the increase in soil pH with Ca addition can accelerate P cycling or desorb P from soil organic matter (Fiorentino et al. 2003; Gonzales 2017). Reports of the reverse effect, however, are less common in tree studies, but have been reported in studies of several herbaceous crop species; increases in Ca or soil pH in these previous P-addition studies could be attributed to the use of triple superphosphate fertilizer, which includes Ca (Reinbott and Blevins 1994; Siedliska et al. 2021). An increase in Ca concentrations in foliar tissues has also been observed following the addition of iron phosphate and monosodium phosphate, however, suggesting that phosphate itself may influence foliar Ca concentrations somehow (Nichols and Beardsell 1981; Li et al. 2004). Our results therefore suggest a synergistic coupling of Ca and P that warrants further investigation.

The increase in litter K with P addition is also unexpected and might suggest a coupling of K and P. The incorporation of P into phospholipid bilayers and susceptibility of K to leaching might suggest a connection between cell membranes and leachability of K. Because it doesn’t form covalent bonds, K is easily leached through cell membranes (Schreeg et al. 2013; Sardans and Peñuelas 2015). Phosphorus nutrition of plants can influence the permeability of phospholipid bilayer cell membranes; in particular, plants grown in low-P soils had more permeable cell membranes than those grown in soils with higher P availability (Knowles et al. 2001). It may be that P addition increased membrane impermeability, decreasing the loss of K through leaching in P-treated litter during senescence. Examining how the different foliar P pools (i.e., Tsujii et al. 2017) change with P addition, and whether P addition in this study led to an increase in the lipid fraction, would help confirm this hypothesis.

Higher foliar Ca and litter Ca at Jeffers Brook compared to Hubbard Brook and Bartlett Experimental Forest is likely due to differences in soil parent material, as Jeffers Brook was chosen for its base-rich soil (derived from Ammonoosuc volcanics) and presumed higher site productivity. Other site differences, such as the higher foliar P at Hubbard Brook (Rangely schist), higher litter K at Bartlett Experimental Forest (Conway and Osceola granites), and changes in which site had the highest foliar and litter Mg, are more difficult to explain. Note that Hubbard Brook and Jeffers Brook were sampled in 2021 but Bartlett was sampled in 2022, which means that differences might instead be due to the year of sampling. Interannual variation in foliar nutrient concentrations may occur due to changes in soil water driven by interannual differences in precipitation (Moore and Ouimet 2006; Braun et al. 2020). Measuring all trees in a single year would alleviate this discrepancy but was not possible given the available resources.

In conclusion, our results provide further evidence to support the hypothesis that these northeastern USA forests are co-limited by N and P, though not in a simple, straightforward way. The 2014-16 responses of foliar N and P to the addition of the other nutrient clearly indicated community co-limitation. The 2021-22 results also indicated co-limitation, but in a different way; they indicated that N addition may influence P availability. The effects of N+P on foliar P in 2021-22 indicated a potential dilution effect consistent with greater tree growth with N+P addition than with N or P addition alone (Ostertag and DiManno 2016). Our results align with recent evidence of N and P co-limitation in tree growth in the MELNHE study (Blumenthal et al. in prep). Changes in foliar Ca with N and P addition suggest interactions with N and P cycling that may be worth investigating further, particularly in light of the known coupling of Ca and P and the effect of P addition.

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CHAPTER 5: SUMMARY OF FINDINGS AND RECOMMENDATIONS

The previous chapters addressed changes in foliar and litter characteristics following nutrient addition. In these chapters, I explored how long-term N and P addition influenced foliar traits, foliar nutrient concentrations, litter nutrient concentrations, and resorption. For two tree species, I explored how N, P, and $CaSiO_3$ addition influenced stomatal density, stomatal length, and carbon isotope composition. I found effects of both N and P addition on foliar or litter characteristics, but these effects differed by species and by characteristics. Nutrient limitation implications of my results were consistent with recent data suggesting that trees ≥ 10 cm in diameter are now co-limited by N and P (Blumenthal et al. in prep) in this study system.

I predicted that the five traits I measured in six northern hardwood tree species in Chapter 2 would co-vary with each other along the leaf economics spectrum, and I outlined a series of predictions for how I thought these traits would change with N and P addition if either N or P was limiting. I tried to determine whether N or P was limiting tree growth in the subset of trees I sampled using changes in relative basal area increment (RBAI) from 2011-19 for each species. These traits were aligned along a principal components axis in a way that reflected the leaf economics spectrum. As for limitation, I did not detect a nutrient effect for four species but detected a slight increase in RBAI with N addition for the two *Betula* (birch) species. Some traits responded to N or P addition, or both, but some did not respond to N or P at all (Table 5-1). Collectively, effects of N and P on traits in species and at the community level suggest that these stands are co-limited by N and P but highlight the different effects that N and P can have on different traits.

The lack of clear correspondence between the trait results and my predictions for the behavior of these traits following N and P addition could suggest that nutrient addition influences traits in different ways among species. Plants use N and P in different ways; for example, N is primarily found in proteins, predominantly in RuBisCO and chlorophyll in foliage (Makino and Osmond 1991), while P serves more diverse metabolic roles and is incorporated into DNA and RNA, lipids, and energy molecules such as ATP (Estiarte et al. 2022). The distinction in metabolic roles associated with N and P could explain why the addition of N or P may influence some traits more than others.

Focusing on traits further in Chapter 3, I explored the effects of N, P, and $CaSiO_3$ addition on stomatal density and $\delta^{13}C$ in two species: *B. alleghaniensis* (yellow birch) and *A. saccharum* (sugar maple). I detected a positive effect of P addition on stomatal density in sugar maple, but it was small. I also detected an increase in $\delta^{13}C$

in sugar maple in response to N and $CaSiO_3$ addition. These results suggest that, by increasing stomatal density, P addition could increase photosynthetic capacity but also increase transpiration, which might explain why I did not detect an effect of P on iWUE. Nitrogen and $CaSiO_3$ addition, however, seemed to increase iWUE, potentially through increases in photosynthetic capacity. These results suggest that the addition of different nutrients may influence photosynthetic capacity through different mechanisms, resulting in different outcomes for water use. These relationships were not observed for yellow birch, possibly due to the high variability of light availability experienced by the sampled leaves. Upon performing a variance partitioning analysis, I found that most of the variability in stomatal density in our sampling design was among impressions on the same leaf and among trees within plots, and variance in stomatal length was highest among stomata within the same image. This sampling design is consistent with other published protocols (e.g., two to three leaves per plant, three locations per leaf), which suggests that protocol modifications to reduce variability at these levels, such as measuring the same trees over time or better standardizing the location of impressions on leaves, may be relevant for other studies as well.

Broadening my perspective from species to the community in Chapter 4, I found evidence for co-limitation by N and P in both foliage and litter in two post-treatment sampling periods, but this evidence differed by year, and the interpretation was not as clear as I had expected. By 2021-22, I observed a decrease in foliar N with P addition and no decrease in foliar P with N addition. This alone could suggest P limitation, and if I had seen a decrease in foliar P with N addition, I would have concluded that we saw community co-limitation. I also, however, observed a smaller increase in foliar P with both N and P addition than P addition alone. If I had observed this in foliar N as well (e.g., a smaller increase in foliar N with both N and P addition than N addition alone), I would have called this biochemically dependent co-limitation (Bracken et al. 2015). My result instead is somewhere in between these scenarios. It seems that N addition increases P availability or uptake, while P addition does not seem to have the same effect on N availability. Therefore, I found evidence of co-limitation in my foliar and litter results, but the interpretation was not as straightforward as expected. Complicating this story further are the interactions of foliar and litter Ca, K, and Mg with N and P addition. Interactions among these nutrients warrant further investigation.

Overall, these studies provide evidence from foliage and litter that trees in these northern hardwood forest stands are co-limited by N and P (e.g., Table 5-1). In addition to being consistent with the most recent MELNHE tree growth results, these results are consistent with meta-analyses suggesting that co-limitation of N and P, or at least a synergistic response when N or P is added, is common across ecosystems (Elser et al. 2007; Harpole et al.

2011; Bracken et al. 2015) and that northeastern North American forests are limited by multiple elements (Vadeboncoeur 2010). Nitrogen and phosphorus both have important biological roles in plants and therefore can affect plant traits in different ways; these effects can also differ by species. Limitation by N and P has been increasing in recent decades as a result of increasing CO_2 concentrations in the atmosphere and is expected to continue increasing over time (Wieder et al. 2015). My research provides more possible insights into how N and P co-limitation can be expressed in foliage and litter, suggests mechanisms that might underlie N and P co-limitation in northern hardwood forests, and in doing so, raises new questions and produces new hypotheses to test.

Limitations

My decision to sample all species at Hubbard Brook and Jeffers Brook in 2021 and Bartlett in 2022 limited my ability to distinguish between effects of year and site. I made this decision for logistical reasons. I wanted to ensure that I would be able to sample from all trees at Jeffers Brook, a remote site accessed by a logging road that is known to close often for repair. Sampling at Hubbard Brook and Jeffers Brook one year and Bartlett the next also streamlined travel and field work for collecting fresh litter. Sampling half of the species across all stands and plots in one year and the remaining species in the next year, which was done by Kara Gonzales and Daniel Hong in 2015 and 2016, would have preserved my ability to compare among sites, but would have obscured my ability to distinguish between species or year effects. Both choices represent a tradeoff, and I chose the option that would simplify logistics and ensure that the nutrient concentrations used to calculate community-weighted means in each stand or site would all come from the same year.

When processing the foliage upon collection, I ran into a potential contamination issue that affected stands C4 and C9. Upon using the Wiley mill to grind samples, I discovered too late that the inside was covered in a light brown residue. This material was subsequently scraped from the inside of the mill and analyzed. It had a relatively higher C:N ratio (39), a low $\delta^{13}C$ of -31, and a Ca concentration near the high end of my foliage samples (14.4 mg g^{-1}). Based on the $\delta^{13}C$ signature, it could have come from a shaded or understory plant (M. Vadeboncoeur pers. comm.). Given the small amount of residue coating the mill, and the relatively large amounts of sample we used for analysis compared to what could have been scraped off into the samples, the influence of this contamination on our samples is likely negligible. I am not aware of any other major sources of contamination.

Chapter 3 is limited in that I measure stomatal density and length in only two of the six species I sampled: yellow birch and sugar maple. Given how time consuming it was to measure stomatal density and length in these samples, I had time to process only two species. The most time-consuming part of this process was measuring length, and I decided to stop measuring length once I completed a preliminary variance partitioning analysis and determined how high the variability was within images, from stomate to stomate. Proceeding with length measurements became infeasible. In addition, my variance partitioning analysis revealed that variability in stomatal density was greatest within leaves, among images. This could be in part due to “edge effects”, as stomatal density might be lower near the edges of leaves, which in our study would correspond with the “top” and “bottom” impressions made on the leaf. While I intended for the top, middle, and bottom slides to be consistent across leaves, having multiple technicians collaborate resulted in inconsistent ordering of impressions on the slides. As a result, it is not possible to test whether there were edge effects in my dataset.

Our lack of observed nutrient-addition effects in yellow birch, but significant relationship between SLA and stomatal density, additionally suggest that variation in light availability in these leaf samples might have been relatively high. Alternative collection methods such as tree climbing and unmanned aerial vehicles (UAVs) offer more control in selecting sunlit leaves, which would reduce this variation and improve our ability to detect a treatment effect. These methods, however, are much more time-intensive than the shotgun method, so they would not enable sampling of as many trees as we were able to sample in this study.

The logistics for collecting litter samples in Chapter 4 for resorption measurements were challenging and revealed further limitations in this study. It is important to pair foliage and litter samples when measuring resorption (Van Heerwaarden et al. 2003), as foliage and litter characteristics differ throughout the canopy due in part to differences in light availability (Young et al. 2023). This is difficult to accomplish in practice, as most foliage samples are taken from sunlit portions of the upper third of the canopy to ensure standardization across samples for ease of comparison, while litter samples taken from suspended nets or from the forest floor are more random. This has led some authors to clarify that their resorption measurements serve more as a comparative index than a true measurement of resorption (e.g., Weand et al. 2010). Pre-treatment and 2014-16 litter collections did not necessarily match the characteristics of the foliage shot the summer before. I tried to match the litter samples to the foliage samples in 2021 and 2022, by selecting leaves one by one and applying the same criteria I would apply when selecting which foliage samples to keep when collecting foliage. This led to me approving 4,692 inspected leaves in total. While this method was an attempt to better match the foliage and litter samples, I acknowledge that this method is somewhat subjective. To help identify potential biases, I photographed all foliage and litter collections from these two years. These photographs will be publicly available on the Environmental Data Initiative. In addition to providing a way to visually compare the foliage and corresponding litter samples in light of resorption analyses, these photographs can also serve as an aid for future researchers who want to repeat these sampling efforts and collect similar samples to mine or compare the samples they collected to mine to check for differences.

I use similar linear mixed-effects models for analysis in all three chapters. These models are useful when working with unbalanced statistical designs, especially those with nested or grouped factors (Logan 2010), which is true of MELNHE. In the MELNHE, the number of stands by site and age are not equal; for example, there are nine stands within Bartlett Experimental Forest (of which I used six), three at Hubbard Brook, and two at Jeffers Brook. Linear mixed-effects models can accommodate an unbalanced design if the ANOVA used to interpret the fixed effects uses Type III sums of squares and the degrees of freedom are estimated using the Satterthwaite method. ANOVA tests run using Type III sums of squares, which estimates the unique effect of a factor above those of other factors and interactions (Logan 2010), are generally considered easier to interpret in unbalanced designs (Kuznetsova et al. 2017). The Satterthwaite method is used to approximate degrees of freedom among groups that do not have equal variances (Satterthwaite 1946). With several of the models in my dissertation, I encountered issues with overfitting the model when including site as a factor, due to the complexity of the model structure compared to the relatively small number of observations. In some cases, I was able to remedy this by removing site from the model. In quite a few other cases, the Satterthwaite degrees of freedom did not match the random-effects structure and the model exhibited pseudoreplication, in which the factors (e.g., age, site, nutrient addition) were not tested at the correct level at which they were measured. My goal was to reduce the incidence of pseudoreplication, even if it meant removing site, to ensure that the remaining model could be interpreted properly without overstating the importance of a "significant" result. In most cases, the site variable was not significant anyway, and in the few cases where site differences were visually observed, I documented these differences in the text. In some cases, however, pseudoreplication was unavoidable, and I indicated when this was the case. It is also important to note that, with 10 stands, the power to detect site and stand age effects is smaller than that to detect treatment effects.

The best way to alleviate this issue is to increase sampling effort by adding more stands to increase the number of replicates, but this is difficult to do. MELNHE is already remarkable for its extent, involving multiple sites with differences in parent material and productivity and stands of multiple ages within those sites, rather than one site and one stand age, and for the size of its plots (among the largest in N x P factorial studies around the world), which allows us to avoid edge effects and may be more informative for assessing community-level responses than small plots. While I sampled from only 10 of the 13 stands in MELNHE, the 10 stands in which foliage had been sampled before, my 2021-22 sampling was time-consuming and resource-intensive; it would be difficult to sample more intensively, especially given the time-sensitive nature of both foliage and litter sampling and the logistical challenges associated with litter collection. While not ideal, I believe that my statistical approach was best suited for the constraints dictated by study design, past sampling efforts, differences in stand composition, and available resources.

Future Directions

My dissertation results raise as many questions as they answer, if not more. Foliage and litter are often used as indirect measurements of nutrient availability and plant function; a clear next step would be to confirm my interpretations with more direct measurements of nutrient availability and plant physiology. I hypothesize in Chapters 2 and 3 that the effects of N and P addition on traits could relate to changes in physiological processes, such as photosynthesis and transpiration. Measuring photosynthetic capacity, transpiration rates, or stomatal conductance would help confirm whether the changes I observed in foliar nutrient concentrations, LDMC, carbon isotope composition, and stomatal density have resulting effects on plant physiology that help explain the biological mechanisms involved during the alleviation of limitation when these nutrients are added.

Several of the observations I make in my dissertation, particularly related to foliar calcium, could relate to changes in soil pH. Measuring soil pH would help confirm the extent to which our N fertilizer may increase soil acidification, with effects on soil base cations. While the effect of ammonium nitrate fertilization on soil pH is well documented globally (Tian and Niu 2015) and locally within northern hardwood forests (Magill et al. 2004; Lovett et al. 2013), the rates used in our study are low relative to other studies in this region (Magill et al. 2004; Finzi 2009). Measuring soil pH across treatments could also aid in future interpretations of changes observed in the Ca and P plots; I would expect $CaSiO_3$ addition to increase soil pH and would not necessarily expect NaH_2PO_4 to change soil pH.

Additional questions that follow from my research seek to expand the scope of our knowledge of foliage and litter in these forests by measuring these characteristics in trees of different species or ontogenetic stages. My study focused on only six species that were dominant or codominant in these forests, and I prioritized measurements of sunlit leaves to enable clearer comparisons across species. Most leaves, however, exist in some degree of shade

(Keenan and Niinemets 2016), rendering the community-weighted means I calculated in Chapters 2 and 4 biased compared to what a "true" community-weighted mean might be. Additional sampling of these same characteristics could be done in intermediate or suppressed trees or seedlings and saplings. Expanding the scope of measurements to include less common species and different ontogenetic stages would help produce a more full picture of how the entire woody plant community is responding to N and P addition. The understory and overstory differ in N and P limitation in tropical secondary forests, for example, with understory plants displaying P limitation and overstory plants displaying N and P co-limitation (Mo et al. 2020). Intrinsic water-use efficiency can also differ with tree ontogeny, which could indicate differences in water-use strategies with tree growth stage (Yan et al. 2022). Physical traits like SLA and LDMC have also been shown to vary with plant ontogeny as well, which could indicate a shift in resource-investment strategies (Dayrell et al. 2018). The amount of light that these plants are exposed to would need to be considered when sampling seedlings, saplings, suppressed trees, and intermediate trees, as light availability can substantially influence a variety of traits (Keenan and Niinemets 2016).

The detection of a P effect on stomatal density in sugar maple could warrant further investigation to see if this effect exists in other species. Pressed leaf samples from four species (white birch, red maple, pin cherry, American beech) are in storage, ready to analyze. To expedite the process, future scholars could consider using automated counting programs, such as StomataCounter, to measure stomata (Fetter et al. 2019). With my images and manually measured data published in EDI (Zukswert et al. 2023a; Zukswert et al. 2023b), it would be relatively straightforward to run one of these models with the existing images and compare these automated counts to our actual counts. If a program can accurately estimate stomatal density in these images, the processing time for measuring stomatal density, from preparing the slides to measuring stomata, would be reduced by 25%, and for measuring both stomatal density and length combined by 57%, based on the average time it took me to make these measurements.

Analyzing these samples, however, still restricts researchers to the number of trees in the original sampling design, meaning that variability in stomatal density and length among trees would still be high. Re-sampling the same trees over time could help reduce tree-level variability and thereby increase the chances of detecting a treatment effect. Increasing the number of trees sampled could also reduce this variability. Measuring edaphic characteristics at the tree level that are known to influence stomatal density could also help explain some of the residual variance. The lack of treatment effects and strong relationship between SLA and stomatal density in yellow birch could suggest that light availability was highly variable, perhaps due to variation in canopy position of sampled leaves. Better standardizing canopy position and thereby irradiance on these samples could also improve detectability of treatment effects.

I reported results of foliar and litter N, P, Ca, Mg, and K, but I also measured aluminum (Al), boron (B), iron (Fe), manganese (Mn), sodium (Na), strontium (Sr), sulfur (S), and zinc (Zn). Given how difficult it was for all elements to pass all ICP-OES quality control measures within 5%, or even 10%, I prioritized the precision and accuracy of P, Ca, K, and Mg. As a result, several of these elements may not be measured as accurately. In particular, Al, B, and Na were highly inaccurate due to contamination or poor calibration curves, so I did not publish these data from 2021-22. Future researchers, however, can explore the effects of N and P on S, Sr, Zn, Fe, and Mn. These elements were also measured in 2016 foliage and litter samples, and S, Sr, and Mn were also measured from 2008 through 2015. These data are available to the public through the Environmental Data Initiative.

Many future research directions generated from my results could come from adding new or improved measurements. For example, my foliar N and P measurements do not enable the exact determination of how N and P are allocated within leaves. It is possible to chemically distinguish different pools of both N and P through fractionation. Sequential extraction of N or P can isolate an easily-soluble pool (e.g., inorganic P, ATP, inorganic N, amino acids), a nucleic acid pool (DNA and RNA), a lipid pool (cell membranes, chlorophyll), and a residual pool (proteins and other unhydrolyzable residues; Chapin and Kedrowski 1983; Tsujii et al. 2017). Incorporating fractionation protocols into future foliar and litter N and P measurements could provide insight into how the added N and P are biochemically allocated among plants and how allocation of N or P changes when addition of the opposite nutrient induces a limitation and changes following senescence.

To further understand the effects of nutrient addition and water-use strategy, beyond the insights that can be gained from ^{13}C and stomatal density measurements, more traits related to water use and gas exchange could be measured. One such trait is vein density. Sugar maple was more difficult to photograph in part because it was difficult to obtain a vein-free image. Our technician who measured sugar maple stomatal density in the control and $CaSiO_3$ treatments remarked that it seemed more difficult to get a clear, vein-free photo in the $CaSiO_3$ plots compared to the control plots; measuring vein density could confirm whether sugar maple leaves in the $CaSiO_3$ plots had higher vein density. Vein density has been shown to relate to nutrient availability, particularly N availability. For example, vein density was negatively correlated with N addition in maple but not birch (Jin et al. 2023) and has been shown to be positively correlated with N resorption, but not P resorption, in 17 tropical tree species in China (Zhang et al. 2015). Adding this measurement could further clarify mechanisms driving plant physiological responses to changes in nutrient availability in their environment.

Further research could be done to better characterize and understand the effects of nutrient addition on resorption and senescence. Resorption of litter changes during senescence; leaves that fall earlier in autumn tend to have higher nutrient concentrations and therefore lower resorption efficiency than leaves that fall later (Niinemets and Tamm 2005). The rate of decrease in nutrient concentrations differs for N and P, with P decreasing proportionally more than N (See et al. 2019). Future researchers could measure the resorption trajectory over time; this includes collecting and measuring fresh litter multiple times throughout the fall to document how resorption changes over time across treatments. Senescence more generally could be influenced by nutrient addition, and previous studies have shown a delay in senescence onset with N addition (Millard and Thomson 1989) and P addition (Wang et al. 2022). Past attempts to evaluate whether N or P addition influence abscission in MELNHE were conducted by comparing canopy photos over time, but the results were inconclusive. Current efforts are underway to analyze the effects of N and P on litterfall that was collected multiple times during the fall of 2016, but preliminary analyses are again inconclusive. Measurements of senescence and abscission over time could also be made with remote sensing, such as with UAV imagery or terrestrial laser scanning.

Resorption can change depending on the production of reproductive structures, i.e., flowers, fruits, and seeds, in a particular year. Fruiting trees have higher resorption rates to account for the high nutrient demand of the fruit, and relationships between soil nutrient availability and resorption efficiency and proficiency become clear when reproductive status is considered (Tully et al. 2013). Comparing our foliar data to litterfall mass (to estimate foliar production), and to fruit or seed nutrient concentrations and production, could therefore present a more complete representation of resorption with nutrient addition in these forests.

As CO_2 concentrations increase, N and P limitations are expected to intensify (Wieder et al. 2015; Wang et al. 2020), which could interact with the N and P addition treatments in MELNHE as the study progresses. There is already evidence for an exacerbation of N limitation in northern hardwood forests, such as Hubbard Brook; the term “oligotrophication” is used to describe this phenomenon (Groffman et al. 2018). Reductions in N cycling rates, increases in forest floor C:N ratios, and increases in microbial respiration have all been observed in recent decades at Hubbard Brook, suggest a decline in N availability following an increase in atmospheric CO_2 uptake by plants

(Groffman et al. 2018). This exacerbation can be consequential for ecosystem carbon storage, reducing global net primary productivity estimates by 25% when accounting for both N and P limitation (Wieder et al. 2015). Foliar N concentrations have been decreasing in European forests at a rate of 0.04 ± 0.004 mg g^{-1} yr^{-1} since 1995 (Mason et al. 2022). CO_2 concentrations have increased by about 7% on average in New Hampshire since the start of sampling in MELNHE, ranging from 389 ppm on average in 2010 to 416 ppm on average in 2022 in Portsmouth (McKain et al. 2023). I observed lower concentrations for several foliar and litter nutrients in MELNHE, including foliar N, from 2008-10 to 2021-22. Community-weighted foliar N was 1.3 mg g^{-1} lower in control plots in 2021-22 than in 2008-10, which is a difference of 0.13 mg g^{-1} yr^{-1}, more than twice what has been reported in European forests (Mason et al. 2022), though the Wilcoxon signed rank test I performed was not statistically significant ($p = 0.11$). Continuing to monitor foliar and litter as MELNHE progresses over time will therefore provide data on how foliage and leaf litter are changing with global climate change, especially providing insight on how N and P limitations are intensifying over time and how changes in nutrient availability intersect with this new reality.

