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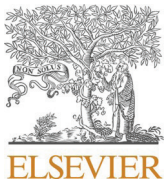

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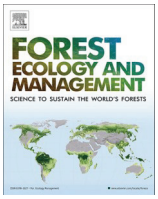

Evidence for P limitation in eight northern hardwood stands: Foliar concentrations and resorption by three tree species in a factorial N by P addition experiment

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A B S T R A C T

Nitrogen and phosphorus, alone or in combination, are the nutrients most often limiting to plants. Resorption is one way plants conserve nutrients, thereby reducing dependence on nutrient uptake from soil. We investigated foliar nutrient concentrations, ratios, and resorption in three northern hardwood species growing in eight stands across three sites and in two age classes as part of a long-term N × P factorial fertilization experiment. We found that neither P nor N addition affected N resorption, but trees in plots receiving P addition exhibited lower P resorption. Foliar N:P ratios often indicated P limitation in the control and N plots, but co-limitation by N and P in plots where P was added, alone or with N. Green leaf N and P concentrations and P resorption were highest at the site with the highest N availability and intermediate P availability. Though these stands are in a region where trees are commonly assumed to be N limited, we found numerous indications of P limitation in these stands, as well as site and species differences in resorption proficiency and efficiency.

1. Introduction

Plants possess traits that afford a competitive advantage when soil nutrients are in short supply, including those favoring nutrient acquisition (e.g., mycorrhizal associations) and conservation of acquired nutrients (e.g., low tissue turnover rates). Resorption, the process by which plants reabsorb nutrients from senescing foliage prior to abscission, is one of the most important mechanisms for nutrient conservation because it reduces plant dependency on soil nutrients (Killingbeck, 1996; Aerts and Chapin, 2000; Yuan and Chen, 2015). The ubiquity of foliar nutrient resorption attests to its adaptive value; despite the energetic costs (Wright and Westoby, 2003), a high proportion of the nutritional content of leaves is resorbed prior to abscission in plants of many phylogenies growing in a multitude of environments around the world (Aerts, 1996; Vergutz et al., 2012).

Nitrogen and phosphorus are the nutrients most often limiting to plant growth in terrestrial ecosystems, and they have received the most attention in studies of nutrient acquisition and conservation mechanisms, including resorption (Aerts and Chapin, 2000; Brant and Chen, 2015). However, the interaction between these macronutrients in the process of foliar resorption is not well understood. As a conservation mechanism, resorption should be highest in nutrient-poor sites (Vitousek, 1982; Stachurski and Zimka, 1975). Yet results from nutrient manipulation experiments (Gonzales and Yanai, 2019), observational studies along nutrient gradients (Côté et al., 2002; Rentería et al., 2005; See et al., 2015), and reviews of larger datasets have noted inconsistent associations between nutrient availability and resorption (Aerts, 1996; Killingbeck, 1996;Yuan and Chen, 2015; He et al., 2020). One possible explanation is that resorption of a specific nutrient depends upon the availability of other nutrients. If plants allocate assets so as to remain simultaneously co-limited by multiple resources (Bloom et al., 1985, Rastetter et al., 2013), then demand for P, for example, could depend not only on P availability, but also on the availability of N. Indeed, foliar P resorption increased with soil N stocks in six unmanipulated northern hardwood stands in our study system (See et al., 2015), and the three youngest stands in the study demonstrated reciprocal sensitivity of N and P resorption to P and N after four years of nutrient addition (Gonzales and Yanai, 2019).

Co-limitation may occur at scales beyond individual plants and may be influenced by factors other than nutrient availability, such as site and stand age. Although N has been assumed to limit primary production in temperate forests in the northeastern United States, the legacy of anthropogenic atmospheric N deposition in this region might be expected to induce greater P limitation (Vitousek et al., 2010). A *meta*-analysis of fertilization studies in the northern hardwood region showed greater growth responses to N than to P but the best growth was in response to multiple nutrients, indicating co-limitation (Vadeboncoeur, 2010). Limitation by P might be most likely in older forests, as N losses following forest harvest could result in greater N limitation in early stages of succession (Rastetter et al., 2013). Thus, both anthropogenic

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influences, such as N deposition and harvest, and environmental factors, such as inherent soil fertility and species composition, might be expected to affect biogeochemical cycling and nutrient limitation.