Table 5-1. Summary of N, P, and $CaSiO_3$ effects on characteristics of six different northern hardwood species measured in 2021-22: relative basal area increment (RBAI), foliar N, foliar P, litter N, litter P, N resorption efficiency (NRE), P resorption efficiency (PRE), specific leaf area (SLA), leaf dry matter content (LDMC), carbon isotope composition ($\delta^{13}C$), and stomatal density. These characteristics are reported in chapters 2, 3, or 4. “N” indicates that RBAI or the trait responded to N in a way consistent with N limitation, “P” indicates that the trait responded to P in a way consistent with P limitation, and “NP” suggests that the trait responded to N and P in a way consistent with N and P co-limitation through synergistic effects. Gray cells indicate that the measurement was not applicable, and a blank cell indicates that there was no effect of N or P addition, or the observed effect could not indicate N or P limitation. Values in parentheses in the Foliar N and Foliar P columns indicate results from Chapter 4 that differ from those in Chapter 2. Inferences made about limitation in Chapter 4 came from models of four species and examining graphs of *A. rubrum* and *P. pensylvanica*.

Species	**RBAI**	**Foliar N**	**Foliar P**	**Litter N**	**Litter P**	**NRE**	**PRE**	**SLA**	**LDMC**	**$\delta^{13}C$**	**Stomatal Density**
Chapter	2	2, 4	2, 4	4	4	4	4	2	2	2, 3	3
P. pensylvanica		P	N	P	N		NP		P		
B. papyrifera	N			P	NP	NP	NP			N	
B. alleghaniensis	N	P	N (NP)	NP	NP	NP	NP				
A. rubrum		P or NP	NP (N)		NP		NP				
A. saccharum			NP	NP	NP	NP	NP		P	N, $CaSiO_3$	P
F. grandifolia		P	N	P		NP	NP				
Community		NP	NP	P	NP		NP			N	

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APPENDIX A: DAMAGED FOLIAGE COMPARISON FOR N, P, AND LDMC (CHAPTER 2)

Table A-1. Results of paired t-tests comparing LDMC, foliar N, and foliar P in 20 undamaged foliage samples to corresponding, damaged samples from the same tree. Trees were sampled from Hubbard Brook and Jeffers Brook in 2021; select trees from the study were selected for this purpose if they had a substantial number of damaged leaves. Damage refers primarily to pest and disease activity, such as herbivory, skeletonization, and gall presence. Mean difference represents the difference between damaged samples and undamaged samples (i.e., if the value is positive, then the damaged sample has a larger value than the undamaged sample).

Trait	**Mean Difference (mg g^{-1})**	***t***	***DF***	***p***
LDMC	0.482	0.033	19	0.97
N	-0.292	-0.706	19	0.49
P	0.077	1.558	19	0.14

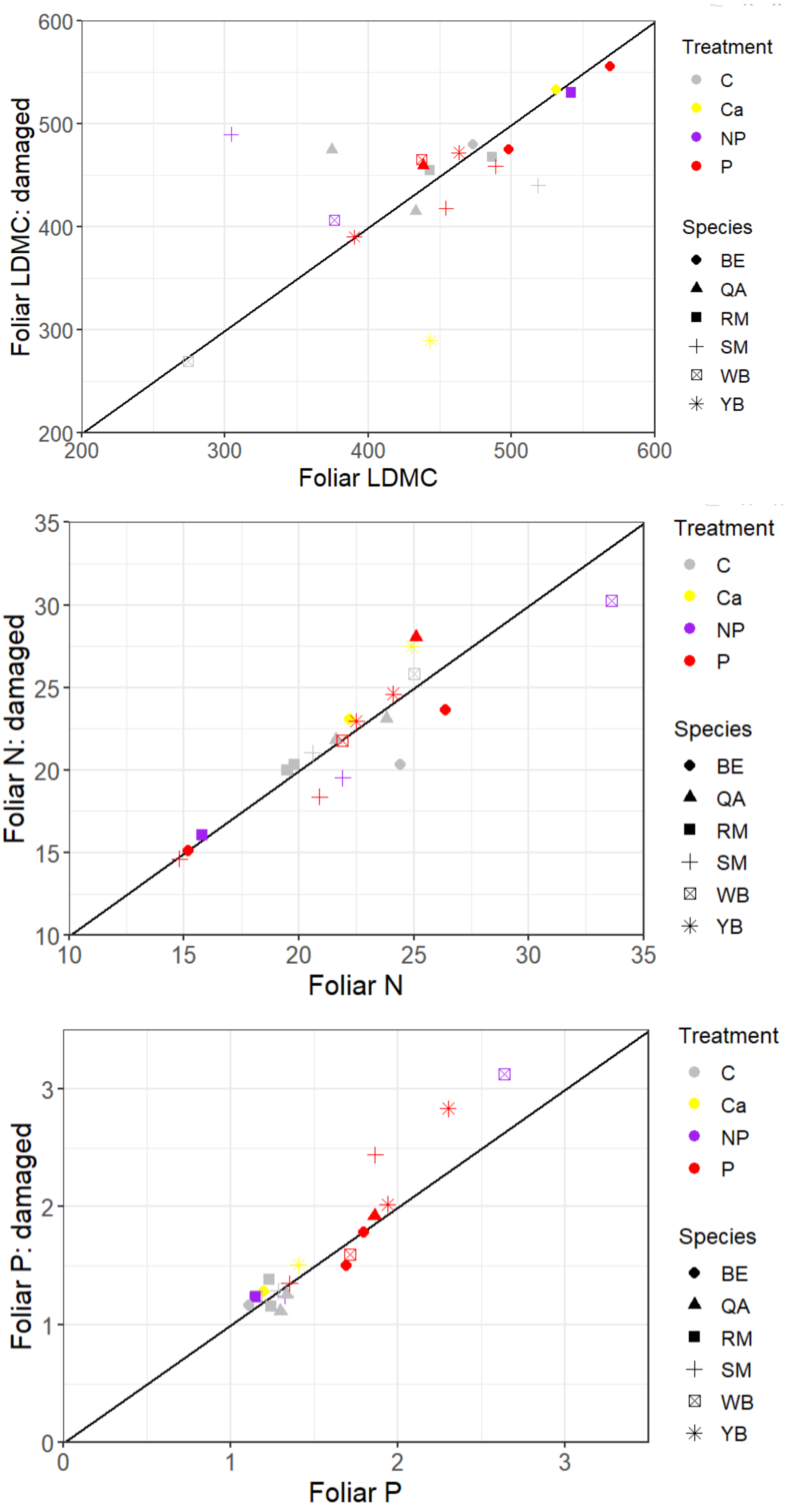

Figure A-1. Comparison of LDMC (top), foliar N (middle), and foliar P (bottom) between damaged and undamaged foliage samples collected from Hubbard Brook and Jeffers Brook in 2021.

APPENDIX B: SPECIES-LEVEL LINEAR MIXED-EFFECTS MODEL RESULTS: RBAI (CHAPTER 2)

Table B-1. Nutrient addition, stand age, and site effects on RBAI in five tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	0.000	1	70	0.017	0.895
P	0.001	1	69	2.313	0.133
Age	0.002	1	5	7.385	0.042
Site	0.000	2	5	0.693	0.540
N x P	0.000	1	69	0.252	0.617
A. rubrum					
N	0.000	1	31	1.000	0.325
P	0.001	1	31	2.381	0.133
Site	0.000	1	3	0.305	0.632
N x P	0.000	1	31	0.870	0.358
A. saccharum					
N	0.000 (0.000)	1	16 (14)	2.172 (1.859)	0.160 (0.195)
P	0.000 (0.000)	1	17 (15)	1.998 (0.669)	0.176 (0.427)
Age	0.003 (0.001)	1	18 (2)	15.813 (15.144)	<0.001 (0.061)
Site	0.000 (0.000)	2	17 (2)	0.523 (0.976)	0.602 (0.506)
N x P	0.001 (0.000)	1	16 (14)	3.665 (4.183)	0.074 (0.060)
B. papyrifera					
N	0.001	1	56	4.763	0.033
P	0.000	1	56	0.004	0.947
Site	0.000	2	3	0.599	0.604
N x P	0.000	1	56	0.671	0.416
B. alleghaniensis					
N	0.002	1	17	13.523	0.002
P	0.000	1	17	0.624	0.440
Age	0.001	1	4	7.834	0.049
Site	0.000	2	4	0.647	0.572
NxP	0.000	1	17	0.078	0.783

APPENDIX C. COMMUNITY- AND SPECIES-LEVEL LINEAR MIXED-EFFECT MODEL RESULTS BY TRAIT (CHAPTER 2)

Table C-1. Nutrient addition, stand age, and site effects on community-weighted means (CWM) of five foliar traits in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Foliar Nitrogen					
N	74.556	1	27	24.818	<0.001
P	13.160	1	27	4.380	0.046
Age	15.069	1	6	5.016	0.067
Site	15.578	2	6	2.593	0.154
N x P	1.634	1	27	0.544	0.467
Foliar Phosphorus					
N	0.253	1	27	10.506	0.003
P	5.328	1	27	221.016	<0.001
Age	0.025	1	6	1.016	0.352
Site	0.269	2	6	5.573	0.043
N x P	0.217	1	27	9.016	0.006
SLA					
N	0.168	1	27	0.087	0.771
P	0.853	1	27	0.441	0.512
Age	0.043	1	6	0.022	0.887
Site	5.232	2	6	1.353	0.327
N x P	0.080	1	27	0.041	0.41
LDMC					
N	15.3	1	27	0.042	0.840
P	112.4	1	27	0.307	0.583
Age	10570.3	1	6	28.912	0.002
Site	54.4	2	6	0.074	0.929
N x P	3.9	1	27	0.011	0.918
$\delta^{13}C$					
N	0.712	1	21	3.731	0.067
P	0.137	1	21	0.720	0.406
Age	0.001	1	4	0.003	0.961
Site	0.109	2	4	0.285	0.766
N x P	0.008	1	21	0.039	0.845

Table C-2. Nutrient addition, stand age, and site effects on the contribution of intraspecific variability to the community-weighted means (CWM_{Intra}) for five foliar traits. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Foliar Nitrogen					
N	63.726	1	33	23.722	<0.001
P	16.415	1	33	6.111	0.019
Age	7.592	1	33	2.826	0.102
Site	5.536	2	33	1.031	0.368
N x P	0.752	1	33	0.280	0.600
Foliar Phosphorus					
N	0.273	1	27	11.748	0.002
P	5.242	1	27	225.337	<0.001
Age	0.003	1	6	0.147	0.715
Site	0.131	2	6	2.823	0.137
N x P	0.177	1	27	7.606	0.010
SLA					
N	0.409	1	27	0.211	0.649
P	1.085	1	27	0.561	0.460
Age	7.476	1	6	3.864	0.097
Site	4.291	2	6	1.109	0.389
N x P	0.202	1	27	0.104	0.749
LDMC					
N	61.8	1	33	0.174	0.680
P	129.7	1	33	0.366	0.549
Age	3083.0	1	33	8.702	0.006
Site	3338.4	2	33	4.712	0.016
N x P	120.7	1	33	0.341	0.563
$\delta^{13}C$					
N	0.610	1	21	4.216	0.053
P	0.015	1	21	0.104	0.751
Age	0.971	1	4	6.709	0.061
Site	0.019	2	4	0.066	0.938
N x P	0.000	1	21	0.000	0.983

Table C-3. Nutrient addition, stand age, and site effects on fixed community-weighted means (CWM_{Inter}) of five foliar traits. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Foliar Nitrogen					
N	0.565	1	27	2.053	0.163
P	0.349	1	27	1.268	0.270
Age	0.717	1	6	2.607	0.158
Site	1.216	2	6	2.210	0.191
N x P	0.169	1	27	0.614	0.440
Foliar Phosphorus					
N	0.002	1	27	1.101	0.303
P	0.002	1	27	1.076	0.309
Age	0.015	1	6	7.603	0.033
Site	0.010	2	6	2.601	0.154
N x P	0.002	1	27	1.080	0.308
SLA					
N	0.002	1	27	0.034	0.855
P	0.001	1	27	0.020	0.889
Age	0.790	1	6	13.336	0.011
Site	0.058	2	6	0.493	0.634
N x P	0.028	1	27	0.469	0.500
LDMC					
N	83.98	1	27	1.481	0.234
P	33.78	1	27	0.596	0.447
Age	1185.04	1	6	20.900	0.004
Site	286.89	2	6	2.530	0.160
N x P	81.12	1	27	1.431	0.242
$\delta^{13}C$					
N	0.012	1	21	1.234	0.279
P	0.059	1	21	5.859	0.025
Age	0.080	1	4	7.968	0.048
Site	0.029	2	4	1.458	0.334
N x P	0.009	1	21	0.894	0.355

Table C-4. Nutrient addition, stand age, and site effects on foliar N in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P. The *A. saccharum* model was re-run without the outlier tree #8419 from stand HBO plot 2; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	38.324	1	23	6.127	0.021
P	19.378	1	23	3.098	0.092
Age	12.478	1	5	1.995	0.217
Site	17.379	2	6	1.109	0.393
N x P	13.876	1	23	2.779	0.110
P. pensylvanica					
N	94.033	1	3	12.949	0.039
P	53.811	1	3	7.140	0.072
N x P	0.169	1	3	0.023	0.889
A. rubrum					
N	3.045	1	12	0.630	0.443
P	23.875	1	12	4.938	0.046
Site	Excluded				
N x P	2.668	1	12	0.552	0.472
A. saccharum					
N	75.968 (76.395)	1	59 (15)	13.632 (17.083)	<0.001 (<0.001)
P	9.293 (4.884)	1	59 (16)	1.668 (1.092)	0.202 (0.312)
Age	4.451 (0.724)	1	2 (2)	0.799 (0.162)	0.460 (0.725)
Site	4.399 (3.557)	2	2 (2)	0.395 (0.795)	0.715 (0.554)
N x P	6.062 (10.284)	1	59 (16)	1.088 (2.300)	0.301 (0.149)
B. papyrifera					
N	104.492	1	15	12.106	0.003
P	3.865	1	15	0.448	0.513
Site	75.569	2	3	4.378	0.114
N x P	0.023	1	15	0.003	0.959
B. alleghaniensis					
N	50.454	1	28	11.029	0.003
P	36.232	1	28	7.920	0.009
Age	0.068	1	8	0.015	0.906
Site	Excluded				
N x P	7.638	1	28	1.670	0.207

Table C-5. Nutrient addition, stand age, and site effects on foliar P in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P. Foliar P values were log-transformed for *F. grandifolia* and *P. pensylvanica*. The *F. grandifolia* model was re-run without the outlier tree #8872 from stand C6 plot 4; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia (ln)					
N	0.095 (0.072)	1	23	4.500 (3.775)	0.045 (0.064)
P	1.806 (1.699)	1	23	85.863 (89.001)	<0.001 (<0.001)
Age	0.003 (0.002)	1	5	0.155 (0.089)	0.710 (0.778)
Site	0.127 (0.160)	2	6	3.020 (4.185)	0.129 (0.077)
N x P	<0.001 (0.002)	1	23	0.006 (0.131)	0.938 (0.721)
P. pensylvanica (ln)					
N	0.157	1	4	8.977	0.040
P	1.186	1	4	67.974	0.001
N x P	0.004	1	4	0.244	0.647
A. rubrum					
N	0.046	1	11	1.842	0.202
P	1.142	1	11	46.015	<0.001
Site	0.017	1	11	0.674	0.429
N x P	0.047	1	11	1.897	0.196
A. saccharum					
N	0.361	1	15	6.600	0.021
P	2.200	1	15	40.230	<0.001
Age	0.009	1	2	0.157	0.729
Site	0.071	2	2	0.650	0.604
N x P	0.383	1	15	7.003	0.018
B. papyrifera (ln)					
N	0.062	1	15	2.794	0.116
P	4.264	1	15	192.349	<0.001
Site	0.086	2	3	1.928	0.281
N x P	0.060	1	15	2.697	0.122
B. alleghaniensis					
N	0.299	1	26	4.466	0.044
P	8.656	1	26	129.314	<0.001
Age	0.002	1	6	0.035	0.858
Site	0.162	2	6	1.209	0.364
N x P	0.286	1	26	4.274	0.049

Table C-6. Nutrient addition, stand age, and site effects on LDMC in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P. The *P. pensylvanica* model was re-run without the outlier tree #2193 from stand C1 plot 3; these results are shown below in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	600.3	1	22	0.762	0.392
P	19.1	1	23	0.024	0.878
Age	4370.7	1	5	5.547	0.064
Site	6136.1	2	6	3.894	0.088
N x P	2744.5	1	22	3.483	0.075
P. pensylvanica					
N	23.33 (157.6)	1	3	0.039 (0.524)	0.856 (0.518)
P	2483.11 (4261.0)	1	3	4.179 (14.178)	0.133 (0.029)
N x P	0.53 (272.6)	1	3	0.001 (0.907)	0.978 (0.407)
A. rubrum					
N	432.6	1	41	0.298	0.588
P	3398.0	1	41	2.340	0.134
Site	997.9	1	41	0.687	0.412
N x P	72.5	1	41	0.050	0.824
A. saccharum					
N	464.8	1	15	0.230	0.638
P	13611.6	1	15	6.748	0.020
Age	4703.4	1	15	2.332	0.147
Site	945.4	2	15	0.469	0.635
N x P	1689.6	1	15	0.838	0.375
B. papyrifera					
N	29.64	1	65	0.017	0.897
P	2156.63	1	65	1.235	0.270
N x P	248.10	1	65	0.142	0.707
Site	Excluded				
B. alleghaniensis					
N	1555.1	1	28	1.618	0.214
P	460.7	1	28	0.479	0.495
Age	8526.5	1	6	8.872	0.025
Site	8657.9	2	6	4.504	0.067
N x P	527.7	1	28	0.549	0.464

Table C-7. Nutrient addition, stand age, and site effects on SLA in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P. SLA was log-transformed for *A. rubrum*. The *B. papyrifera* model was re-run without the outlier tree #8513 from stand HBM plot 3; these results are shown below in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	0.195	1	23	0.016	0.900
P	18.322	1	23	1.512	0.231
Age	5.114	1	5	0.422	0.545
Site	18.472	2	5	0.762	0.513
N x P	0.506	1	23	0.042	0.840
P. pensylvanica					
N	0.023	1	19	0.003	0.959
P	8.390	1	19	0.987	0.333
N x P	1.071	1	19	0.126	0.727
A. rubrum (ln)					
N	0.062	1	41	1.875	0.179
P	0.097	1	41	2.934	0.094
Site	0.077	1	41	2.309	0.137
N x P	0.017	1	41	0.526	0.472
A. saccharum					
N	1.226	1	15	0.091	0.767
P	10.117	1	15	0.750	0.400
Age	117.227	1	4	8.694	0.047
Site	Excluded				
N x P	1.296	1	15	0.096	0.761
B. papyrifera					
N	0.841 (0.001)	1	63 (18)	0.210 (<0.001)	0.648 (0.986)
P	21.732 (4.992)	1	63 (18)	5.424 (2.506)	0.023 (0.131)
Site	1.422 (2.366)	2	63 (19)	0.177 (0.594)	0.838 (0.562)
N x P	2.137 (0.085)	1	63 (18)	0.533 (0.043)	0.468 (0.839)
B. alleghaniensis					
N	9.381	1	27	1.068	0.310
P	0.255	1	27	0.029	0.866
Age	58.417	1	8	6.648	0.032
Site	Excluded				
N x P	1.719	1	27	0.196	0.662

Table C-8. Nutrient addition, stand age, and site effects on $\delta^{13}C$ in six tree species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	2.089	1	18	3.023	0.100
P	0.067	1	19	0.097	0.760
Age	0.781	1	3	1.131	0.368
Site	1.187	2	3	0.859	0.506
N x P	0.73	1	18	1.069	0.315
P. pensylvanica					
N	5.820	1	1	8.220	0.214
P	0.267	1	2	0.377	0.605
N x P	0.001	1	3	0.001	0.981
A. rubrum					
N	0.069	1	17	0.062	0.806
P	0.152	1	17	0.137	0.716
Site	Excluded				
N x P	0.011	1	17	0.010	0.921
A. saccharum					
N	1.427	1	17	3.296	0.087
P	0.071	1	17	0.165	0.690
Age	4.424	1	17	10.222	0.005
Site	0.783	2	17	0.905	0.423
N x P	0.111	1	17	0.256	0.619
B. papyrifera					
N	3.538	1	9	4.183	0.070
P	1.530	1	9	1.809	0.211
Site	Excluded				
N x P	1.778	1	9	2.102	0.180
B. alleghaniensis					
N	0.819	1	79	1.021	0.316
P	0.769	1	78	0.959	0.330
Age	0.857	1	4	1.069	0.359
Site	0.932	2	4	0.581	0.601
N x P	0.007	1	78	0.009	0.927

APPENDIX D: SPECIES-LEVEL LINEAR MIXED-EFFECTS MODEL RESULTS USING THE SAME TREES AS THE RBAI ANALYSES (CHAPTER 2)

Table D-1. Nutrient addition, stand age, and site effects on foliar N in five tree species using the same trees for which relative basal area increment was calculated. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	51.981	1	20	9.045	**0.007**
P	5.300	1	20	0.922	0.349
Age	11.646	1	4	2.027	0.224
Site	19.255	2	5	1.675	0.271
N x P	12.833	1	20	2.233	0.151
A. rubrum					
N	0.096	1	12	0.018	0.894
P	23.259	1	12	4.468	0.055
Site	Excluded				
N x P	3.755	1	13	0.721	0.411
A. saccharum					
N	88.720	1	55	17.325	<0.001
P	22.367	1	55	4.368	0.041
Age	2.418	1	2	0.472	0.560
Site	4.284	2	2	0.418	0.703
N x P	0.351	1	55	0.069	0.794
B. papyrifera					
N	51.223	1	16	5.841	0.028
P	3.489	1	15	0.398	0.537
Site	78.766	2	4	4.491	0.111
N x P	0.002	1	16	0.000	0.988
B. alleghaniensis					
N	96.182	1	27	19.744	<0.001
P	35.823	1	27	6.621	0.017
Age	2.943	1	6	0.928	0.381
Site	31.802	2	5	2.732	0.169
N x P	10.638	1	28	2.434	0.132

Table D-2. Nutrient addition, stand age, and site effects on foliar P in five tree species using the same trees for which relative basal area increment was calculated. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia (ln)					
N	0.049	1	19	2.151	0.159
P	1.732	1	18	75.449	<0.001
Age	0.002	1	6	0.104	0.759
Site	0.078	2	8	1.710	0.241
N x P	0.000	1	18	0.000	0.998
A. rubrum					
N	0.000	1	10	0.001	0.973
P	1.010	1	10	35.673	<0.001
Site	0.014	1	11	0.494	0.497
N x P	0.049	1	11	1.748	0.215
A. saccharum					
N	0.352	1	15	0.000	0.024
P	2.230	1	15	39.330	<0.001
Age	0.005	1	2	0.087	0.794
Site	0.076	2	2	0.685	0.590
N x P	0.343	1	15	6.164	0.026
B. papyrifera (ln)					
N	0.118	1	15	5.166	0.038
P	4.654	1	14	203.918	<0.001
Site	0.088	2	3	1.925	0.282
N x P	0.054	1	15	2.369	0.145
B. alleghaniensis					
N	0.255	1	23	3.385	0.079
P	9.447	1	23	125.514	<0.001
Age	0.003	1	5	0.033	0.864
Site	0.177	2	5	1.178	0.387
N x P	0.606	1	23	8.053	0.009

Table D-3. Nutrient addition, stand age, and site effects on SLA in five tree species using the same trees for which relative basal area increment was calculated. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P. The *B. papyrifera* model was re-run without the outlier tree #8513 in stand HBM plot 3; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	0.042	1	19	0.003	0.956
P	8.134	1	19	0.614	0.443
Age	2.500	1	4	0.189	0.685
Site	9.790	2	6	0.370	0.706
N x P	0.810	1	19	0.061	0.807
A. rubrum					
N	0.060	1	30	1.884	0.180
P	0.021	1	30	0.679	0.417
Site	0.044	1	30	1.406	0.245
N x P	0.002	1	30	0.061	0.807
A. saccharum (ln)					
N	0.007	1	15	0.138	0.716
P	0.089	1	16	1.952	0.182
Age	0.128	1	4	2.819	0.176
Site	Excluded				
N x P	0.006	1	15	0.126	0.727
B. papyrifera					
N	0.873 (0.096)	1	61 (19)	0.216 (0.048)	0.643 (0.829)
P	21.210 (4.732)	1	61 (18)	5.258 (2.368)	0.025 (0.141)
Site	0.914 (2.666)	2	61 (19)	0.113 (0.667)	0.893 (0.525)
N x P	0.468 (0.063)	1	61 (18)	0.116 (0.031)	0.734 (0.861)
B. alleghaniensis					
N	0.138	1	20	0.019	0.891
P	5.019	1	21	0.703	0.412
Age	41.961	1	7	5.876	0.046
Site	Excluded				
N x P	0.008	1	21	0.011	0.974

Table D-4. Nutrient addition, stand age, and site effects on LDMC in five tree species using the same trees for which relative basal area increment was calculated. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. “N x P” refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	1243.6	1	20	1.706	0.206
P	120.0	1	19	0.165	0.689
Age	2962.7	1	5	4.63	0.105
Site	8874.0	2	7	6.085	**0.031**
N x P	3562.3	1	20	4.886	**0.039**
A. rubrum					
N	1377.63	1	29	1.279	0.267
P	328.82	1	29	0.305	0.585
Site	377.93	1	2	0.351	0.609
N x P	131.12	1	29	0.122	0.730
A. saccharum					
N	130.3	1	59	0.000	0.801
P	14352.2	1	59	39.330	**0.010**
Age	3819.3	1	59	0.087	0.176
Site	1984.9	2	59	0.685	0.617
N x P	1995.5	1	59	6.164	0.327
B. papyrifera					
N	19.27	1	61	0.011	0.918
P	1621.96	1	61	0896	0.345
Site	1153.18	2	61	0.319	0.728
N x P	0.00	1	61	0.000	0.999
B. alleghaniensis					
N	261.6	1	24	0.285	0.598
P	2.6	1	24	0.003	0.958
Age	7361.9	1	5	8.030	**0.039**
Site	6355.6	2	4	3.466	0.126
N x P	354.6	1	24	0.389	0.540

Table D-5. Effects of nutrient addition, stand age, and site on $\delta^{13}C$ in five tree species using the same trees for which relative basal area increment was calculated. Stands C2 and C6 were excluded from these analyses due to lack of $\delta^{13}C$ data. Sum of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom (DF) are displayed. "N x P" refers to the interaction of N and P.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
F. grandifolia					
N	0.399	1	15	0.506	0.488
P	0.217	1	13	0.275	0.609
Age	0.063	1	2	0.080	0.807
Site	2.174	2	2	1.378	0.421
N x P	0.353	1	13	0.448	0.515
A. rubrum					
N	0.009	1	14	0.007	0.933
P	0.000	1	14	0.000	0.991
Site	Excluded				
N x P	0.051	1	14	0.041	0.843
A. saccharum					
N	1.568	1	16	3.794	**0.069**
P	0.128	1	17	0.311	0.584
Age	3.401	1	17	8.226	**0.011**
Site	0.912	2	17	1.103	0.355
N x P	0.051	1	16	0.124	0.729
B. papyrifera					
N	4.244	1	9	5.483	**0.043**
P	1.606	1	9	1.990	0.194
Site	2.867	2	2	1.776	0.360
N x P	2.082	1	9	2.580	0.144
B. alleghaniensis					
N	1.336	1	69	1.784	0.186
P	0.000	1	69	0.001	0.993
Age	0.572	1	4	0.764	0.432
Site	1.217	2	4	0.812	0.510
N x P	0.004	1	69	0.006	0.939

APPENDIX E: PSEUDO R^2 VALUES FOR LINEAR MIXED-EFFECT MODELS (CHAPTER 3)

Table E-1. Fixed, random and model pseudo R^2 values calculated for four linear mixed-effect models of *stomatal density*. R^2 was approximated using the function 'rsq.lmm' in the 'rsq' package (Zhang et al. 2022)

Analysis	R^2: Fixed	R^2: Random	R^2: Model
Sugar Maple NxP	3.0%	45.4%	48.5%
Sugar Maple Ca	1.0%	32.7%	33.7%
Yellow Birch NxP	3.1%	61.3%	64.4%
Yellow Birch Ca	7.1%	60.6%	67.7%

Table E-2. Fixed, random, and model pseudo R^2 values calculated for four linear mixed-effect models of $\delta^{13}C$. R^2 was approximated using the function 'rsq.lmm' in the 'rsq' package (Zhang et al. 2022)

Analysis	R^2: Fixed	R^2: Random	R^2: Model
Sugar Maple NxP	36.2%	16.0%	52.2%
Sugar Maple Ca	35.4%	18.5%	53.9%
Yellow Birch NxP	15.7%	15.9%	31.6%
Yellow Birch Ca	16.7%	8.4%	25.1%

Zhang D (2022) Package 'rsq'. R package version 4.2-0. https://cran.r-project.org/web/packages/rsq/rsq.pdf

APPENDIX F: LINEAR MIXED-EFFECTS MODEL RESULTS FOR SPECIFIC LEAF AREA IN YELLOW BIRCH (CHAPTER 3)

To help further explain yellow birch results, we also examined the relationship between SLA and stand age pooling the trees across all stands in this study using a general linear mixed model with stand age as a fixed effect and both stand and plot within stand as random effects. Site was excluded to prevent singularity errors.

Table F-1. Linear mixed-effect model results for a test stand age on specific leaf area (SLA) in yellow birch.

Fixed Effect	**Coefficient ± SE**	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Yellow Birch						
Age	Mid-successional: 2.427 ± 0.839	70.805	1	10	8.366	0.017

APPENDIX G: DAMAGED FOLIAGE COMPARISON (CHAPTER 4)

Table G-1. Results of paired t-tests comparing foliar chemistry in undamaged foliage samples to corresponding, damaged samples from the same tree. Twenty trees were sampled from Hubbard Brook and Jeffers Brook for this comparison in 2021; trees were selected for this comparison if they had a substantial number of damaged leaves. Damage refers primarily to pest and disease activity, such as herbivory, skeletonization, and gall presence. Mean difference represents the difference between damaged samples and undamaged samples (i.e., if the value is positive, then the damaged sample has a larger value than the undamaged sample).

Trait	**Mean Difference (mg g^{-1})**	***t***	**DF**	***p***
Ca	-0.724	-2.797	19	0.01
Mg	-0.082	-2.370	19	0.03
S	-0.034	-1.785	19	0.09
P	0.077	1.558	19	0.14
Mn	-0.049	-1.511	19	0.15
Zn	-0.004	-1.240	19	0.23
K	0.254	1.1425	19	0.27
Sr	-0.0009	-1.022	10	0.32
N	-0.292	-0.706	19	0.49
Fe	0.001	0.467	19	0.65

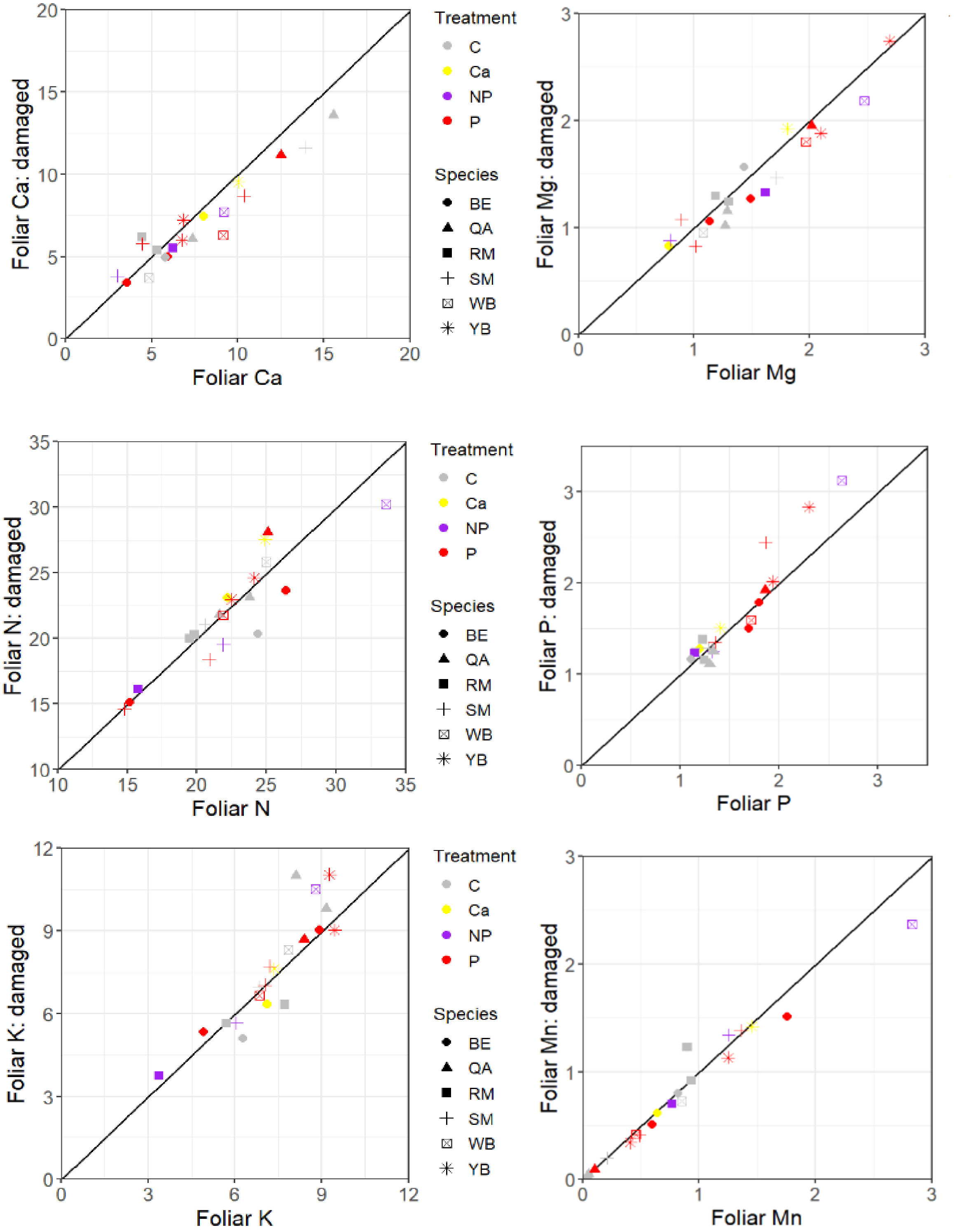

Figure G-1. Comparison of undamaged (X-axis) to damaged (Y-axis) foliar chemistry values for 20 trees for which both damaged and undamaged foliage was collected and processed. Elements studied include Ca (top left), Mg (top right), N (middle left), P (middle right), K (bottom left), and Mn (bottom right).

APPENDIX H: SPECIES-LEVEL ANALYSES (CHAPTER 4)

Table H-1. Direction of N, P, and N+P (NP) addition effects on foliar and litter N and P concentrations, N resorption efficiency (NRE), and P resorption efficiency (PRE) in six northern hardwood species in 2014-16 and 2021. Results for *B. alleghaniensis*, *B. papyrifera*, *F. grandifolia*, and *A. saccharum* were determined from linear mixed-effect models. Results for *A. rubrum* and *P. pensylvanica* were obtained from graphs and should be treated with caution. ↑ indicates an increase with nutrient addition, ↓ indicates a decrease with nutrient addition, ↗ and ↘ indicate an increase or decrease (respectively) with N+P addition that is not as great as with either N or P, and = indicates no detectable or consistent effect of nutrient addition. Results in parentheses are for models re-run without outliers if the direction of the results differ.

Species	**Sampling Period**	**Foliar N**	**Foliar P**	**Litter N**	**Litter P**	**NRE**	**PRE**
B. alleghaniensis	2014-16	↑N, = P	↓N, ↑P	↑N, ↓P	↓N, ↑P	= N, ↑P	= N, ↓P, = NP
	2021-22	↑N, ↓P	= N, ↑P, ↗NP	↑N, = P, ↗NP	= N, ↑P, ↗NP	↓N, = P, ↘NP	= N, ↓P, ↘NP
B. papyrifera	2014-16	↑N, = P	= N, ↑P	↑N, ↓P	↓N, ↑P	= N, ↑P	= N, ↓P, = NP
	2021-22	↑N, = P	= N, ↑P	↑N, ↓P	= N, ↑P, ↗NP	= N, ↑P	= N, ↓P, ↘NP
F. grandifolia	2014-16	↑N, = P (↑N, ↓ P, ↑NP)	= N, ↑P	= N, ↓P	= N, ↑P, ↗NP	= N, = P	= N, ↓P, = NP
	2021-22	↑N, ↓P	↓N, ↑P	↑N, ↓P	= N, ↑P	↓N, = P, ↘NP	= N, ↓P, ↘NP
A. saccharum	2014-16	↑N, = P	= N, ↑P	↑N, = P	= N, = P	= N, = P	= N, = P
	2021-22	↑N, = P	= N, ↑P, ↗NP	↑N, ↑P	= N, ↑P, ↗NP	↓N, = P, ↘NP	= N, ↓P, ↘NP
A. rubrum	2014-16	↑N, ↓P	↑N, ↑P	= N, = P	= N, ↑P, ↗NP	= N, = P	= N, ↓P, ↘NP
	2021-22	↑N, ↓P	↓N, ↑P	= N, = P	= N, ↑P, ↗NP	↓N, ↓ P	= N, ↓P, ↘NP
P. pensylvanica	2014-16	↑N, = P	↓N, ↑P	↑N, ↓P	= N, ↑P, ↗NP	↓N, = P	= N, ↓P, = NP
	2021-22	↑N, ↓P	↓N, ↑P	↑N, ↓P	↓N, ↑P	↓N, = P	= N, ↓P, ↘NP

Table H-2. Interpretation of limitation based on effects of N and P on foliar and litter N and P concentrations, N resorption efficiency (NRE), and P resorption efficiency (PRE) in 2014-16 and 2021-22. “N” indicates that the response variable responded to N in a way consistent with N limitation, “P” indicates that the response variable responded to P in a way consistent with P limitation, and “NP” suggests that the response variable responded to N and P in a way consistent with N and P co-limitation through synergistic effects.

Species	**Sampling Period**	**Foliar N**	**Foliar P**	**Litter N**	**Litter P**	**NRE**	**PRE**
B. alleghaniensis	2014-16		N	P	N	P	
	2021-22	P	NP	NP	NP	NP	NP
B. papyrifera	2014-16			P	N	P	
	2021-22			P	NP	NP	NP
F. grandifolia	2014-16	none (P)		P	NP		
	2021-22		N	P		NP	NP
A. saccharum	2014-16						
	2021-22		NP	NP	NP	NP	NP
A. rubrum	2014-16		NP		NP		NP
	2021-22	P	N		NP		NP
P. pensylvanica	2014-16		N	P	NP		
	2021-22	P	N	P	N		NP

Table H-3. Direction of N, P, and N+P (NP) addition effects on foliar and litter Ca, K, and Mg concentrations in six northern hardwood species in 2014-16 and 2021. Results for *B. alleghaniensis*, *B. papyrifera*, *F. grandifolia*, and *A. saccharum* were determined from linear mixed-effect models. Results for *A. rubrum* and *P. pensylvanica* were obtained from graphs and should be treated with caution. ↑ indicates an increase with nutrient addition, ↓ indicates a decrease with nutrient addition, ↗ and ↘ indicate an increase or decrease (respectively) with N+P addition that is not as great as with either N or P, and = indicates no detectable or consistent effect of nutrient addition.

Species	**Sampling Period**	**Foliar Ca**	**Foliar K**	**Foliar Mg**	**Litter Ca**	**Litter K**	**Litter Mg**
B. alleghaniensis	2014-16	↓N, = P	= N, = P	↓N, = P	↓N, = P	= N, = P	↓N, = P
	2021-22	↓N, = P	= N, = P	↓N, = P	↓N, = P	= N, = P	↓N, = P
B. papyrifera	2014-16	= N, ↑P	= N, = P	= N, = P	= N, = P	= N, = P	= N, = P
	2021-22	↓N, ↑P	= N, = P	= N, ↑P	↓N, ↑P	= N, = P	↓N, = P
F. grandifolia	2014-16	↓N, = P	= N, = P, ↑NP	= N, = P	= N, = P	↓N, ↑P	= N, = P
	2021-22	↓N, ↑P	↓N, = P	= N, = P	↓N, ↑P	= N, ↑P	= N, = P
A. saccharum	2014-16	↓N, = P	↓N, = P	= N, = P	= N, = P	= N, = P	= N, = P
	2021-22	↓N, = P	= N, = P, ↓NP	= N, = P	= N, = P	= N, = P	= N, = P
A. rubrum	2014-16	= N, = P	= N, = P	↑N, ↑P	↑N, = P	= N, = P	= N, = P
	2021-22	= N, = P	= N, = P	= N, = P	= N, ↓P	↑ N, = P	= N, = P
P. pensylvanica	2014-16	= N, = P	= N, = P	= N, = P	↓N, ↑P	= N, = P	= N, = P
	2021-22	= N, ↑P	↓N, ↑P	= N, = P	↑N, ↑P, ↓ NP	= N, ↓P	= N, = P

Table H-4. The 3-to-5-year foliar N response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar N, site, and the interaction of N and P. Stand was a random effect. The *F. grandifolia* model was repeated without stand C1 to observe the effects of outliers on the results; these model results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	23.990	1	11	59.625	<0.001
P	0.087	1	11	0.217	0.650
Age	0.573	1	1	1.424	0.425
Pre-N	0.297	1	11	0.738	0.408
Site	5.358	2	2	6.658	0.131
N x P	0.010	1	11	0.024	0.879
B. alleghaniensis					
N	47.117	1	25	12.476	0.002
P	7.162	1	25	1.897	0.181
Age	1.402	1	5	0.371	0.571
Pre-N	31.031	1	27	8.217	0.008
Site	4.973	2	5	0.659	0.554
N x P	0.240	1	25	0.064	0.803
B. papyrifera					
N	39.817	1	14	12.298	0.003
P	4.359	1	14	1.346	0.265
Pre-N	59.321	1	16	18.322	0.001
Site	40.919	2	3	6.319	0.079
N x P	0.078	1	14	0.024	0.879
F. grandifolia					
N	67.243 (78.460)	1	27 (24)	25.837 (45.550)	<0.001 (<0.001)
P	3.000 (7.461)	1	27 (24)	1.153 (4.332)	0.293 (0.048)
Age	17.395 (22.377)	1	8 (7)	6.684 (12.991)	0.032 (0.009)
Pre-N	0.033 (0.282)	1	31 (19)	0.013 (0.164)	0.911 (0.690)
Site	4.570 (14.652)	2	7 (5)	0.878 (4.253)	0.459 (0.078)
N x P	4.338 (8.518)	1	27 (25)	1.667 (4.945)	0.208 (0.036)

Table H-5. The 10-to-11-year foliar N response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar N, site, and the interaction of N and P. Stand was a random effect. The model for *B. alleghaniensis* has random effects equal to zero and should be interpreted with caution. Foliar N was log-transformed for *B. alleghaniensis* to meet assumptions of residual normality.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	27.177	1	14	14.505	0.002
P	5.681	1	14	5.634	0.104
Age	0.158	1	3	0.001	0.793
Pre-N	2.045	1	15	3.035	0.312
Site	2.963	2	2	1.505	0.558
N x P	1.771	1	14	0.231	0.347
B. alleghaniensis (ln)					
N	0.053	1	31	8.380	0.007
P	0.048	1	31	7.646	0.009
Age	0.002	1	31	0.246	0.624
Pre-N	0.030	1	31	4.751	0.037
Site	0.004	2	31	0.345	0.711
N x P	0.009	1	31	1.443	0.239
B. papyrifera					
N	50.144	1	15	12.577	0.003
P	2.699	1	15	0.677	0.424
Pre-N	5.375	1	15	1.348	0.263
Site	29.748	2	4	3.731	0.134
N x P	0.009	1	14	0.002	0.963
F. grandifolia					
N	22.014	1	24	6.082	0.021
P	8.171	1	24	2.258	0.146
Age	3.954	1	7	1.092	0.333
Pre-N	0.271	1	21	0.075	0.787
Site	5.645	2	5	0.780	0.506
N x P	9.840	1	24	2.719	0.112

Table H-6. The 3-to-5-year foliar P response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar P, site, and the interaction of N and P. Stand was a random effect. Foliar P was log-transformed for *A. saccharum* to meet assumptions of residual normality

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum (ln)					
N	0.015	1	11	1.399	0.262
P	0.466	1	11	43.645	<0.001
Age	0.007	1	1	0.690	0.596
Pre-P	0.003	1	12	0.239	0.635
Site	0.004	2	2	0.196	0.836
N x P	0.022	1	11	2.061	0.178
B. alleghaniensis					
N	0.268	1	24	9.530	0.005
P	3.441	1	24	122.403	<0.001
Age	0.000	1	5	0.001	0.976
Pre-P	0.743	1	27	26.433	<0.001
Site	0.093	2	5	1.648	0.277
N x P	0.032	1	24	1.121	0.300
B. papyrifera					
N	0.001	1	14	0.093	0.765
P	0.986	1	14	71.352	<0.001
Pre-P	0.171	1	17	12.355	0.003
Site	0.074	2	3	2.672	0.200
N x P	0.040	1	14	2.670	0.124
F. grandifolia					
N	0.001	1	28	0.163	0.690
P	0.553	1	27	82.938	<0.001
Age	0.003	1	7	0.387	0.555
Pre-P	0.049	1	30	7.316	0.011
Site	0.053	2	7	3.958	0.074
N x P	0.012	1	27	1.869	0.183

Table H-7. The 10-to-11-year foliar P response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar P, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *B. alleghaniensis* model to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.461	1	15	7.422	0.016
P	2.662	1	16	43.014	<0.001
Age	0.000	1	3	0.008	0.934
Pre-P	0.221	1	9	3.562	0.092
Site	Excluded				
N x P	0.257	1	15	4.142	0.059
B. alleghaniensis					
N	0.258	1	26	5.163	0.032
P	6.429	1	26	128.807	<0.001
Age	0.000	1	8	0.006	0.981
Pre-P	0.183	1	33	3.666	0.064
Site	Excluded				
N x P	0.225	1	26	4.509	0.044
B. papyrifera					
N	0.048	1	14	2.903	0.110
P	3.653	1	14	220.863	<0.001
Pre-P	0.102	1	16	6.187	0.024
Site	0.053	2	3	1.610	0.326
N x P	0.004	1	14	0.227	0.641
F. grandifolia					
N	0.176	1	24	6.453	0.018
P	2.176	1	23	79.849	<0.001
Age	0.047	1	5	1.718	0.244
Pre-P	0.148	1	25	5.446	0.028
Site	0.154	2	5	2.827	0.151
N x P	0.000	1	23	0.001	0.982

Table H-8. The 3-to-5-year foliar Ca response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar Ca, site, and the interaction of N and P. Stand was a random effect. Foliar Ca was log-transformed for *B. alleghaniensis* and *B. papyrifera* to meet assumptions of residual normality

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	3.086	1	11	4.857	0.050
P	0.472	1	11	0.743	0.407
Age	1.953	1	3	3.073	0.175
Pre-Ca	4.060	1	12	6.390	0.027
Site	Excluded				
N x P	0.540	1	11	0.850	0.377
B. alleghaniensis (ln)					
N	0.279	1	25	6.411	0.018
P	0.002	1	25	0.049	0.826
Age	0.030	1	6	0.692	0.439
Pre-Ca	0.650	1	21	14.952	0.001
Site	Excluded				
N x P	0.009	1	24	0.197	0.661
B. papyrifera (ln)					
N	0.051	1	16	2.348	0.145
P	0.094	1	15	4.348	0.054
Pre-Ca	0.216	1	12	10.024	0.008
Site	0.060	2	3	1.399	0.365
N x P	0.001	1	15	0.030	0.864
F. grandifolia					
N	1.554	1	26	4.494	0.044
P	0.130	1	26	0.375	0.546
Age	0.252	1	7	0.729	0.420
Pre-Ca	4.877	1	29	14.107	<0.001
Site	2.316	2	6	3.349	0.104
N x P	0.417	1	26	1.205	0.282