The goal of this study was to investigate nutrient limitation and nutrient conservation in three common northern hardwood species occurring in stands of different ages and on sites differing in fertility. We quantified N and P concentrations in both green leaves and leaf litter. The litter nutrient concentration is defined as resorption proficiency, such that a low litter nutrient concentration corresponds to high resorption proficiency (Killingbeck, 1996). We also calculated resorption efficiency, which is the proportion resorbed of green leaf nutrient concentrations (Aerts, 1996). Finally, we computed foliar N:P and N:P resorption, because these may indicate ecosystem nutrient limitation and the relative availability of N and P (Koerselman and Meuleman, 1996; Güsewell, 2004; Reed et al., 2012). We expected that all three species would show greater foliar N under N addition and greater foliar P under P addition, and also that additions of one nutrient would exacerbate limitation by the other nutrient as indicated by foliar concentrations and resorption proficiency and efficiency. Additionally, we hypothesized that successional forests would show signs of limitation by N and mature forests would be more limited by P. Finally, we expected that site-level differences in nutrient availability would affect foliar concentrations and resorption.

2. Methods

2.1. Site description

In three forested sites in the White Mountains of central New Hampshire, experimental plots were established in 13 stands as part of a study of Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE; Fisk et al., 2014); eight of these stands were sampled for the present study (Table 1). Each of the sites includes successional forests and mature forests, all regenerated naturally following forest harvest. Of the eight stands sampled for this study, four were mid-successional following clearcut harvest in the 1970s and 1980s and were 30–44 years old at the time of sampling in 2015; these stands are "third growth," having been intensively harvested twice in the past. The other four stands were mature, second-growth, and > 100 years old in 2015. Two stands of each age class were located in Bartlett Experimental Forest (BEF) and one stand of each age class was located at each of Hubbard Brook Experimental Forest (HB) and Jeffers Brook (JB).

The sites were chosen to provide a broader range of soil fertility than is usually achieved in field experiments of this kind. Specifically, the soils at JB are influenced by base-rich amphibolite, a metamorphosed basalt, whereas base-poor metamorphic and igneous rocks dominate at HB and BEF. Resin-available N measured in untreated (control plot) surface soils (Fisk et al., 2022) at JB (12.8 ± 2.5 μg d^{-1}; n = 2 stands; Table 1) was more than double that measured at BEF (3.12 ± 0.82 μg d^{-1}; n = 4 stands) or HB (5.21 ± 2.4 μg d^{-1}; n = 2 stands; $p < 0.001$ for the main effect of site in ANOVA). Resin-available P (Fisk et al., 2022) ranged from 0.17 ± 0.05 μg d^{-1} at HB to 0.80 ± 0.69 μg d^{-1} at JB, but did not differ significantly among sites or stands ($p \geq 0.31$; Table 1). Pretreatment potential nitrification, net N mineralization, and exchangeable Ca also were highest at JB (Bae et al., 2015).

The species composition of the eight stands reflects a typical successional sequence of northern hardwoods. A mixture of red maple (*Acer rubrum* L.), sugar maple (*A. saccharum* Marsh.), white birch (*Betula papyrifera* Marsh.), yellow birch (*B. alleghaniensis* Britt.), pin cherry (*Prunus pennsylvanica* L.f.), and beech (*Fagus grandifolia* Ehrh.) in the successional stands transitions to a forest dominated by beech, sugar maple, and yellow birch in the mature stands. Soils in our study sites are well drained Spodosols (Typic Haplorthods) formed in glacial drift. The climate is humid continental. Between 1955 and 2014, temperatures at HB ranged from a mean January low of −8°C to a mean July high of 18 °C (Bailey 2003; USDA Forest Service 2022). Average annual precipitation at HB is approximately 140 cm (Campbell et al., 2010). Nitrogen deposition in this region exceeded 8 kg N ha^{-1} y^{-1} for much of the 1980s and 90s, but declined in the early 21st century to about 6.5 kg N ha^{-1} y^{-1} (Yanai et al., 2013). Atmospheric deposition of P in the area is estimated to be about 0.04 kg P ha^{-1} y^{-1} (Yanai, 1992).