Table H-9. The 10-to-11-year foliar Ca response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar Ca, site, and the interaction of N and P. Stand was a random effect. The models for *B. alleghaniensis* and *B. papyrifera* have random effects equal to zero and should be interpreted with caution.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	9.8595	1	14	11.219	0.004
P	1.412	1	14	1.651	0.220
Age	0.624	1	2	0.730	0.495
Pre-Ca	29.008	1	16	33.919	<0.001
Site	2.115	2	2	1.237	0.447
N x P	0.180	1	14	0.210	0.654
B. alleghaniensis					
N	31.331	1	31	13.369	0.001
P	1.980	1	31	0.845	0.365
Age	1.800	1	31	0.768	0.388
Pre-Ca	11.058	1	31	4.718	0.038
Site	9.073	2	31	1.936	0.161
N x P	0.679	1	31	0.290	0.594
B. papyrifera					
N	6.451	1	17	4.001	0.062
P	17.127	1	17	10.624	0.005
Pre-Ca	17.200	1	17	10.669	0.005
Site	0.398	2	17	0.124	0.884
N x P	0.388	1	17	0.241	0.630
F. grandifolia					
N	4.010	1	23	5.362	0.026
P	4.892	1	23	6.858	0.015
Age	0.250	1	6	0.351	0.574
Pre-Ca	5.099	1	27	7.148	0.013
Site	2.147	2	6	1.505	0.301
N x P	0.390	1	23	0.547	0.467

Table H-10. The 3-to-5-year foliar K response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar K, site, and the interaction of N and P. Stand was a random effect. The *F. grandifolia* model was repeated without stand JBO to observe the effects of outliers on the results; these model results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	2.012	1	11	8.352	0.015
P	0.495	1	11	2.053	0.179
Age	0.085	1	3	0.351	0.595
Pre-K	0.316	1	12	1.311	0.274
Site	Excluded	2			
N x P	0.002	1	11	0.007	0.937
B. alleghaniensis					
N	1.993	1	25	0.765	0.390
P	4.927	1	24	1.891	0.182
Age	4.142	1	5	1.590	0.264
Pre-K	120.264	1	28	46.160	<0.001
Site	8.612	2	6	1.653	0.267
N x P	4.816	1	25	1.849	0.186
B. papyrifera					
N	1.853	1	14	1.282	0.276
P	0.352	1	14	0.244	0.629
Pre-K	12.502	1	17	8.649	0.009
Site	1.520	2	3	0.526	0.634
N x P	0.698	1	14	0.075	0.786
F. grandifolia					
N	0.116 (0.842)	1	25 (23)	0.124 (1.347)	0.728 (0.258)
P	0.351 (1.204)	1	25 (23)	0.373 (1.926)	0.547 (0.179)
Age	0.781 (0.031)	1	5 (6)	0.829 (0.049)	0.406 (0.832)
Pre-K	5.034 (8.128)	1	18 (18)	5.341 (13.002)	0.033 (0.002)
Site	3.966 (Excluded)	2	5 (NA)	2.104 (NA)	0.225 (NA)
N x P	2.644 (0.785)	1	25 (23)	2.805	0.106 (0.274)

Table H-11. The 10-to-11-year foliar K response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar K, site, and the interaction of N and P. Stand was a random effect. The model for *B. alleghaniensis* has random effects equal to zero and should be interpreted with caution. Site was removed from the *F. grandifolia* model to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	3.441	1	15	6.618	0.022
P	0.029	1	15	0.056	0.816
Age	1.173	1	2	2.257	0.267
Pre-K	0.319	1	13	0.613	0.448
Site	4.228	2	2	4.066	0.197
N x P	1.696	1	14	3.261	0.092
B. alleghaniensis					
N	6.644	1	31	2.344	0.136
P	1.943	1	31	0.685	0.414
Age	0.056	1	31	0.020	0.889
Pre-K	14.632	1	31	5.161	0.030
Site	0.226	2	31	0.080	0.923
N x P	1.701	1	31	0.600	0.444
B. papyrifera					
N	0.392	1	14	0.498	0.491
P	0.442	1	14	0.561	0.466
Pre-K	10.454	1	15	13.255	0.002
Site	Excluded				
N x P	0.698	1	14	0.885	0.362
F. grandifolia					
N	11.478	1	25	6.530	0.017
P	0.218	1	25	0.124	0.728
Age	2.437	1	7	1.387	0.278
Pre-K	11.742	1	19	6.681	0.018
Site	Excluded				
N x P	0.739	1	24	0.421	0.523

Table H-12. The 3-to-5-year foliar Mg response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar Mg, site, and the interaction of N and P. Stand was a random effect. Foliar Mg was log-transformed for *B. alleghaniensis* to meet assumptions of residual normality.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.008	1	11	0.196	0.666
P	0.002	1	11	0.050	0.827
Age	0.101	1	3	2.579	0.209
Pre-Mg	0.038	1	13	0.965	0.344
Site	Excluded				
N x P	0.010	1	11	0.260	0.620
B. alleghaniensis (ln)					
N	0.188	1	25	5.420	0.028
P	0.000	1	26	0.003	0.961
Age	0.002	1	4	0.068	0.806
Pre-Mg	0.432	1	12	12.463	0.004
Site	0.137	2	4	1.979	0.255
N x P	0.015	1	25	0.441	0.513
B. papyrifera					
N	0.036	1	15	1.418	0.252
P	0.022	1	16	0.848	0.371
Pre-Mg	0.304	1	14	11.795	0.004
Site	0.004	2	3	0.068	0.935
N x P	0.064	1	15	2.488	0.136
F. grandifolia					
N	0.003	1	26	0.170	0.684
P	0.003	1	26	0.189	0.668
Age	0.006	1	6	0.398	0.553
Pre-Mg	0.048	1	29	3.268	0.081
Site	0.071	2	6	2.397	0.176
N x P	0.000	1	27	0.000	0.994

Table H-13. The 10-to-11-year foliar Mg response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar Mg, site, and the interaction of N and P. Stand was a random effect. The model for *B. papyrifera* has random effects equal to zero and should be interpreted with caution. Site was removed from the *B. alleghaniensis* model to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.002	1	14	0.073	0.791
P	0.024	1	14	0.866	0.368
Age	0.052	1	1	1.883	0.358
Pre-Mg	0.332	1	14	12.010	0.004
Site	0.138	2	2	2.507	0.285
N x P	0.010	1	14	0.359	0.559
B. alleghaniensis					
N	0.295	1	25	3.613	0.069
P	0.001	1	26	0.011	0.919
Age	0.001	1	7	0.010	0.923
Pre-Mg	0.864	1	23	10.561	0.003
Site	Excluded				
N x P	0.002	1	25	0.021	0.887
B. papyrifera					
N	0.004	1	17	0.059	0.811
P	0.202	1	17	3.178	0.092
Pre-Mg	0.391	1	17	6.165	0.024
Site	0.067	2	17	0.530	0.598
N x P	0.023	1	17	0.355	0.559
F. grandifolia					
N	0.018	1	23	0.382	0.543
P	0.071	1	23	1.508	0.232
Age	0.003	1	4	0.056	0.825
Pre-Mg	0.005	1	24	0.122	0.730
Site	0.708	2	5	7.489	0.037
N x P	0.739	1	23	0.421	0.047

Table H-14. The 3-to-5-year litter N response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter N, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	20.975	1	9	11.886	0.008
P	2.310	1	8	1.309	0.287
Pre-N	0.349	1	1	0.198	0.791
Site	6.630	2	2	1.879	0.347
N x P	0.096	1	8	0.054	0.822
B. alleghaniensis					
N	13.381	1	25	16.618	<0.001
P	12.969	1	24	16.107	<0.001
Age	1.761	1	6	2.187	0.190
Pre-N	0.998	1	30	1.239	0.275
Site	2.480	2	7	1.540	0.280
N x P	0.940	1	25	1.168	0.290
B. papyrifera					
N	6.261	1	14	4.964	0.043
P	9.319	1	14	7.387	0.017
Pre-N	0.106	1	15	0.053	0.949
Site	0.134	2	4	0.084	0.776
N x P	1.242	1	14	0.985	0.338
F. grandifolia					
N	2.381	1	23	1.288	0.268
P	9.619	1	23	5.203	0.032
Age	0.333	1	5	0.180	0.689
Pre-N	1.792	1	27	0.969	0.334
Site	0.382	2	5	0.103	0.904
N x P	0.424	1	23	0.229	0.637

Table H-15. The 10-to-11-year litter N response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter N, site, and the interaction of N and P. Stand was a random effect. Litter N was log-transformed for *A. saccharum* and *B. alleghaniensis* to meet assumptions of residual normality.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum (ln)					
N	0.318	1	14	14.675	0.002
P	0.097	1	14	4.484	0.052
Age	0.034	1	5	1.556	0.267
Pre-N	0.058	1	18	2.676	0.119
Site	Excluded				
N x P	0.008	1	14	0.373	0.551
B. alleghaniensis (ln)					
N	0.352	1	25	23.702	<0.001
P	0.276	1	25	18.497	<0.001
Age	0.027	1	6	1.794	0.229
Pre-N	0.000	1	30	0.024	0.877
Site	0.053	2	7	1.790	0.236
N x P	0.075	1	25	5.051	0.034
B. papyrifera					
N	13.406	1	14	20.730	<0.001
P	23.345	1	14	36.100	<0.001
Pre-N	5.012	1	17	7.751	0.013
Site	0.856	2	4	0.662	0.568
N x P	3.088	1	14	4.774	0.050
F. grandifolia					
N	13.304	1	23	18.719	<0.001
P	3.110	1	23	4.377	0.048
Age	0.967	1	5	1.361	0.298
Pre-N	0.002	1	26	0.003	0.957
Site	1.870	2	5	1.315	0.350
N x P	0.056	1	23	0.078	0.782

Table H-16. The 3-to-5-year litter P response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter P, site, and the interaction of N and P. Stand was a random effect. Litter P was log-transformed for *B. alleghaniensis* to meet assumptions of residual normality.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.029	1	8	0.230	0.645
P	0.126	1	8	0.998	0.346
Age					
Pre-P	0.221	1	9	1.750	0.219
Site	0.107	2	2	0.423	0.703
N x P	0.153	1	8	1.211	0.303
B. alleghaniensis (ln)					
N	0.454	1	20	3.082	0.094
P	4.741	1	20	32.149	<0.001
Age	0.053	1	5	0.359	0.577
Pre-P	0.020	1	30	0.134	0.717
Site	Excluded				
N x P	0.151	1	22	1.021	0.323
B. papyrifera					
N	0.356	1	16	11.154	0.004
P	0.811	1	15	25.427	<0.001
Pre-P	0.092	1	11	0.912	0.360
Site	Excluded				
N x P	0.111	1	15	3.464	0.083
F. grandifolia					
N	0.112	1	23	5.496	0.028
P	0.247	1	24	12.094	0.002
Age	0.013	1	5	0.630	0.464
Pre-P	0.015	1	27	0.754	0.393
Site	0.036	2	5	0.891	0.466
N x P	0.103	1	25	5.053	0.034

Table H-17. The 10-to-11-year litter P response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter P, site, and the interaction of N and P. Stand was a random effect. Litter P was log-transformed for *F. grandifolia* to meet assumptions of residual normality. The model for *B. papyrifera* has random effects equal to zero and should be interpreted with caution. Site was removed from the *B. alleghaniensis* model to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	1.813	1	13	12.043	0.004
P	7.252	1	15	48.184	<0.001
Age	0.001	1	1	0.006	0.953
Pre-P	0.008	1	6	0.053	0.824
Site	0.488	2	2	1.622	0.381
N x P	1.656	1	13	11.001	0.006
B. alleghaniensis					
N	4.010	1	25	32.220	<0.001
P	28.585	1	25	229.654	<0.001
Age	0.475	1	8	3.817	0.087
Pre-P	0.031	1	31	0.248	0.622
Site	Excluded				
N x P	3.177	1	26	25.524	<0.001
B. papyrifera					
N	0.603	1	17	6.345	0.022
P	8.817	1	17	92.795	<0.001
Pre-P	0.271	1	17	2.848	0.110
Site	0.000	2	17	0.001	0.999
N x P	0.289	1	17	3.044	0.099
F. grandifolia (ln)					
N	0.378	1	28	1.943	0.174
P	22.909	1	28	117.648	<0.001
Age	0.121	1	28	0.623	0.437
Pre-P	0.593	1	28	3.046	0.092
Site	0.042	2	28	0.108	0.898
N x P	0.154	1	28	0.792	0.381

Table H-18. The 3-to-5-year litter Ca response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter Ca, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.705	1	9	0.264	0.619
P	2.059	1	9	0.772	0.402
Pre-Ca	13.701	1	9	5.138	0.050
Site	3.018	2	9	0.566	0.587
N x P	0.570	1	9	0.214	0.655
B. alleghaniensis					
N	47.960	1	25	27.401	<0.001
P	2.157	1	25	1.233	0.277
Age	2.459	1	6	1.405	0.278
Pre-Ca	1.685	1	29	0.963	0.335
Site	8.189	2	6	2.339	0.176
N x P	0.010	1	25	0.006	0.624
B. papyrifera					
N	4.244	1	17	1.889	0.187
P	5.924	1	17	2.592	0.126
Pre-Ca	3.081	1	17	1.371	0.258
Site	13.303	2	17	2.961	0.079
N x P	5.929	1	17	2.639	0.123
F. grandifolia					
N	0.002	1	24	0.001	0.973
P	0.096	1	23	0.047	0.830
Age	1.709	1	6	0.839	0.394
Pre-Ca	2.722	1	28	1.336	0.258
Site	4.919	2	6	1.207	0.367
N x P	2.662	1	24	1.306	0.265

Table H-19. The 10-to-11-year litter Ca response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter Ca, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *B. alleghaniensis* model to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	10.624	1	14	2.829	0.114
P	0.796	1	14	0.212	0.652
Age	0.932	1	3	0.248	0.654
Pre-Ca	11.966	1	16	3.187	0.093
Site	9.634	2	4	1.283	0.377
N x P	1.284	1	15	0.342	0.568
B. alleghaniensis					
N	58.738	1	24	15.766	<0.001
P	7.879	1	25	2.115	0.158
Age	2.779	1	8	0.746	0.413
Pre-Ca	0.830	1	31	0.223	0.640
Site	Excluded				
N x P	0.918	1	24	0.246	0.624
B. papyrifera					
N	26.513	1	14	19.162	<0.001
P	10.392	1	15	7.511	0.015
Pre-Ca	0.077	1	17	0.056	0.817
Site	2.992	2	3	2.163	0.247
N x P	0.560	1	14	0.404	0.535
F. grandifolia					
N	8.058	1	23	10.849	0.003
P	6.503	1	23	8.756	0.007
Age	0.339	1	5	0.456	0.528
Pre-Ca	0.519	1	25	0.699	0.411
Site	2.059	2	5	1.386	0.332
N x P	1.157	1	23	1.558	0.225

Table H-20. The 3-to-5-year litter K response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter K, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.760	1	9	1.569	0.242
P	0.347	1	9	0.717	0.419
Age	Excluded				
Pre-K	3.330	1	9	6.880	0.028
Site	11.727	2	9	12.115	0.003
N x P	0.207	1	9	0.428	0.529
B. alleghaniensis					
N	4.103	1	23	0.840	0.369
P	0.080	1	25	0.016	0.899
Age	0.387	1	4	0.079	0.792
Pre-K	9.138	1	26	1.871	0.183
Site	72.136	2	4	7.386	0.045
N x P	6.4447	1	22	1.320	0.263
B. papyrifera					
N	0.814	1	14	0.563	0.465
P	0.000	1	14	0.000	0.997
Pre-K	10.175	1	16	7.031	0.017
Site	19.547	2	3	6.753	0.067
N x P	14.041	1	15	9.702	0.007
F. grandifolia					
N	6.974	1	23	3.417	0.077
P	7.319	1	23	3.586	0.071
Age	0.017	1	5	0.009	0.930
Pre-K	0.005	1	27	0.003	0.960
Site	16.279	2	6	3.989	0.083
N x P	0.914	1	23	0.448	0.510

Table H-21. The 10-to-11-year litter K response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter K, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *A. saccharum*, *B. papyrifera*, and *F. grandifolia* models to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.735	1	14	1.162	0.300
P	0.330	1	14	0.522	0.482
Age	1.439	1	4	2.274	0.215
Pre-K	1.289	1	17	2.038	0.172
Site	Excluded	2			
N x P	1.489	1	14	2.354	0.148
B. alleghaniensis					
N	1.994	1	25	1.607	0.217
P	0.275	1	26	0.221	0.642
Age	0.075	1	7	0.061	0.813
Pre-K	9.378	1	30	7.556	0.010
Site	7.917	2	7	3.190	0.105
N x P	0.205	1	25	0.165	0.688
B. papyrifera					
N	0.228	1	15	0.117	0.737
P	0.974	1	15	0.501	0.490
Pre-K	14.902	1	15	7.670	0.014
Site	Excluded				
N x P	5.772	1	16	2.971	0.104
F. grandifolia					
N	3.745	1	24	1.789	0.205
P	19.814	1	23	9.464	0.006
Age	9.769	1	8	4.666	0.068
Pre-K	9.977	1	16	4.765	0.035
Site	Excluded				
N x P	0.086	1	23	0.033	0.858

Table H-22. The 3-to-5-year litter Mg response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter Mg, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.020	1	9	0.507	0.494
P	0.024	1	9	0.573	0.468
Pre-Mg	0.471	1	9	11.950	0.007
Site	0.058	2	9	0.731	0.508
N x P	0.004	1	9	0.093	0.767
B. alleghaniensis					
N	1.044	1	24	12.468	0.002
P	0.028	1	24	0.339	0.566
Age	0.048	1	5	0.569	0.482
Pre-Mg	0.001	1	30	0.006	0.937
Site	0.391	2	5	2.335	0.187
N x P	0.157	1	24	1.871	0.184
B. papyrifera					
N	0.068	1	17	4.807	0.376
P	0.177	1	17	0.425	0.161
Pre-Mg	0.388	1	17	0.019	0.045
Site	0.563	2	17	3.310	0.057
N x P	0.192	1	17	1.220	0.146
F. grandifolia					
N	0.059	1	24	0.670	0.421
P	0.106	1	25	1.197	0.284
Age	0.025	1	5	0.286	0.617
Pre-Mg	0.002	1	28	0.027	0.872
Site	0.013	2	5	0.076	0.928
N x P	0.027	1	24	0.303	0.587

Table H-23. The 10-to-11-year litter Mg response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter Mg, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *A. saccharum* and *B. alleghaniensis* models to remove the singularity and prevent pseudoreplication.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.030	1	15	0.811	0.382
P	0.054	1	14	1.436	0.251
Age	0.090	1	3	2.404	0.209
Pre-Mg	0.376	1	14	10.005	0.007
Site	Excluded				
N x P	0.009	1	15	0.227	0.640
B. alleghaniensis					
N	1.740	1	25	10.862	0.003
P	0.006	1	25	0.037	0.848
Age	0.087	1	5	0.543	0.497
Pre-Mg	0.477	1	15	2.976	0.105
Site	2.487		5	7.762	0.034
N x P	0.002	1	24	0.015	0.903
B. papyrifera					
N	0.419	1	15	4.807	0.045
P	0.037	1	15	0.425	0.524
Pre-Mg	0.002	1	11	0.019	0.894
Site	0.577	2	3	3.310	0.184
N x P	0.106	1	14	1.220	0.288
F. grandifolia					
N	0.006	1	23	0.131	0.721
P	0.104	1	24	2.154	0.155
Age	0.066	1	4	1.367	0.311
Pre-Mg	0.002	1	28	0.038	0.847
Site	0.234	2	4	2.414	0.202
N x P	0.007	1	22	0.143	0.709

Table H-24. The 3-to-5-year nitrogen resorption efficiency (NRE) response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment NRE, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	74.771	1	8	2.258	0.170
P	56.680	1	9	1.712	0.223
Pre-NRE	3.376	1	1	0.102	0.792
Site	313.605	2	2	4.736	0.174
N x P	1.242	1	9	0.038	0.851
B. alleghaniensis					
N	13.942	1	22	2.732	0.112
P	35.084	1	22	6.876	0.016
Age	5.731	1	5	1.123	0.337
Pre-NRE	0.004	1	25	0.001	0.977
Site	6.548	2	5	0.642	0.562
N x P	11.517	1	22	2.257	0.147
B. papyrifera					
N	0.016	1	14	0.001	0.973
P	55.218	1	14	4.166	0.061
Pre-NRE	1.762	1	15	0.133	0.721
Site	0.915	2	3	0.035	0.966
N x P	8.820	1	14	0.665	0.428
F. grandifolia					
N	35.974	1	23	1.898	0.182
P	28.095	1	23	1.482	0.236
Age	2.307	1	5	0.122	0.742
Pre-NRE	11.416	1	26	0.602	0.445
Site	2.012	2	5	0.053	0.949
N x P	28.152	1	24	1.485	0.235

Table H-25. The 10-to-11-year nitrogen resorption efficiency (NRE) response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment NRE, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *A. saccharum* model to remove the singularity and prevent pseudoreplication. The *A. saccharum* model was repeated without stand C9 and the *B. alleghaniensis* model repeated without stand C1 to observe the effects of outliers on the results; these model results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	77.012 (14.800)	1	14 (11)	1.768 (1.138)	0.233 (0.309)
P	55.281 (0.000)	1	14 (11)	1.269 (0.000)	0.324 (0.996)
Age	59.155 (10.899)	1	4 (3)	1.358 (0.838)	0.309 (0.426)
Pre-NRE	13.142 (3.941)	1	18 (14)	0.302 (0.303)	0.547 (0.591)
Site	Excluded				
N x P	4.790 (2.751)	1	15 (11)	0.110 (0.212)	0.876 (0.654)
B. alleghaniensis					
N	131.324 (43.028)	1	25 (22)	4.364 (5.077)	0.047 (0.035)
P	84.760 (6.358)	1	24 (21)	2.816 (0.750)	0.106 (0.396)
Age	40.531 (9.915)	1	6 (5)	1.347 (1.170)	0.288 (0.331)
Pre-NRE	0.010 (1.658)	1	29 (25)	0.000 (0.196)	0.986 (0.662)
Site	26.463 (58.593)	2	7 (5)	0.440 (3.457)	0.662 (0.111)
N x P	60.064 (13.619)	1	24 (21)	1.996 (1.607)	0.171 (0.219)
B. papyrifera					
N	18.542	1	14	1.382	0.259
P	211.729	1	14	15.779	0.001
Pre-NRE	91.714	1	15	6.835	0.020
Site	24.503	2	4	0.913	0.475
N x P	27.980	1	14	2.085	0.171
F. grandifolia					
N	49.302	1	22	3.248	0.085
P	5.413	1	22	0.357	0.556
Age	7.649	1	4	0.504	0.515
Pre-NRE	2.162	1	28	0.142	0.709
Site	63.978	2	4	2.107	0.227
N x P	24.901	1	23	1.641	0.213

Table H-26. The 3-to-5-year phosphorus resorption efficiency (PRE) response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment PRE, site, and the interaction of N and P. Stand was a random effect. The *B. alleghaniensis* model was repeated without stand C2 to observe the effects of outliers on the results; these model results are shown in parentheses. The full *F. grandifolia* model could not be run using REML, so the model without stand C2, an outlier, is reported here.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	0.00	1	8	0.000	0.997
P	57.77	1	9	0.422	0.533
Pre-PRE	226.10	1	9	1.650	0.231
Site	482.65	2	2	1.761	0.362
N x P	48.60	1	8	0.355	0.568
B. alleghaniensis					
N	0.20 (26.60)	1	21 (19)	0.002 (0.376)	0.961 (0.547)
P	1051.68 (827.26)	1	20 (18)	12.763 (11.679)	0.002 (0.003)
Age	0.24 (17.66)	1	4 (4)	0.003 (0.249)	0.960 (0.645)
Pre-PRE	6.48 (0.80)	1	26 (23)	0.079 (0.011)	0.781 (0.916)
Site	Excluded				
N x P	103.50 (203.30)	1	20 (18)	1.256 (2.870)	0.276 (0.107)
B. papyrifera					
N	838.09	1	16	6.825	0.019
P	1010.76	1	14	8.231	0.012
Pre-PRE	36.19	1	16	0.295	0.595
Site	832.88	2	4	3.391	0.151
N x P	569.19	1	14	4.635	0.049
F. grandifolia					
N	435.53	1	21	6.250	0.021
P	352.57	1	21	5.060	0.036
Age	195.75	1	4	2.809	0.172
Pre-PRE	102.53	1	21	1.471	0.239
Site	255.98	2	4	1.837	0.268
N x P	698.35	1	21	10.022	0.005