All stands in BEF and the mature stands at HB and JB contain four 50 m × 50 m plots; in the successional stands at HB and JB, the treatment plots are 30 m × 30 m. All plots were treated annually from 2011 to 2015 with either N (30 kg N ha^{-1} y^{-1} as NH_4NO_3), P (10 kg P ha^{-1} y^{-1} as NaH_2PO_4), both N and P together (same rates), or neither N nor P (control).

2.2. Leaf sampling

Beech and maple were selected for study because they occurred in all the stands; the species of maple collected depended on forest composition (Table 1). All sampling was conducted in the plot interiors to avoid edge effects, with a 10 m buffer in the 50 m × 50 m plots and a 5 m buffer in the 30 m × 30 m plots. Pre-treatment foliar sampling occurred in 2008–2010, prior to the initial fertilizer application in 2011. Post-treatment sampling occurred in 2015.

Green leaves were collected from the mid-canopy or higher of sampled trees with a shotgun during the first half of August in each sampling year. Trees were selected that were dominant or codominant in the canopy with diameters > 10 cm at breast height. Pretreatment, not every species was sampled in each plot each year; for example, beech was sampled in 17 plots in 2008 and in all 32 plots in 2010. Pretreatment, from 2008 to 2010, maple and beech were sampled at least once in every plot with the exception of one plot (C4-4) in which maple was never sampled. When a species was sampled in a plot, leaves were collected from an average of three trees. Post-treatment, maple and beech were sampled in all 32 plots in 2015. Three trees of a species were sampled in all but five plots where forest composition precluded sampling more than one or two trees of a species.

Litter was collected pretreatment in autumn 2009 and 2010 with net traps hung at three locations within each plot (See et al., 2015). Litter

Table 1
Characteristics of the eight sampled stands. Soil N and P are mean and standard error (n = 4 subplots) of resin-available concentrations in control plots sampled in 2012 and/or 2013 (Fisk et al. 2022). Values for N are the sum of available N from NO3 and NH4 resin strips. Basal area was measured in 2014 (Goswami et al. 2018).

Site	Stand	Last cut	Elevation (m)	Aspect	Slope (%)	Soil N (μg d^{-1})	Soil P (μg d^{-1})	Basal area (m^2 ha^{-1}) * BA	BE	PC	RM	SM	WB	YB
BEF	C4	1978	410	NE	20–25	1.09 (0.26)	0.26 (0.10)	7.2	1.4	0.5	2.0	0.03	10.2	0.9
	C6	1975	460	NNW	13–20	5.05 (0.62)	0.29 (0.14)	0.2	4.8	0.3	9.0	1.2	8.5	8.1
	C8	1883	330	NE	5–35	2.80 (1.29)	0.55 (0.17)	0	21.7	0	0.5	19.0	0.2	6.3
	C9	~1890	440	NE	10–35	3.54 (0.17)	0.88 (0.39)	0	8.6	0	0	16.6	0	5.9
HB	HBM	1971	500	S	10–25	7.62 (1.52)	0.22 (0.04)	0.3	1.9	0.1	1.4	1.6	2.4	9.5
	HBO	~1910	500	S	25–35	2.80 (0.53)	0.12 (0.03)	0	8.5	0	0	3.9	0	22.8
JB	JBM	1985	730	WNW	25–35	15.31 (1.65)	0.11 (0.03)	0.2	0.1	0.4	0	2.1	2.3	5.1
	JBO	~1900	730	WNW	30–40	10.33 (1.40)	1.49 (1.04)	0	1.7	0	0	35.1	0	7.6

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

was composited by species for each plot. For post-treatment collections, senesced leaves of the study species were collected from the ground in each plot during the period of peak litterfall on October 3–4 and October 10–11, 2015. At each of these two sampling intervals, we combined leaf litter into one composite sample per species per plot. We analyzed the litter from the second sampling interval except for three samples where data from the first interval were used to replace unusually high values for P concentrations on the later date.

For both green and senesced leaves, only leaves with petioles attached and free of visible damage from disease or insects were used for subsequent analysis. We photographed the green and senesced leaves and determined leaf surface area with ImageJ software so that foliar nutrient concentrations could be expressed on an area basis. Leaves were weighed before and after drying at 60 °C to constant mass and then ground in a Wiley mill using a #40 mesh screen.

2.3. Chemical analysis

We determined N concentrations with a FlashEA 1112 analyzer (Thermo Fisher Scientific, Waltham, MA). Aspartic acid and apple leaves (NIST 1515) were used as standards. For P, ~0.25 g of ground tissue was ashed overnight at 470 °C, hot plate-digested with 10 mL of 6 N nitric acid, and diluted to 50 mL. Phosphorus concentrations were ascertained by inductively coupled plasma optical emission spectroscopy (Optima 5300 DV ICP-OES, Perkin-Elmer, Waltham, MA). Each round of ashing and digestion included one blank, one replicate, and two apple leaf standards. During ICP analysis, we ran a blank after every ten samples and an in-house standard after every-five. Samples were not analyzed unless the standard was within 5 % of certified values.

2.4. Statistical analysis

Green leaf data from individual trees were averaged by species within each plot, resulting in plot-level averages for all variables; thus, plot was considered the experimental unit. We calculated nutrient concentrations on both leaf mass and leaf area bases as well as the nutrient content per leaf, recognizing that each metric supplies different information (van Heerwaarden et al., 2003). We focus here on mass-based concentrations because mass loss was less than area loss between green and senesced leaves. Additionally, using mass-based