Table H-27. The 10-to-11-year phosphorus resorption efficiency (PRE) response to nutrient addition, site, and stand age, analyzed with linear mixed-effects models for each of four species. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment PRE, site, and the interaction of N and P. Stand was a random effect. Site was removed from the *A. saccharum* model to remove the singularity and prevent pseudoreplication. The *F. grandifolia* model was repeated without stand C2 to observe the effects of outliers on the results; these model results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
A. saccharum					
N	1102.7	1	15	6.675	0.021
P	10993.6	1	15	66.546	<0.001
Age	368.9	1	4	2.233	0.203
Pre-PRE	101.1	1	17	0.612	0.445
Site	Excluded				
N x P	1026.5	1	15	6.214	0.025
B. alleghaniensis					
N	3298.2	1	24	21.204	<0.001
P	31218.1	1	24	200.696	<0.001
Age	516.4	1	6	3.320	0.121
Pre-PRE	109.7	1	28	0.705	0.408
Site	327.4	2	5	1.052	0.411
N x P	3074.8	1	24	19.767	<0.001
B. papyrifera					
N	981.6	1	17	8.277	0.010
P	10122.2	1	17	85.350	<0.001
Pre-PRE	479.6	1	17	4.044	0.060
Site	108.2	2	17	0.456	0.641
N x P	835.9	1	17	7.048	0.017
F. grandifolia					
N	532.2 (1051.1)	1	24 (24)	0.996 (3.239)	0.328 (0.085)
P	21878.9 (14704.3)	1	24 (24)	40.956 (45.304)	<0.001 (<0.001)
Age	934.0 (393.4)	1	5 (24)	1.749 (1.212)	0.245 (0.282)
Pre-PRE	33.1 (33.9)	1	22 (24)	0.062 (0.104)	0.806 (0.749)
Site	516.3 (274.8)	2	5 (24)	0.483 (0.423)	0.642 (0.660)
N x P	795.3 (1669.8)	1	25 (24)	1.489 (5.145)	0.234 (0.033)

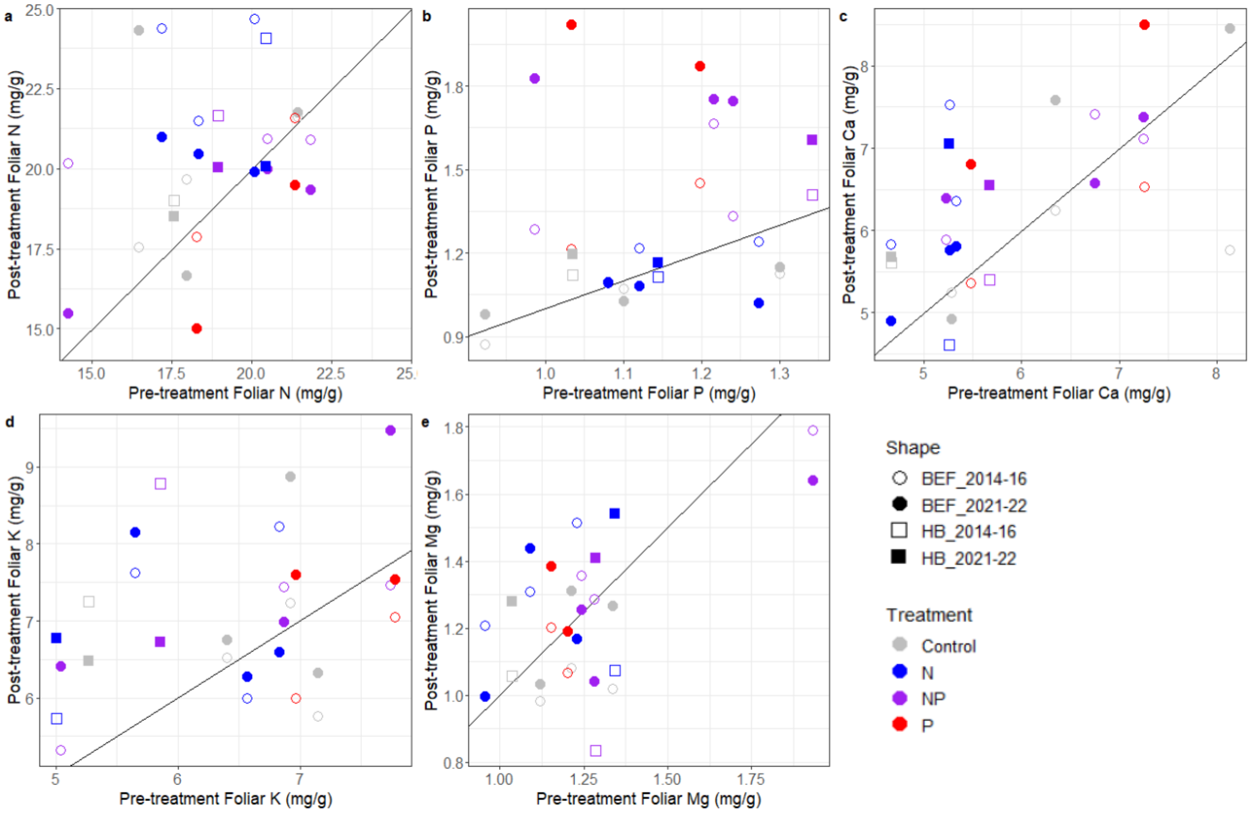

Figure H-1. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Acer rubrum* (red maple) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

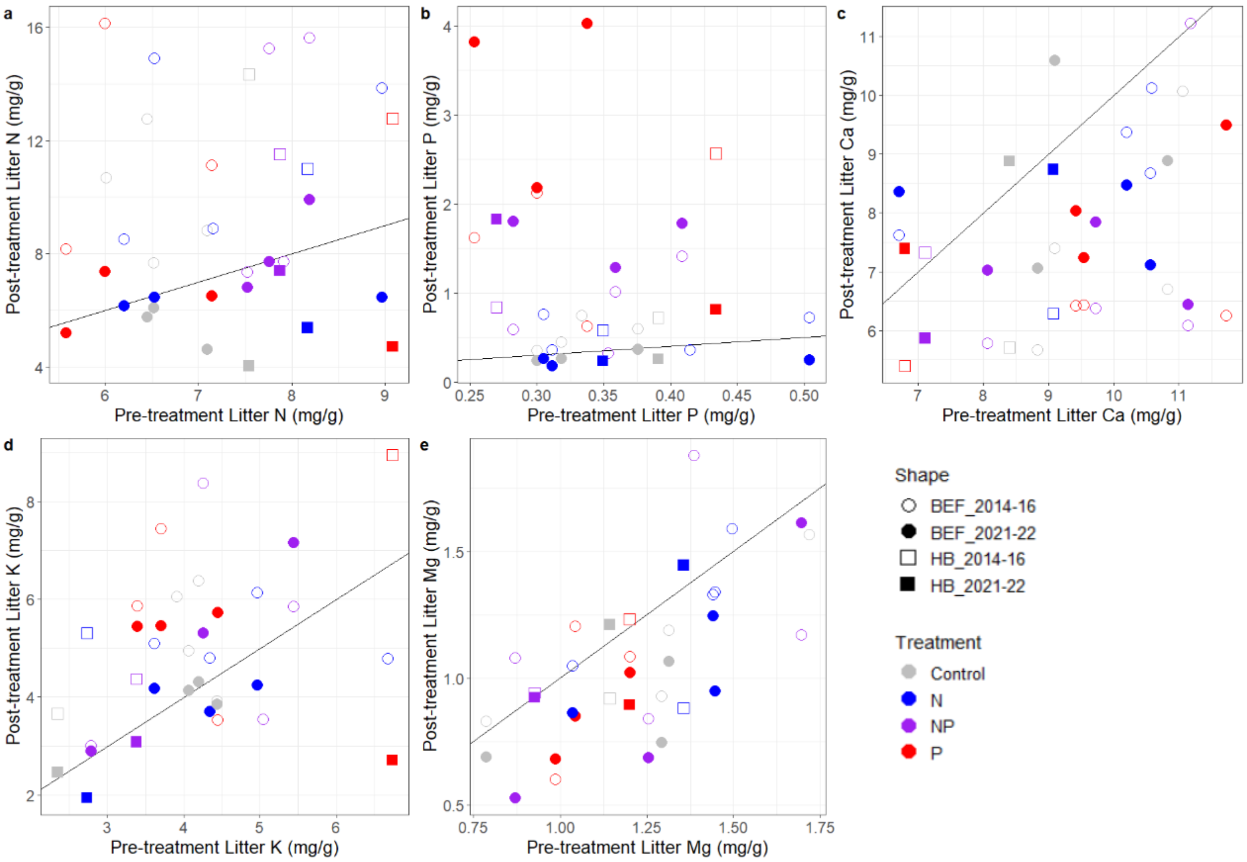

Figure H-2. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Acer rubrum* (red maple) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

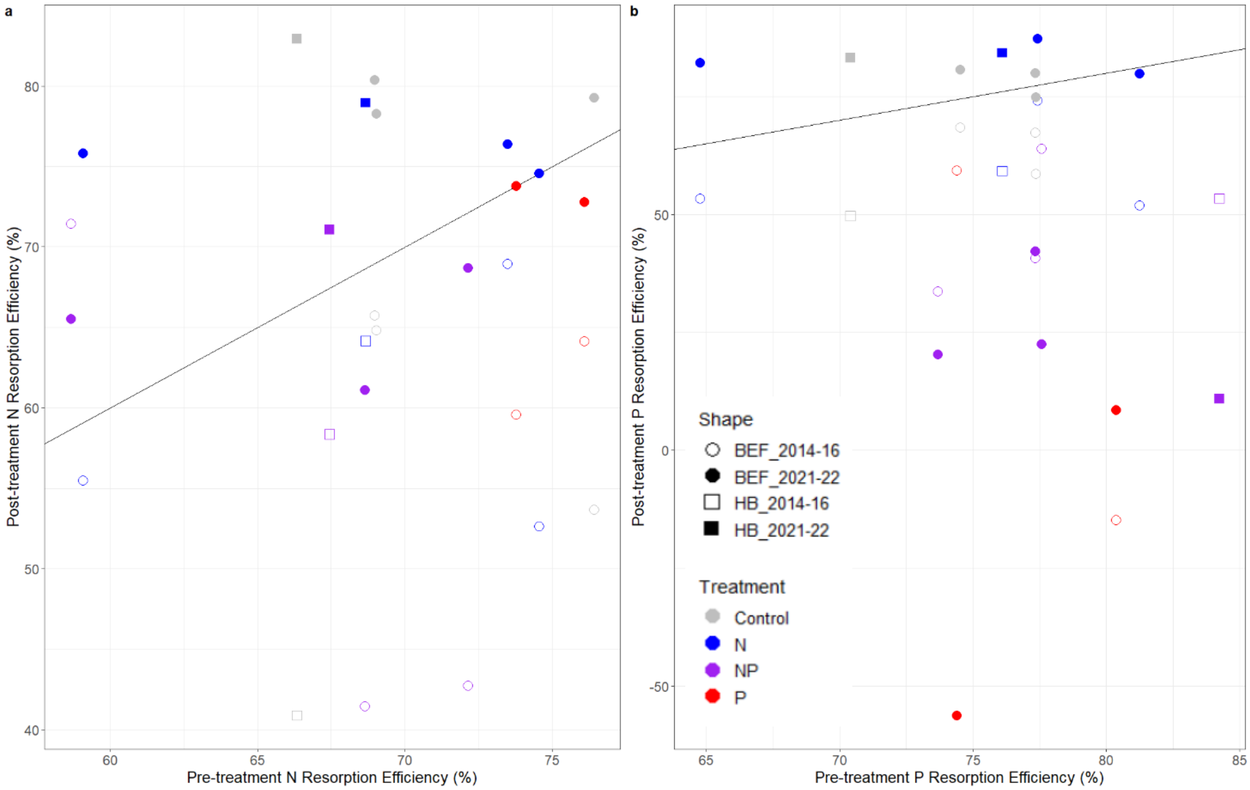

Figure H-3. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Acer rubrum* (red maple) before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-22 ("post-treatment). Each point is one plot.

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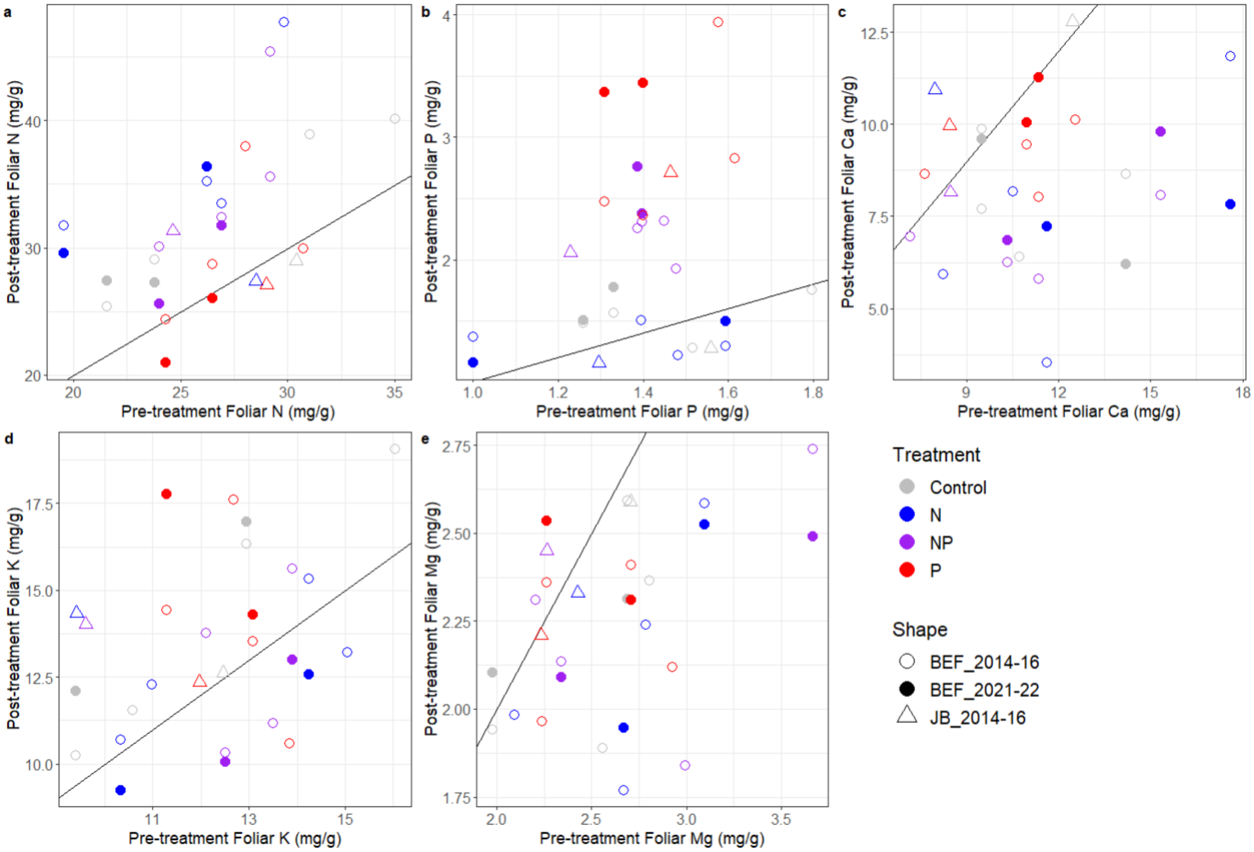

Figure H-4. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Prunus pensylvanica* (pin cherry) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

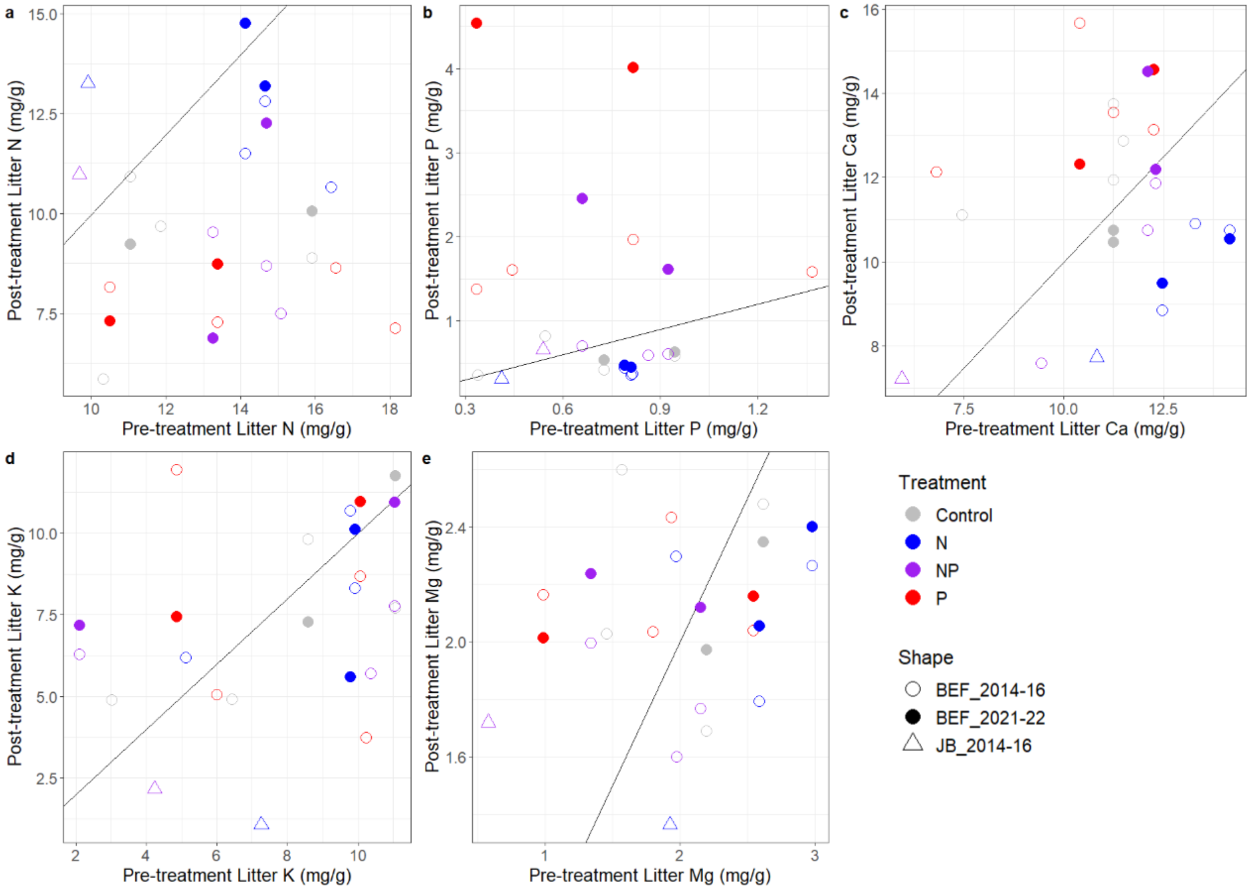

Figure H-5. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Prunus pensylvanica* (pin cherry) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

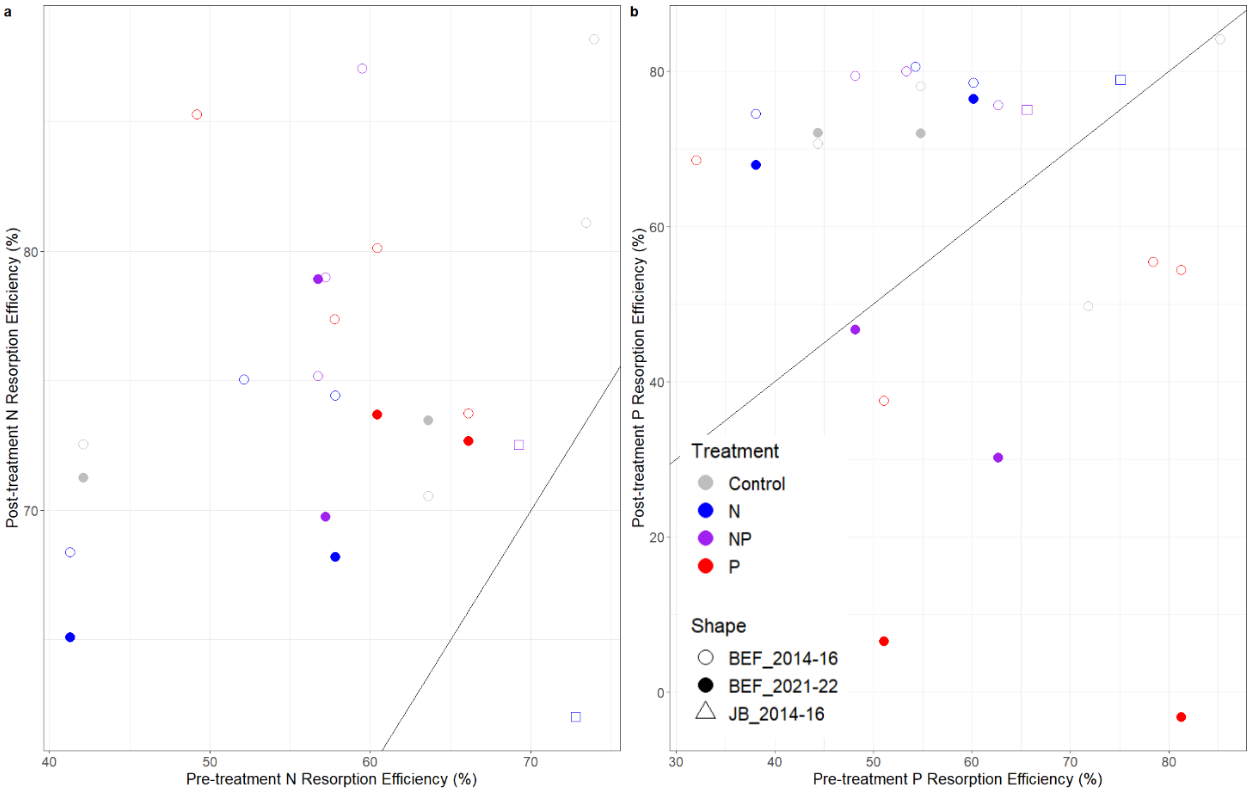

Figure H-6. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Prunus pensylvanica* (pin cherry) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

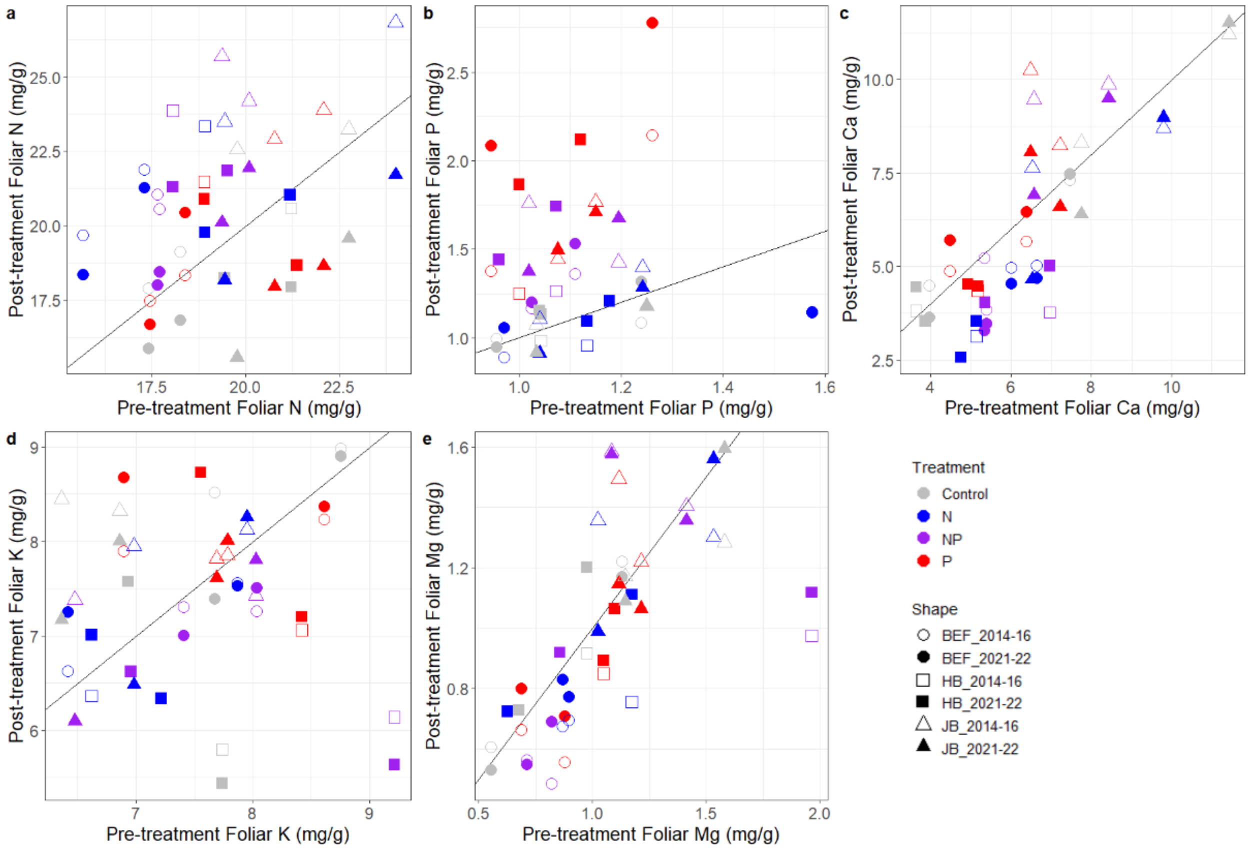

Figure H-7. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Acer saccharum* (sugar maple) before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-22 ("post-treatment). Each point is one plot.

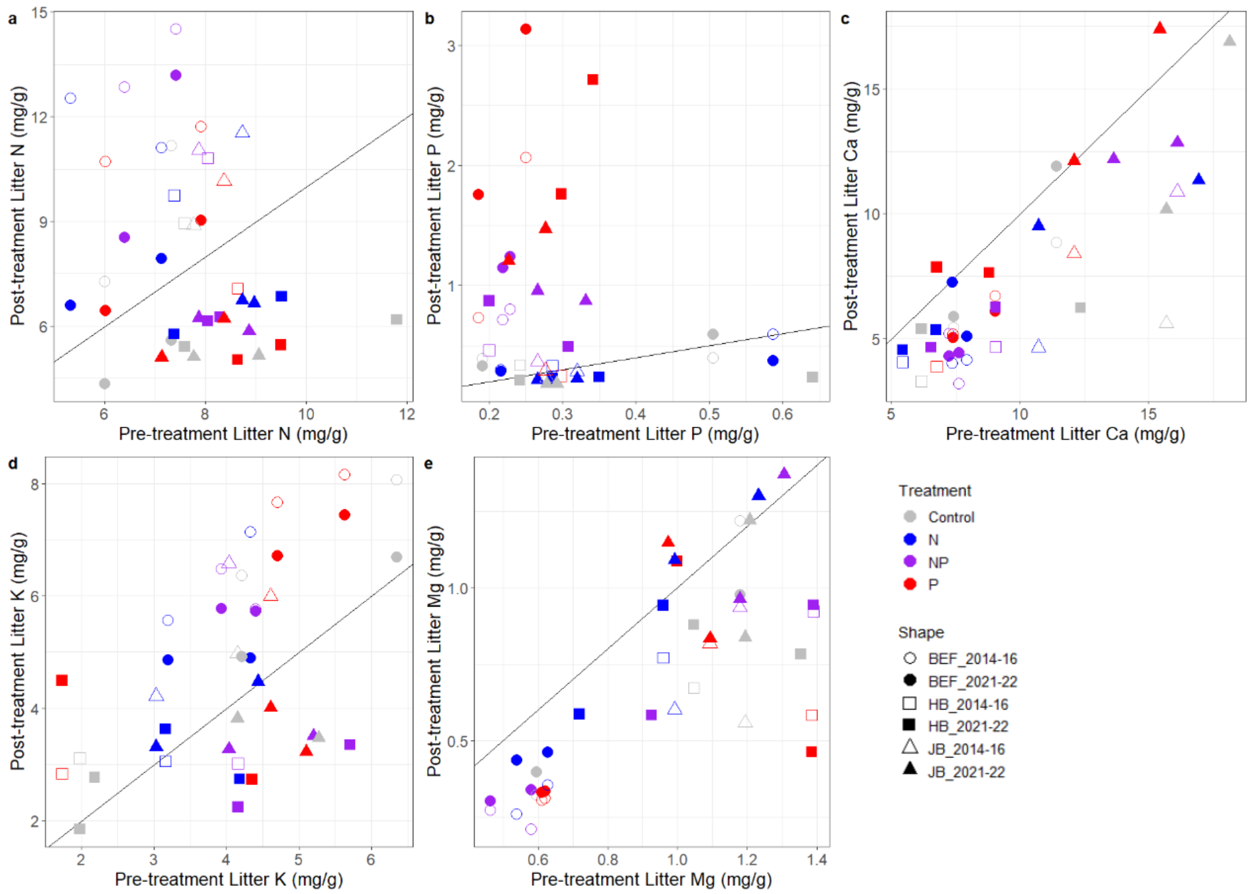

Figure H-8. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Acer saccharum* (sugar maple) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

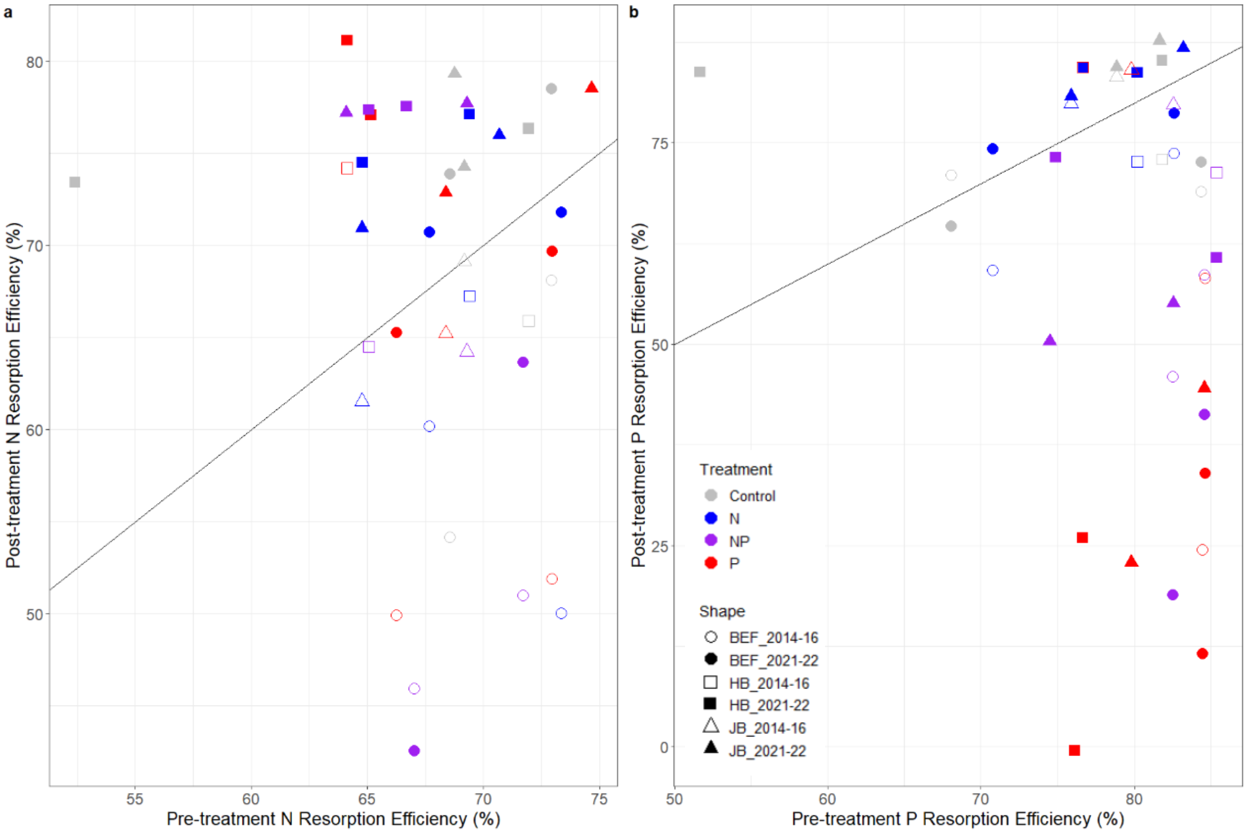

Figure H-9. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Acer saccharum* (sugar maple) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

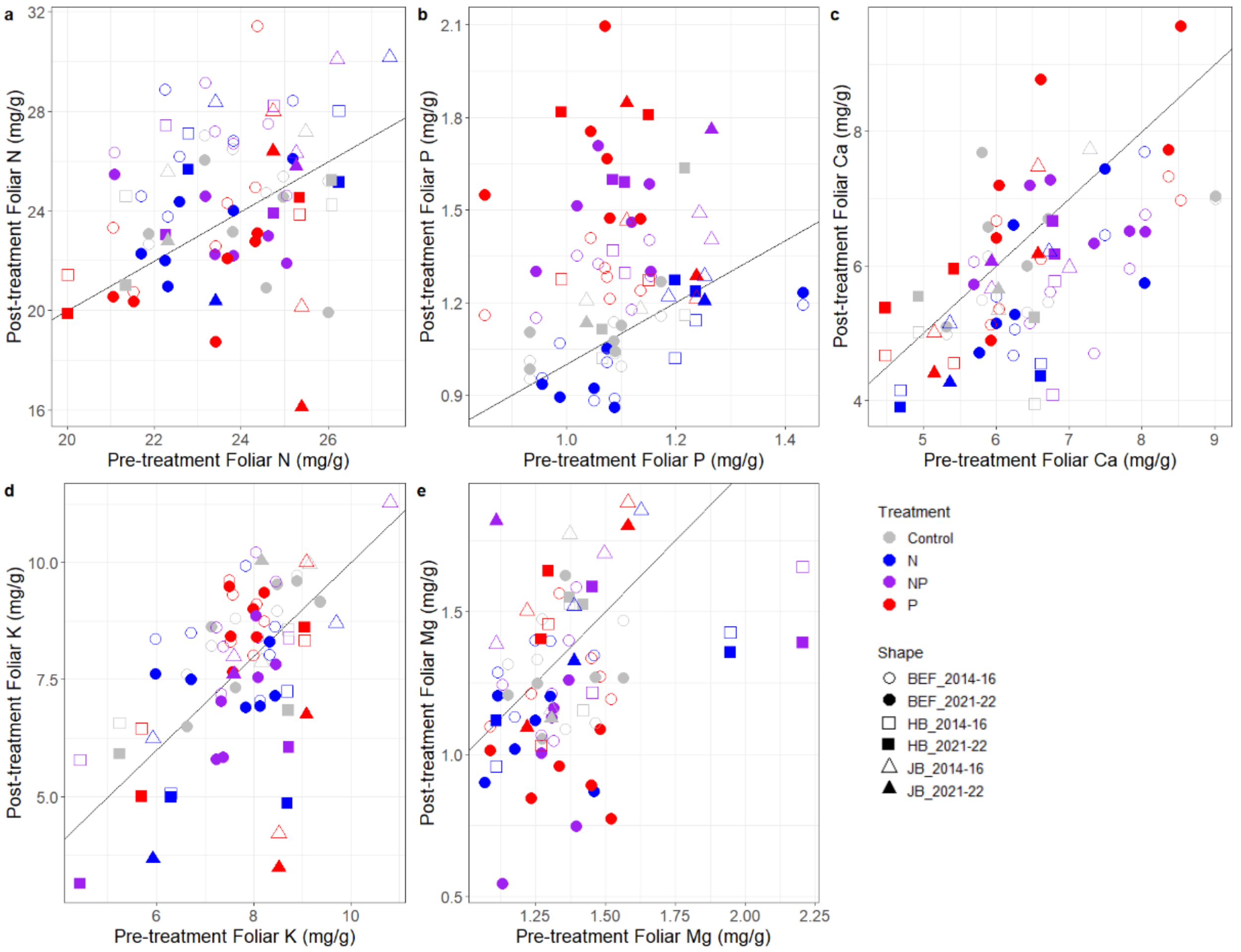

Figure H-10. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Fagus grandifolia* (American beech) before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-22 ("post-treatment). Each point is one plot.

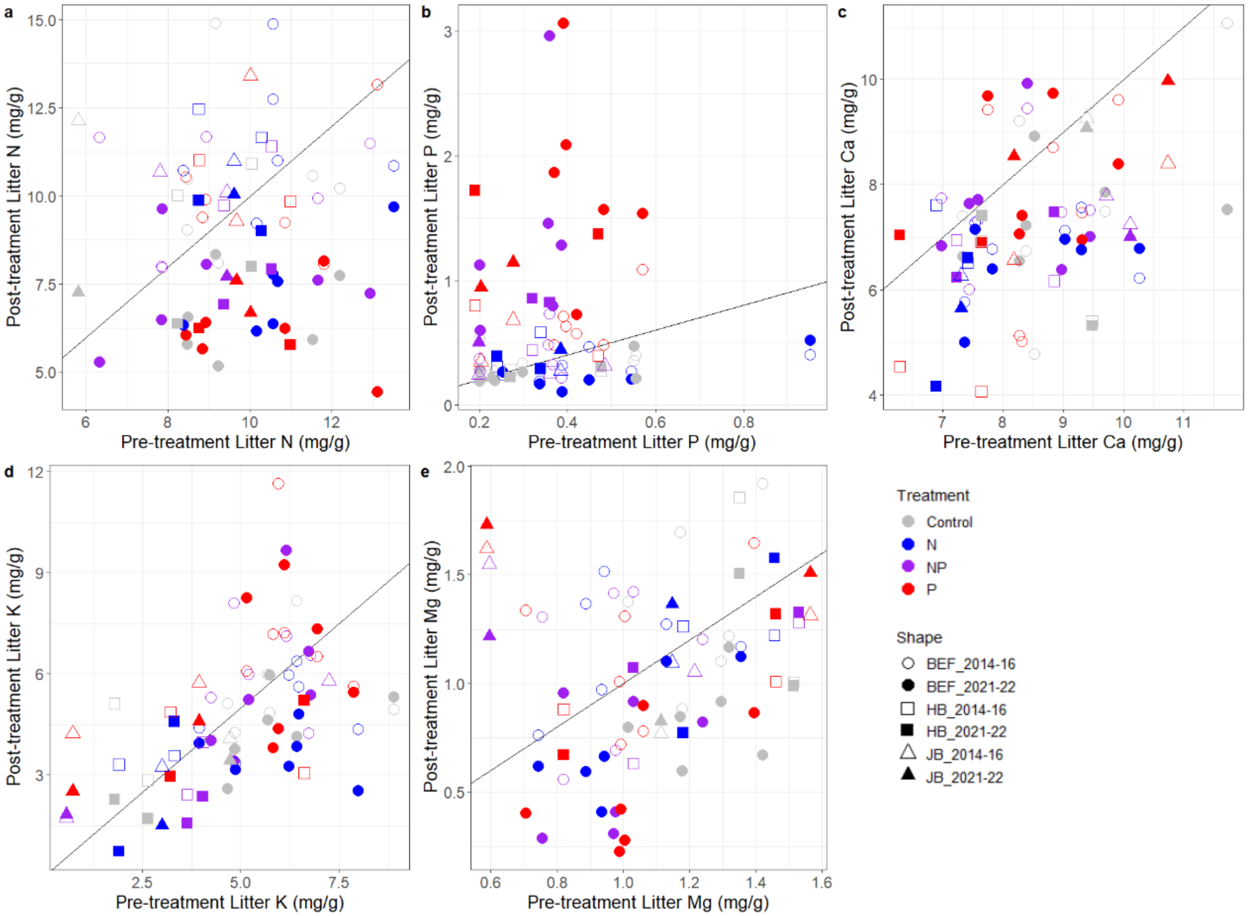

Figure H-11. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Fagus grandifolia* (American beech) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

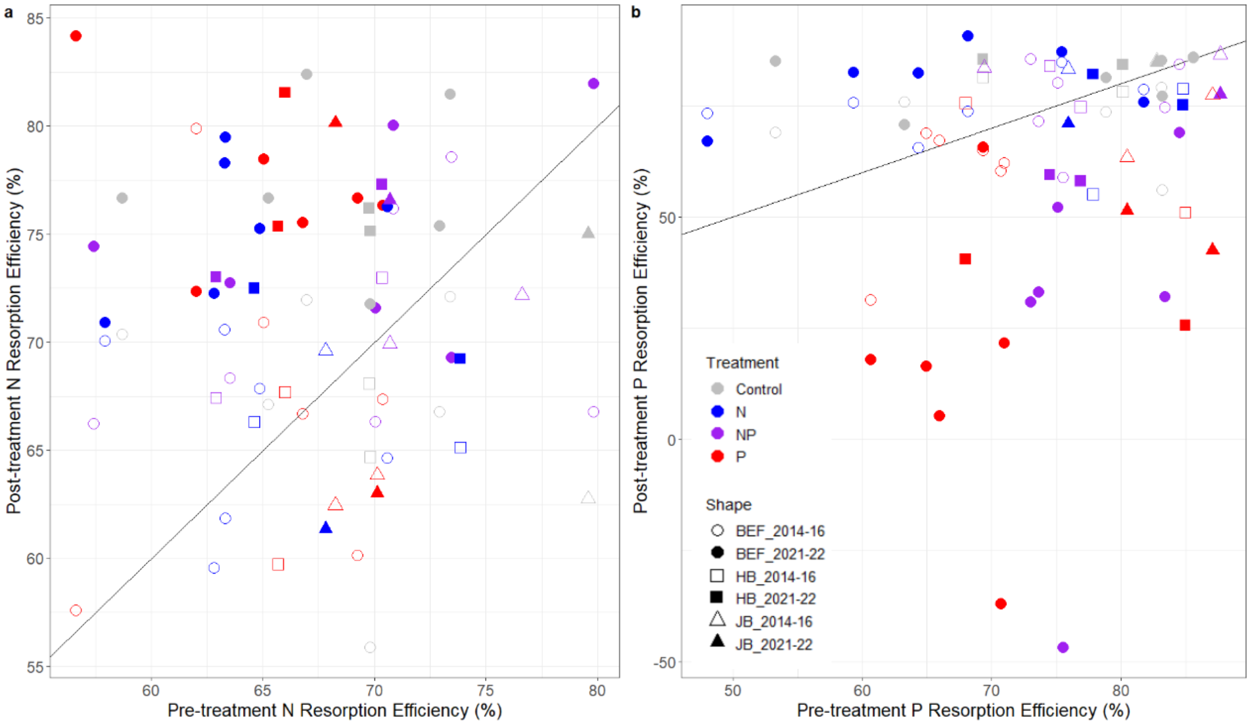

Figure H-12. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Fagus grandifolia* (American beech) before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-22 ("post-treatment). Each point is one plot.

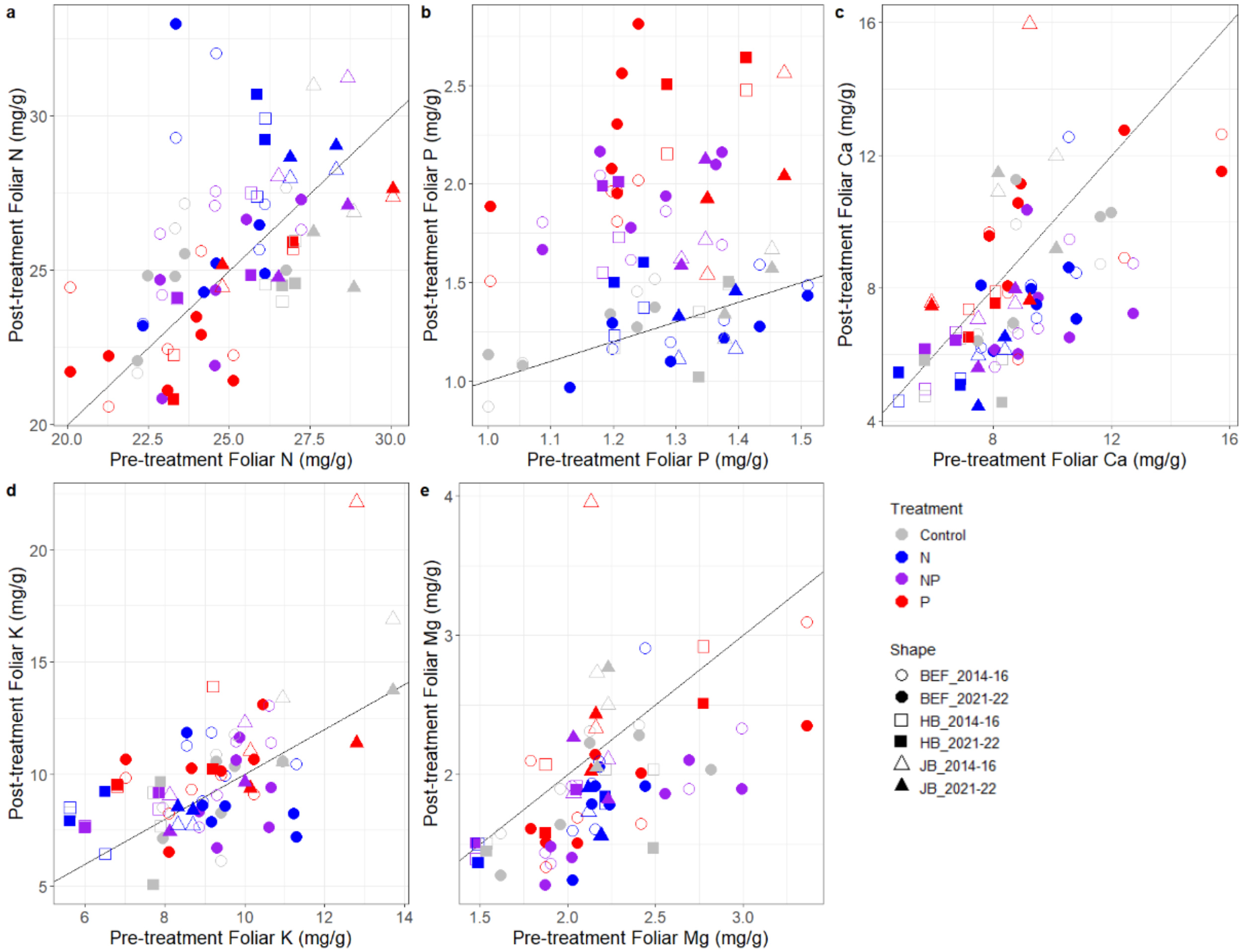

Figure H-13. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Betula alleghaniensis* (yellow birch) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment”). Each point is one plot.

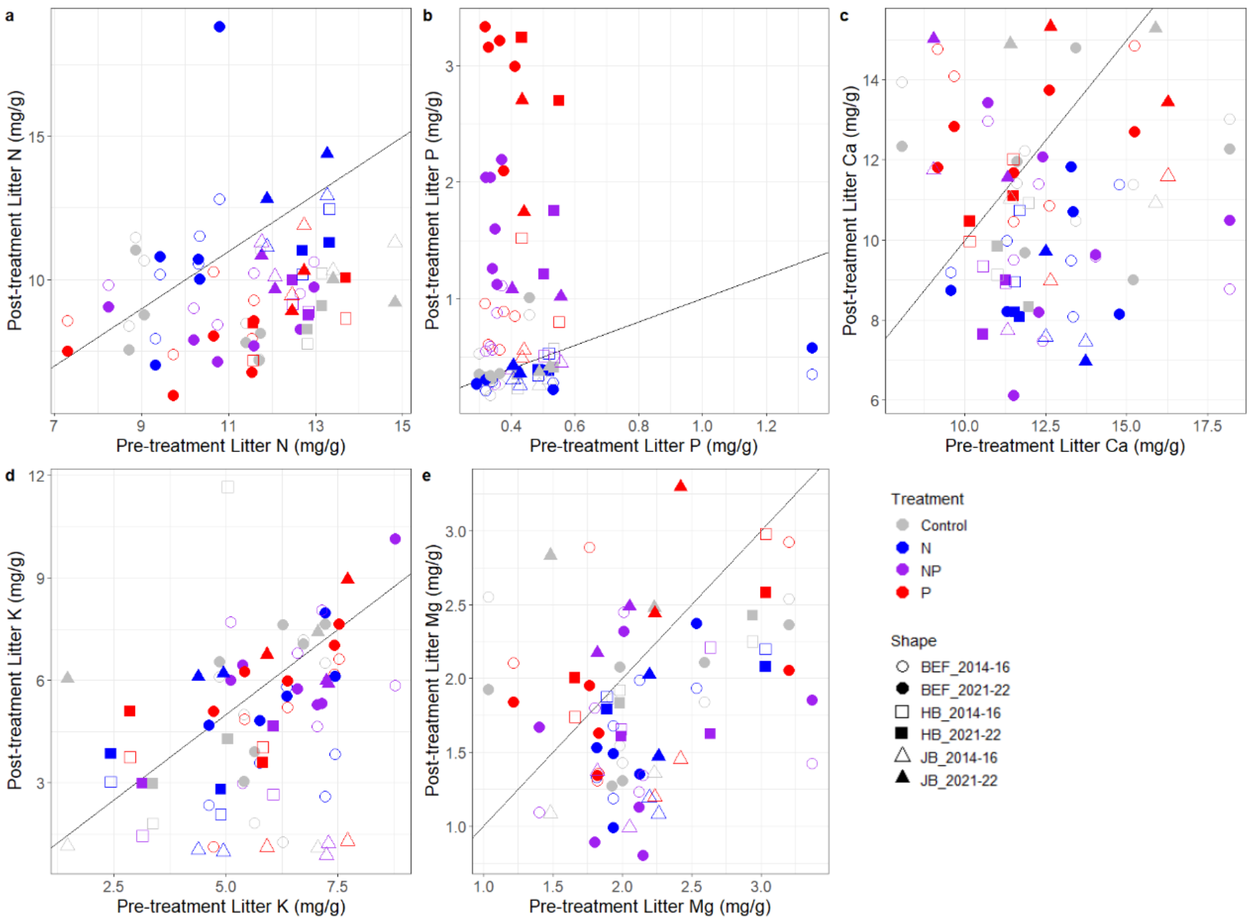

Figure H-14. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Betula alleghaniensis* (yellow birch) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

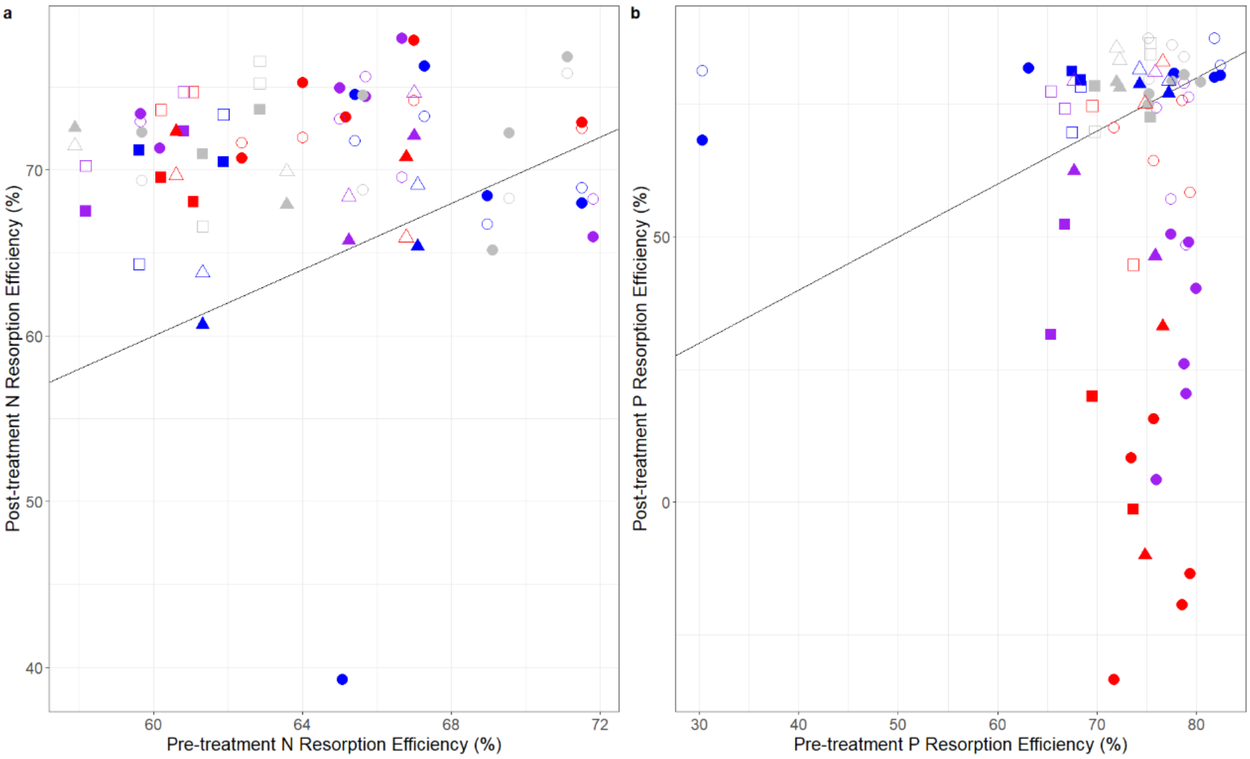

Figure H-15. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Betula alleghaniensis* (yellow birch) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

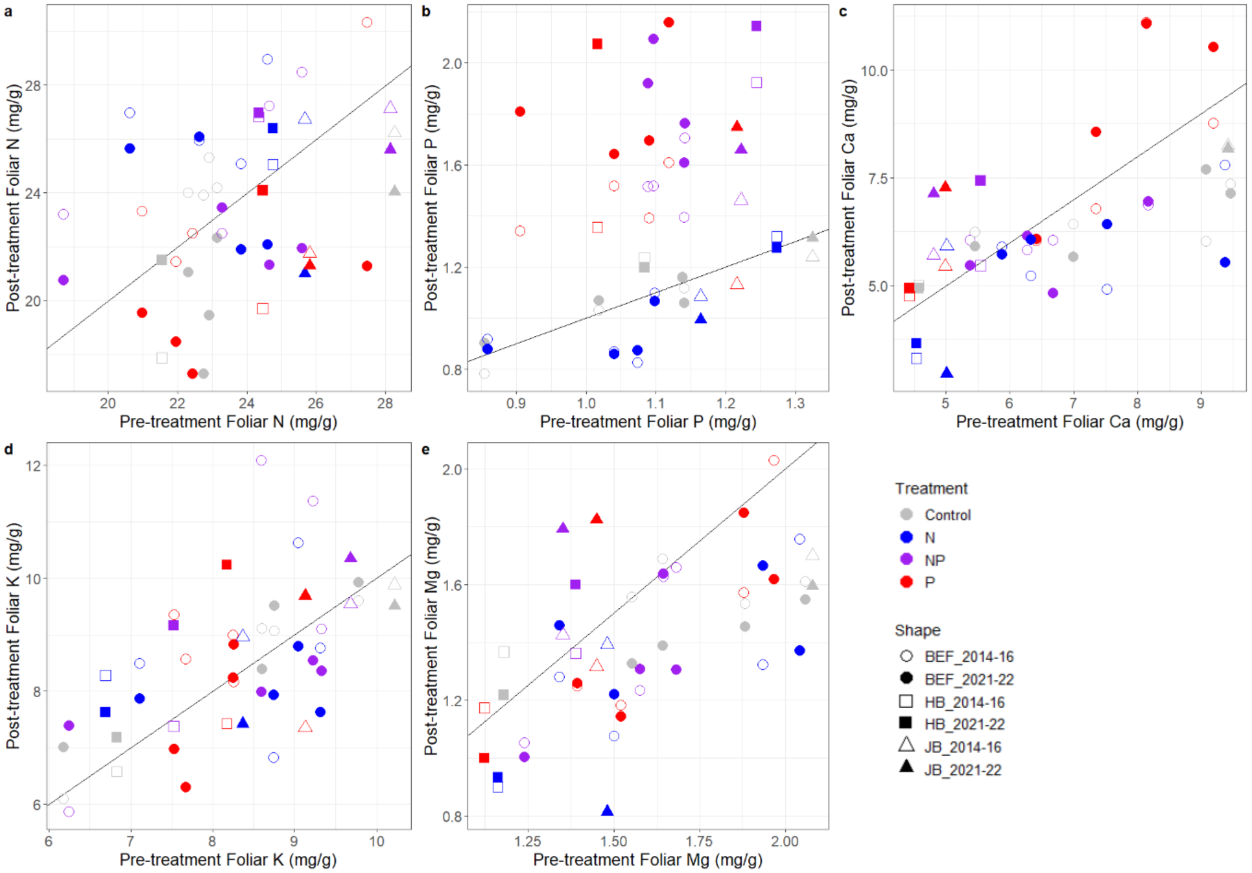

Figure H-16. Mean foliar N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Betula papyrifera* (white birch) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

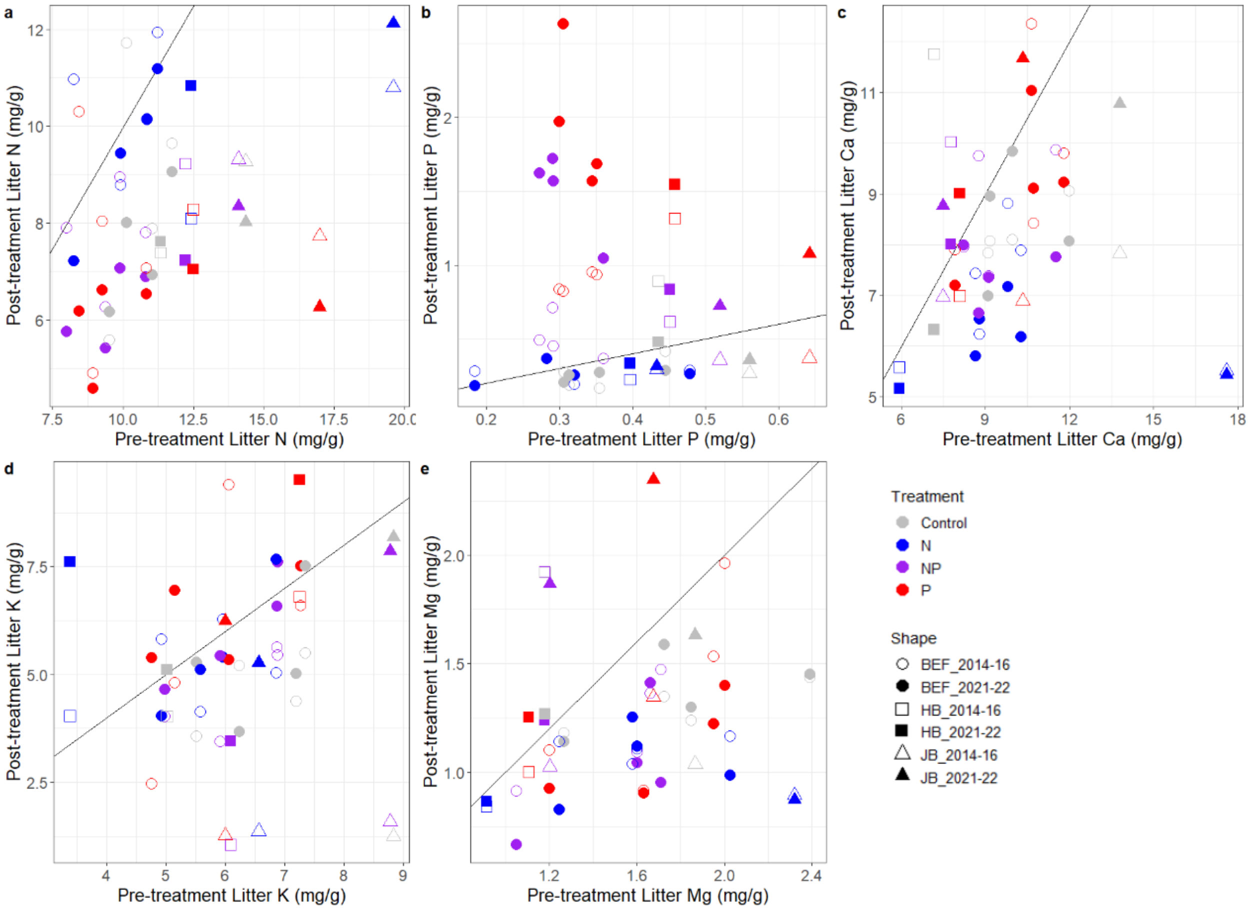

Figure H-17. Mean litter N (a), P (b), Ca (c), K (d) and Mg (e) concentrations in *Betula papyrifera* (white birch) before nutrient addition in 2008-10 (“pre-treatment”) and after in 2014-16 and 2021-22 (“post-treatment). Each point is one plot.

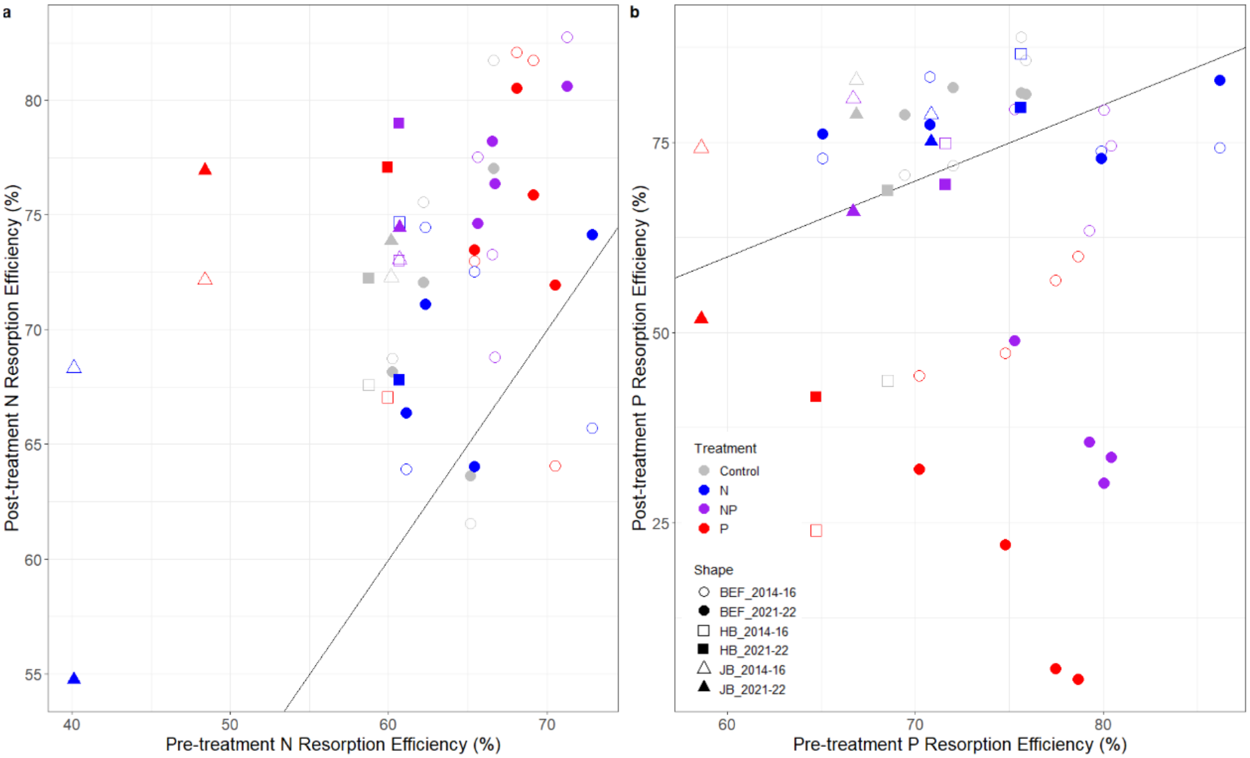

Figure H-18. Mean N resorption efficiency (a) and P resorption efficiency (b) in *Betula papyrifera* (white birch) before nutrient addition in 2008-10 ("pre-treatment") and after in 2014-16 and 2021-22 ("post-treatment). Each point is one plot.

APPENDIX I: ANALYSES OF INTERSPECIFIC AND INTRASPECIFIC COMMUNITY-WEIGHTED MEANS (CWM_{Inter} AND CWM_{Intra}; CHAPTER 4)

Table I-1. The 3-to-5-year response of interspecific community-weighted means (CWM_{Inter}) of foliar nitrogen , phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar CWM_{Inter}, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	0.005	1	27	0.042	0.838
P	0.183	1	27	1.447	0.239
Age	0.038	1	7	0.301	0.600
Pre-N	6.743	1	28	53.480	<0.001
Site	0.185	2	6	0.733	0.519
N x P	0.067	1	27	0.535	0.471
Phosphorus					
N	0.001	1	27	1.655	0.209
P	0.002	1	26	3.094	0.090
Age	0.000	1	9	0.002	0.970
Pre-P	0.037	1	30	50.736	<0.001
Site	0.002	2	7	1.215	0.351
N x P	0.000	1	27	0.373	0.546
Calcium					
N	0.015	1	28	0.966	0.334
P	0.113	1	27	7.320	0.012
Age	0.086	1	8	5.583	0.047
Pre-Ca	1.173	1	15	75.905	<0.001
Site	0.907	2	7	29.337	<0.001
NxP	0.005	1	28	0.353	0.557
Potassium					
N	0.002	1	27	0.032	0.858
P	0.162	1	27	3.228	0.084
Age	0.055	1	7	1.098	0.328
Pre-K	2.825	1	23	56.394	<0.001
Site	0.312	2	6	3.111	0.119
NxP	0.021	1	28	0.423	0.521
Magnesium					
N	0.000	1	28	0.179	0.675
P	0.05	1	27	6.206	0.019
Age	0.000	1	9	0.100	0.759
Pre-Mg	0.263	1	19	107.168	<0.001
Site	0.049	2	7	10.085	0.010
NxP	0.003	1	28	1.285	0.267

Table I-2. The 10-to-11-year response of interspecific community-weighted means ($\text{CWM}_{\text{Inter}}$) of foliar nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar $\text{CWM}_{\text{Inter}}$, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	0.004	1	27	0.034	0.855
P	0.041	1	27	0.367	0.550
Age	0.012	1	8	0.110	0.749
Pre-N	7.490	1	28	66.477	<0.001
Site	0.509	2	6	2.258	0.182
N x P	0.003	1	28	0.026	0.874
Phosphorus					
N	0.001	1	27	1.035	0.318
P	0.000	1	26	0.201	0.658
Age	0.001	1	8	0.638	0.448
Pre-P	0.025	1	31	23.469	<0.001
Site	0.001	2	7	0.261	0.778
N x P	0.000	1	27	0.155	0.697
Calcium					
N	0.000	1	27	0.000	0.999
P	0.010	1	27	0.554	0.450
Age	0.090	1	8	5.314	0.050
Pre-Ca	0.732	1	31	43.478	<0.001
Site	0101	2	7	3.047	0.114
NxP	0.002	1	28	0.111	0.729
Potassium					
N	0.015	1	27	0.303	0.587
P	0.012	1	27	0.252	0.620
Age	0.049	1	7	1.032	0.342
Pre-K	1.227	1	31	25.618	<0.001
Site	0.053	2	6	0.550	0.602
NxP	0.009	1	28	0.180	0.676
Magnesium					
N	0.001	1	27	0.506	0.483
P	0.002	1	27	0.959	0.336
Age	0.002	1	9	0.844	0.382
Pre-Mg	0.153	1	30	70.266	<0.001
Site	0.004	2	7	0.915	0.444
NxP	0.000	1	28	0.049	0.826

Table I-3. The 3-to-5-year response of intraspecific community-weighted means (CWM_{Intra}) of foliar nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects included N addition, P addition, the interaction of N and P, stand age, pre-treatment foliar CWM_{Intra}, and site. Stand was included as a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	77.033	1	26	72.617	<0.001
P	5.569	1	26	5.207	0.031
Age	0.035	1	6	0.033	0.861
Pre-N	16.552	1	32	15.477	<0.001
Site	0.981	2	7	0.459	0.650
N x P	0.221	1	26	0.207	0.653
Phosphorus					
N	0.096	1	26	9.993	0.004
P	2.266	1	26	237.259	<0.001
Age	0.002	1	5	0.159	0.706
Pre-P	0.101	1	29	10.600	0.003
Site	0.022	2	5	1.166	0.380
N x P	0.002	1	26	0.182	0.673
Calcium					
N	2.698	1	27	6.489	0.016
P	4.537	1	27	10.913	0.003
Age	0.158	1	5	0.380	0.563
Pre-Ca	13.691	1	25	32.931	<0.001
Site	6.735	2	7	8.100	0.017
NxP	0.035	1	27	0.083	0.775
Potassium					
N	0.272	1	28	0.411	0.527
P	1.734	1	27	2.617	0.117
Age	0.755	1	6	1.139	0.326
Pre-K	11.130	1	28	16.796	0.003
Site	0.939	2	8	0.708	0.522
NxP	0.206	1	27	0.312	0.581
Magnesium					
N	0.097	1	28	4.092	0.053
P	0.040	1	27	1.683	0.206
Age	0.006	1	6	0.239	0.644
Pre-Mg	1.079	1	11	45.515	<0.001
Site	0.161	2	6	3.398	0.108
NxP	0.001	1	27	0.039	0.846

Table I-4. The 10-to-11-year response of intraspecific community-weighted means (CWM_{Intra}) of foliar nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment foliar CWM_{Intra}, site, and the interaction of N and P. Stand was a random effect. The K model was re-run without HBO to remove outliers; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	63.778	1	34	25.560	<0.001
P	13.461	1	34	5.395	0.026
Age	3.954	1	34	1.585	0.217
Pre-N	11.449	1	34	4.588	0.039
Site	Excluded				
N x P	0.855	1	34	0.343	0.562
Phosphorus					
N	0.291	1	26	12.448	0.002
P	5.475	1	26	234.339	<0.001
Age	0.001	1	8	0.027	0.874
Pre-P	0.079	1	33	3.400	0.074
Site	Excluded				
N x P	0.154	1	26	6.590	0.016
Calcium					
N	7.107	1	32	9.007	0.005
P	9.622	1	32	12.195	0.001
Age	1.001	1	32	1.269	0.268
Pre-Ca	23.143	1	32	29.332	<0.001
Site	1.515	2	32	0.960	0.394
N x P	0.011	1	32	0.013	0.909
Potassium					
N	2.521 (3.658)	1	32 (28)	3.847 (7.166)	0.059 (0.012)
P	0.418 (0.156)	1	32 (28)	0.639 (0.306)	0.430 (0.585)
Age	0.049 (0.172)	1	32 (28)	0.075 (0.337)	0.787 (0.566)
Pre-K	8.761 (4.930)	1	32 (28)	13.369 (9.656)	<0.001 (0.004)
Site	0.331 (1.446)	2	32 (28)	0.252 (1.416)	0.778 (0.259)
N x P	0.064 (0.119)	1	32 (28)	0.098 (0.232)	0.756 (0.634)
Magnesium					
N	0.044	1	28	1.204	0.282
P	0.017	1	27	0.475	0.470
Age	0.024	1	5	0.657	0.451
Pre-Mg	0.924	1	10	25.349	<0.001
Site	0.298	2	6	4.087	0.081
N x P	0.003	1	27	0.082	0.777

Table I-5. The 3-to-5-year response of interspecific community-weighted means (CWM_{Inter}) of litter nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter CWM_{Inter}, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	0.021	1	25	1.787	0.193
P	0.003	1	25	0.279	0.602
Age	0.098	1	6	8.502	0.030
Pre-N	0.327	1	31	28.407	<0.001
Site	0.007	2	4	0.310	0.748
N x P	0.002	1	26	0.157	0.696
Phosphorus					
N	0.000	1	26	0.001	0.980
P	0.001	1	26	0.841	0.368
Age	0.001	1	7	1.562	0.250
Pre-P	0.001	1	29	2.791	0.106
Site	0.000	2	6	0.031	0.970
N x P	0.000	1	26	0.068	0.797
Calcium					
N	0.070	1	26	0.962	0.336
P	0.028	1	26	0.379	0.544
Age	1.163	1	6	15.966	0.007
Pre-Ca	2.991	1	29	41.054	<0.001
Site	0.233	2	7	1.596	0.267
N x P	0.118	1	26	1.621	0.214
Potassium					
N	0.010	1	27	0.419	0.523
P	0.016	1	27	0.681	0.416
Age	0.027	1	9	1.164	0.310
Pre-K	0.091	1	26	3.933	0.058
Site	0.461	2	6	9.978	0.014
N x P	0.000	1	28	0.015	0.903
Magnesium					
N	0.000	1	27	0.016	0.901
P	0.004	1	26	1.422	0.244
Age	0.007	1	8	2.370	0.162
Pre-Mg	0.155	1	31	53.464	<0.001
Site	0.005	2	7	0.880	0.458
N x P	0.010	1	27	3.456	0.074

Table I-6. The 10-to-11-year response of interspecific community-weighted means (CWM_{Inter}) of litter nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter CWM_{Inter}, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	0.000	1	27	0.003	0.956
P	0.037	1	27	1.061	0.312
Age	0.003	1	7	0.010	0.762
Pre-N	1.685	1	21	47.768	<0.001
Site	0.399	2	6	5.652	0.044
N x P	0.002	1	28	0.050	0.825
Phosphorus					
N	0.000	1	27	0.063	0.803
P	0.001	1	26	0.579	0.454
Age	0.004	1	9	1.669	0.227
Pre-P	0.022	1	32	9.639	0.004
Site	0.020	2	6	4.305	0.071
N x P	0.000	1	27	0.186	0.670
Calcium					
N	0.014	1	26	0.489	0.490
P	0.018	1	26	0.613	0.441
Age	0.001	1	6	0.051	0.829
Pre-Ca	2.298	1	31	79.619	<0.001
Site	0.010	2	7	0.179	0.840
N x P	0.002	1	26	0.052	0.821
Potassium					
N	0.056	1	27	1.847	0.185
P	0.011	1	27	0.363	0.552
Age	0.033	1	8	1.086	0.326
Pre-K	1.324	1	28	43.479	<0.001
Site	0.012	2	6	0.190	0.832
N x P	0.000	1	27	0.000	0.985
Magnesium					
N	0.009	1	27	4.203	0.050
P	0.006	1	26	2.829	0.104
Age	0.001	1	9	0.713	0.421
Pre-Mg	0.300	1	32	143.690	<0.001
Site	0.000	2	7	0.047	0.954
N x P	0.002	1	27	0.888	0.354

Table I-7. The 3-to-5-year response of intraspecific community-weighted means (CWM_{Intra}) of litter nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter CWM_{Intra}, site, and the interaction of N and P. Stand was a random effect. The model for K was repeated without HBM, an outlier; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	18.002	1	24	23.094	<0.001
P	3.159	1	24	4.052	0.055
Age	0.031	1	5	0.040	0.850
Pre-N	4.561	1	32	5.852	0.021
Site	1.878	2	5	1.205	0.374
N x P	2.678	1	24	3.436	0.076
Phosphorus					
N	0.458	1	25	11.701	0.002
P	1.619	1	26	41.314	<0.001
Age	0.001	1	7	0.021	0.888
Pre-P	0.022	1	33	0.555	0.461
Site	Excluded				
N x P	0.418	1	25	10.664	0.003
Calcium					
N	4.268	1	28	3.985	0.056
P	4.451	1	28	4.156	0.051
Age	3.360	1	6	3.138	0.127
Pre-Ca	9.410	1	27	8.788	0.006
Site	7.001	2	7	3.269	0.100
N x P	0.633	1	27	0.591	0.449
Potassium					
N	3.336 (0.036)	1	24 (24)	2.088 (0.086)	0.160 (0.772)
P	0.408 (2.781)	1	23 (23)	0.256 (6.649)	0.617 (0.017)
Age	1.589 (1.589)	1	5 (5)	0.994 (3.800)	0.357 (0.111)
Pre-K	4.943 (7.637)	1	28 (28)	3.094 (18.256)	0.091 (<0.001)
Site	17.058 (5.639)	2	6 (6)	5.338 (6.741)	0.043 (0.032)
N x P	0.517 (2.444)	1	23 (24)	0.324 (5.843)	0.574 (0.024)
Magnesium					
N	0.103	1	28	4.095	0.053
P	0.049	1	30	1.954	0.172
Age	0.019	1	6	0.737	0.426
Pre-Mg	0.433	1	13	17.223	0.001
Site	0.237	2	6	4.715	0.062
N x P	0.034	1	27	1.344	0.257

Table I-8. The 10-to-11-year response of intraspecific community-weighted means (CWM_{Intra}) of litter nitrogen, phosphorus, calcium, potassium, and magnesium in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment litter CWM_{Intra}, site, and the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
Nitrogen					
N	26.030	1	26	24.403	<0.001
P	2.574	1	26	2.413	0.132
Age	0.666	1	6	0.624	0.458
Pre-N	16.539	1	24	15.506	<0.000
Site	5.433	2	7	2.547	0.149
N x P	2.562	1	26	2.402	0.133
Phosphorus					
N	2.501	1	26	25.203	<0.001
P	21.630	1	27	217.966	<0.001
Age	0.026	1	6	0.265	0.627
Pre-P	0.038	1	21	0.378	0.545
Site	0.456	2	6	2.298	0.187
N x P	2.027	1	27	20.426	<0.001
Calcium					
N	13.524	1	27	9.221	0.005
P	13.436	1	27	9.161	0.005
Age	0.489	1	6	0.334	0.583
Pre-Ca	34.137	1	18	23.274	<0.001
Site	Excluded				
N x P	0.156	1	26	0.107	0.747
Potassium					
N	0.125	1	27	0.202	0.656
P	6.573	1	26	10.620	0.003
Age	0.005	1	7	0.007	0.934
Pre-K	11.207	1	19	18.109	<0.001
Site	Excluded				
N x P	1.212	1	26	1.958	0.174
Magnesium					
N	0.191	1	28	3.963	0.056
P	0.004	1	30	0.077	0.784
Age	0.028	1	6	0.572	0.481
Pre-Mg	0.260	1	13	5.378	0.037
Site	0.593	2	6	6.149	0.038
N x P	0.000	1	27	0.001	0.983

Table I-9. The 3-to-5-year response of N resorption efficiency (NRE) and P resorption efficiency (PRE) interspecific and intraspecific community-weighted means (CWM_{Inter} and CWM_{Intra}) in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment resorption efficiency CWM_{Inter} or CWM_{Intra}, site, the interaction of N and P. Stand was a random effect.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
NRE CWM_{Inter}					
N	0.003	1	27	0.039	0.844
P	0.309	1	27	4.704	0.039
Age	0.009	1	8	0.140	0.718
Pre-NRE	1.238	1	20	18.823	<0.001
Site	3.124	2	6	23.749	0.002
N x P	0.006	1	28	0.096	0.759
PRE CWM_{Inter}					
N	0.571	1	26	0.993	0.328
P	1.374	1	26	2.390	0.134
Age	1.570	1	8	2.730	0.140
Pre-PRE	2.465	1	30	4.286	0.047
Site	6.726	2	6	5.847	0.039
N x P	0.142	1	26	0.247	0.623
NRE CWM_{Intra}					
N	5.014	1	26	0.418	0.524
P	0.477	1	26	0.040	0.844
Age	34.477	1	6	2.874	0.142
Pre-NRE	16.067	1	28	1.339	0.257
Site	29.525	2	6	1.230	0.355
N x P	27.470	1	26	2.290	0.142
PRE CWM_{Intra}					
N	725.37	1	26	10.468	0.003
P	1662.58	1	27	23.993	<0.001
Age	1.67	1	8	0.024	0.881
Pre-PRE	48.13	1	34	0.695	0.410
Site	Excluded				
N x P	831.42	1	26	11.999	0.002

Table I-10. The 10-to-11-year response of N resorption efficiency (NRE) and P resorption efficiency (PRE) interspecific and intraspecific community-weighted means (CWM$_{\text{Inter}}$ and CWM$_{\text{Intra}}$) in trees to nutrient addition, site, and stand age, analyzed using linear mixed-effects modeling. Sums of squares (SS) and numerator (Num) and denominator (Den) degrees of freedom are displayed. Fixed effects were N addition, P addition, stand age, pre-treatment resorption efficiency CWM$_{\text{Inter}}$ or CWM$_{\text{Intra}}$, site, and the interaction of N and P. Stand was a random effect. The NRE CWM$_{\text{Intra}}$ model was rerun without C9 and the PRE CWM$_{\text{Intra}}$ model was rerun without C2; these results are shown in parentheses.

Fixed Effect	**SS**	**Num. DF**	**Den. DF**	***F***	***p***
NRE CWM$_{\mathit{Inter}}$					
N	0.000	1	27	0.000	0.992
P	0.081	1	27	0.843	0.367
Age	0.000	1	8	0.003	0.957
Pre-NRE	2.022	1	15	21.116	<0.001
Site	4.005	2	5	20.920	0.003
N x P	0.060	1	28	0.623	0.437
PRE CWM$_{\mathit{Inter}}$					
N	1.297	1	27	1.574	0.221
P	0.507	1	26	0.615	0.440
Age	1.280	1	8	1.553	0.247
Pre-PRE	2.410	1	32	2.924	0.097
Site	8.310	2	6	5.041	0.052
N x P	0.108	1	26	0.131	0.720
NRE CWM$_{\mathit{Intra}}$					
N	64.195 (36.577)	1	24 (22)	4.640 (4.523)	0.042 (0.045)
P	0.128 (6.987)	1	24 (22)	0.009 (0.864)	0.924 (0.363)
Age	22.910 (0.190)	1	4 (5)	1.655 (0.024)	0.267 (0.884)
Pre-NRE	145.764 (46.907)	1	32 (27)	10.529 (5.800)	0.003 (0.012)
Site	75.205 (27.488)	2	5 (5)	2.716 (1.699)	0.163 (0.269)
N x P	11.320 (27.631)	1	24 (22)	0.818 (3.417)	0.375 (0.078)
PRE CWM$_{\mathit{Intra}}$					
N	2004.0 (2105.3)	1	26 (28)	14.400 (28.996)	<0.001 (<0.001)
P	26879.6 (20540.0)	1	27 (28)	193.151 (282.895)	<0.001 (<0.001)
Age	74.7 (8.3)	1	5 (28)	0.537 (0.113)	0.495 (0.738)
Pre-PRE	61.4 (74.0)	1	21 (28)	0.442 (1.019)	0.514 (0.321)
Site	764.6 (490.7)	2	5 (28)	2.747 (3.379)	0.151 (0.048)
N x P	1683.4 (1812.8)	1	26 (28)	12.097 (24.968)	0.002 (<0.001)

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- School of Forest Resources, University of Maine, Orono, ME June - August 2019
  Research Assistant
- Penobscot Experimental Forest, University of Maine, Bradley, ME March - May 2019
  Ecologist
- Cooperative Forestry Research Unit, University of Maine, Orono, ME December 2017 - February 2019
  Research & Communications Coordinator
- The Arnold Arboretum of Harvard University, Boston, MA June 2016 - November 2017
  Living Collections Fellow
- Faculty of Forestry, The University of British Columbia, Vancouver, BC September 2013 - May 2016
  M.Sc. Student and Graduate Research Assistant
- Faculty of Science, The University of British Columbia, Vancouver, BC May - August 2015
  Graduate Research Assistant, Carl Wieman Science Education Initiative
- Department of Biological Sciences, Smith College, Northampton, MA June 2012 - August 2013
  Summer Undergraduate Research Fellow and Honors Student
- Department of Botany, Smithsonian Museum of Natural History, Washington, DC June - August 2011
  Laboratory Intern

- Office of Sustainability, Smith College, Northampton, MA November 2010 - January 2012
  Energy Conservation Intern
- Smith College Botanic Garden, Northampton, MA September 2009 - May 2011
  Student Research in Departments (STRIDE) Scholar
- Hubbard Brook Experimental Forest, Plymouth State University, West Thornton, NH June - August 2010
  NSF Research Experiences for Undergraduates (REU) Student
- Department of Biological Sciences, Smith College, Northampton, MA January - April 2010
  Volunteer

PUBLICATIONS

Peer-Reviewed Manuscripts:

- Zukswert, J.M., Vadeboncoeur, M.A., & Yanai, R.D. (2023). Responses of stomatal density and carbon isotope composition of sugar maple and yellow birch foliage to N, P, and $CaSiO_3$ fertilization. *Tree Physiology*, tpad142.
- Hecking, M.J., Zukswert, J.M., Drake, J.E., Dovciak, M., & Burton, J.I. (2022). Montane temperate-boreal forests retain the leaf economic spectrum despite intraspecific variability. *Frontiers in Forests and Global Change 4*: 754063.
- Yanai, R.D., Mann, T.A, Hong, S.D, Pu, G., & Zukswert, J.M. (2021). The current state of uncertainty reporting in ecosystem studies: A systematic evaluation of peer-reviewed literature. *Ecosphere 12*(6): e03535.
- Zukswert, J.M., Hallett, R.A., Bailey, S.W. & Sonti, N.F. (2021). Using regional forest nutrition data to inform urban tree management in the northeastern United States. *Urban Forestry & Urban Greening 57*: 126917.
- Yanai, R.D., Wayson, C., Lee, D., Espejo, A.B., Campbell, J.L., Green, M.B., Zukswert, J.M., Yoffe, S.B., Aukema, J.E., Lister, A.J., Kirchner, J.W., & Garmarra, J.G.P. (2021). Improving uncertainty in forest carbon accounting for REDD+ mitigation efforts. *Environmental Research Letters 15*: 124002
- Crandall, M.S., Costanza, K.K.L, Zukswert, J.M., Kenefic, L.S., & Leahy, J.E. (2020). An adaptive and evidence-based approach to building and retaining gender diversity within a university forestry education program: A case study of SWIFT. *Journal of Forestry*, fvz072.
- Zukswert, J. M., Barker, M. K., & McDonnell, L. M. (2019). Identifying troublesome jargon in biology: Discrepancies between student performance and perceived understanding. *CBE – Life Sciences Education 18*(1). DOI: 10.1187/cbe.17-07-0118
- Zukswert, J. M. & Prescott, C. E. (2017). Relationships among leaf functional traits, litter traits, and mass loss during early phases of leaf litter decomposition in 12 woody plant species. *Oecologia* 185(2): 305-316.
- Prescott, C. E., Corrao, K., Reid, A. M., Zukswert, J. M., & Addo-Danso, S. D. (2017). Changes in mass, carbon, nitrogen and phosphorus in logs decomposing for 30 years in three Rocky Mountain coniferous forests. *Canadian Journal of Forest Research* 47(10): 1418-1423.
- Prescott, C. E. & Zukswert, J. M. (2016). Invasive plant species and litter decomposition: Time to challenge assumptions. *New Phytologist* 209(1): 5-7.
- Zukswert, J. M., Bellemare, J., Rhodes, A. L., Sweezy, T., Gallogly, M., Acevedo, S., & Taylor, R. S. (2014). Forest community structure differs, but not processes, 25 years after Eastern Hemlock removal. *Southeastern Naturalist* 13(Special Issue 6): 61-87.

Conference Abstracts, Posters, and Presentations (* = presented at conference):

- Zukswert, J.M., Fahey, T.J., Vadeboncoeur, M.A., & Yanai, R.D.* (2023). Foliar chemistry and resorption following 10 years of experimental nitrogen and phosphorus addition in New Hampshire forests. Talk, Ecological Society of America's Annual Meeting, Portland, OR.

- Zukswert, J.M.* (2023). Differences in foliar traits across forest stands of varying ages following experimental nitrogen and phosphorus addition. Talk, CNY Conference on Environmental Science and Studies, Syracuse, NY.
- Zukswert, J.M* & Yanai, R.D. (2023). Stomatal density is highly variable within leaves and among trees, but may be higher with phosphorus fertilization in sugar maple trees in New Hampshire northern hardwood forests. Poster, New England Society of American Foresters Annual Meeting, Nashua, NH.
- Zukswert, J.M.*, Hallett, R.A., Bailey, S.W., & Sonti, N.F. (2020). Forest nutrition-tree health relationships inform urban tree management strategies. Poster and talk, New York Society of American Foresters Annual Meeting, Syracuse, NY.
- Zukswert, J. M.* & Prescott, C. E. (2015). Associations between physical functional traits and leaf litter decomposition rates of 16 plant species native to British Columbia. Poster, Soil Science Society of America's Annual Meeting, Minneapolis, MN.
- Zukswert, J. M.* & Prescott, C. E. (2015). Relationships between plant functional traits and short-term leaf litter decomposition rates in 16 plant species native to British Columbia. Poster, Ecological Society of America's Annual Meeting, Baltimore, MD.
- Zukswert, J. M.* & Prescott, C. E. (2015). Exploring the role of plant functional traits and anatomy in litter decomposition. Poster, 6th annual CONFORWest meeting, San Juan Island, WA.
- Zukswert, J. M.*, Bellemare, J., & Rhodes, A. L. (2014). Forest community structure, but not processes, differs 25 years after Eastern Hemlock removal. Poster, Ecological Society of America Annual Meeting, Sacramento, CA.
- Zukswert, J. M.*, Bellemare, J., & Rhodes, A. L. (2013). Changes in nutrient cycling as a result of eastern hemlock removal. Talk, 13th Northeast Natural History Conference, Springfield, MA.
- Sweezy, T.*, Rhodes, A. L., Zukswert, J. M., & Dwyer, C. H. (2013). Soil biogeochemistry of forest succession following logging of eastern hemlock at the MacLeish Field Station, West Whately, MA. Poster, Northeastern Section of the Geological Society of America's Annual Meeting, Bretton Woods, NH.
- Zukswert, J. M.*, Rhodes, A. L, Dwyer, C. H. & Sweezy, T. (2012). Comparison of throughfall chemistry in a mature hemlock forest and an early-successional deciduous forest resulting from salvage logging in Whately, Massachusetts. Poster, American Geophysical Union's Annual Fall Meeting, San Francisco, CA.
- Rhodes, A. L.*, Sweezy, T., Zukswert, J. M. & Dwyer, C. H. (2012). Effects of forest succession on exchangeable cation concentrations and nitrogen mineralization rates in soils following logging of eastern hemlock forest, Whately, Massachusetts. Poster, American Geophysical Union's Annual Fall Meeting, San Francisco, CA.
- Erickson, D. L., Zukswert, J. M.*, Kress, W. J., Novotny, V., Weiblen, G. & Hreck, J. (2011). Molecular reconstruction of tri-trophic interactions within a plant-herbivore-parasitoid-predator food web. Poster, Ecological Society of America's Annual Meeting, Austin, TX. (Note: Not a co-author on the published abstract; joined project after abstract had been submitted.)
- Zukswert, J. M.* & Green, M. B. (2011). Exploring relationships between soil physics and root distribution in two diverse watersheds. Poster, National Conference on Undergraduate Research, Ithaca, NY.

TEACHING AND OUTREACH EXPERIENCE

- Long-Term Ecological Research (LTER) Network, Remote September 2022 - present
  Graduate Writing Fellow

- Envirobites: Environmental Research for Everyone, Remote January 2020 - April 2022
  Author and Peer Editor (Volunteer)

- SUNY College of Environmental Science and Forestry, Syracuse, NY January 2020 - December 2021
  Graduate Teaching Assistant

  - FOR 345/545: Introduction to Forest Soils (Fall 2021)
  - FOR 232: Natural Resources Ecology (Spring 2021)
  - SRE 335: Renewable Energy (Spring 2020)
  - SRE 441: Biomass Energy (Spring 2020)

- Let's Talk Science, The University of British Columbia, Vancouver, BC — October 2013 - April 2016
  Participant (Volunteer), Modules in Ecology and Evolution Development (MEED) program, 2015-16
  Author (Volunteer), CurioCity, 2014-15
  Mentor (Volunteer), Scientific Methods and Research Techniques (SMaRT) program, 2013-14

- Stanley Park Ecology Society, Vancouver, BC — June - August 2015
  Eco Ranger (Volunteer)

- The University of British Columbia, Vancouver, BC — September 2014 - December 2015
  Graduate Teaching Assistant
  - FRST 430: Advanced Biometrics
  - CONS 330: Conservation Science and Sustainability
  - BIOL 101: Introduction to Modern Biology

- Jacobson Center, Smith College, Northampton, MA — September 2012 - May 2013
  Peer Content Tutor
  - BIO 154: Biodiversity, Ecology, and Conservation

- Center for Community Collaboration, Smith College, Northampton, MA — January 2012
  - HHMI Interterm High School Science Teaching Intern

SCHOLARSHIPS, AWARDS, AND HONORS

- Edna B. Sussman Fellowship Special Merit Award, National ($2,000 USD) — 2022
- C. Eugene Farnsworth Fellowship, SUNY ESF ($10,000 USD) — 2021
- Albert L. Leaf Memorial Award, SUNY ESF — 2021
- Bob Marshall Memorial Fund Scholarship, SUNY ESF ($1,225 USD) — 2021
- Albert & Barbara Cline Silviculture Scholarship, SUNY ESF — 2020
- 3rd Place in Graduate Student Poster Contest, New York Society of American Foresters — 2020
- NSF Graduate Research Fellowship Program Honorable Mention, National — 2019
- Donald S. McPhee Fellowship, Faculty of Forestry, UBC ($1,750 CAD) — 2015-16
- VanDusen Graduate Fellowship in Forestry, Faculty of Forestry, UBC — 2015-16
- Best Poster, CONFORWest — 2015
- Mary and David Macaree Fellowship, Faculty of Forestry, UBC ($2,400 CAD) — 2014-16
- Cordula and Gunter Paetzold Fellowship, UBC ($18,000 CAD) — 2014-15
- George S. Allen Memorial Scholarship, Faculty of Forestry, UBC ($1,100 CAD) — 2014-15
- Strategic Recruitment Fellowship (SRF), Faculty of Forestry, UBC ($20,000 CAD) — 2013-15
- International Tuition Award, UBC ($8,533 CAD) — 2013-15
- R. Howard Webster Foundation Fellowship, Green College, UBC ($2,500 CAD) — 2013-14
- Amey Randall Brown Award (best essay on a botanical subject), Smith College — 2013
- Harriet R. Foote Memorial Prize (outstanding work in botany), Smith College — 2013
- Sigma Xi, Smith College Chapter — elected 2013
- Phi Beta Kappa, Zeta Chapter of Massachusetts — elected 2012
- Barry M. Goldwater Scholarship, National ($7,500 USD) — 2012
- CRC Press Award for Achievement in Introductory Chemistry, Smith College — 2010

- First Group Scholar (top 10% of class), Smith College 2010-12
- Student Research in Departments (STRIDE) Scholarship, Smith College ($60,000 USD) 2009-13

LEADERSHIP

- Supporting Women in Forestry Today (SWIFT), University of Maine, Orono, ME 2018-19
  Faculty/Staff Representative, Planning Board
- Pacific Regional Society of Soil Science (PRSSS), Vancouver, BC 2014-16
  Website and Social Media Coordinator
- Department of Biological Sciences, Smith College, Northampton, MA 2012-13
  Student Liaison
- Committee on Sustainability, Smith College, Northampton, MA 2011-12
  Student Representative

SERVICE

- Conference Abstract Peer Reviewer, Ecological Society of America 2022-23
- Academic Peer Reviewer 2017-23

  Ecological Applications, *Plant and Soil*, *Acta Oecologia*, *Biology and Fertility of Soils*, *Dendrobiology*, *Ecosphere*, *Journal of Ecology*, *New Phytologist*, *PeerJ*, *Forest Ecology and Management*
- Undergraduate Mentor, SUNY College of Environmental Science and Forestry 2019-23
  - Carley Sackett: Spring 2023, litterfall error checking and sorting
  - Sydney Weimer: Spring 2022, stomatal density measurements
  - Emelia Sargent: Summer 2021, leaf litter decomposition
  - Savannah Crowe: Fall 2019, leaf litter decomposition

LECTURES

- Zukswert, J.M. (2021). “Decomposition and Nutrient Cycling.” Guest lecture. *FOR 232: Natural Resources Ecology. SUNY ESF.*
- Zukswert, J. M. (2019, 2018). “Research and Communications Coordinator, CFRU.” Guest lecture. *SFR 222: Environmental Communication Skills. University of Maine.*
- Zukswert, J. M. (2015). “Leaves in Laundry Baskets: How Science Can Be (and is) Done Using Everyday Objects.” In ‘Addressing Myths and Stereotypes in Science.’ *Green College Member Series.*

CERTIFICATIONS

- Climate Models, Downscaling and Assessments, South Central Climate Adaptation Science Center, 2020
- First Aid, University of Maine, 2018
- Certificate of Technical Ability in Composting, Maine Compost School, 2016
- Biology Program TA Professional Development Certificate, UBC, 2015
- International Tutoring Program Certification Level 1, College Reading and Learning Association, 2013