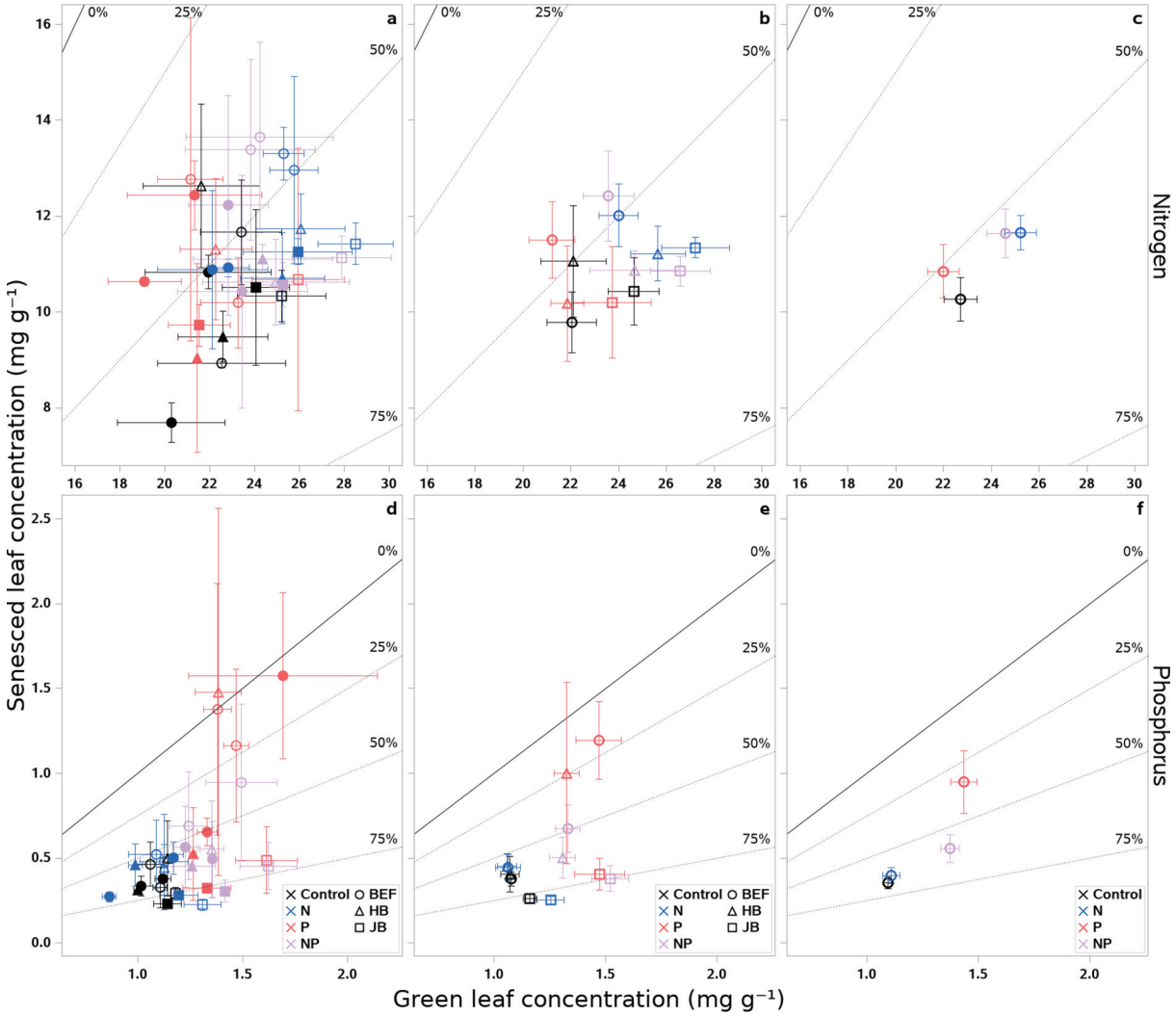

Fig. 1. Concentrations of N (a-c) and P (d-f) in senesced (y axis) and green leaves (x axis). Diagonal lines indicate resorption efficiency; for example, trees with a green leaf N concentration of 20 mg/g and a senesced leaf N concentration of 10 mg/g would fall on the 50 % diagonal line. Panels a and d show stand means (n = 2 species per stand); open symbols indicate successional stands and filled symbols indicate mature stands. Panels b and e show site means (n = 8 for BEF (two species in four stands) and 4 for HB and JB (two species in two stands)). Panels c and f show treatment means for all stands and sites combined (n = 16 (two species in eight stands)). Error bars in each panel indicate the standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations permitted the inclusion of pre-treatment data as a covariate in statistical analyses.

The experimental design was a randomized complete block design with a factorial of N treatment, P treatment, and species. Stand, nested within age and site, was the blocking factor. The ten dependent variables evaluated were green and senesced leaf N and P concentrations; resorption efficiency of N and P; and the N:P ratios of green leaves, litter, and resorption efficiency. Treatment effects were assessed using analysis of covariance (ANCOVA), with site, age, stand, N treatment, P treatment, and species as predictor variables in a model reflecting the factorial design of N and P treatment. For N and P concentrations, ratios, and mass-based calculations of resorption, we used plot-level pre-treatment concentrations, ratios, and resorption values for each species as the covariate.

For effects significant at $\alpha = 0.05$, means were compared by performing Tukey's post-hoc tests. Reported means are least squares means. All statistical tests were performed using SAS 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Nutrient concentrations and resorption

Not surprisingly, green leaves sampled from plots where nutrient addition occurred had elevated concentrations of the added nutrient. Green leaf N concentrations were 12 % higher with N addition (Fig. 1c; main effect of N, $p < 0.001$) and green leaf P concentrations were 25 % higher with P addition (Fig. 1f; main effect of P, $p < 0.001$). Additionally, green leaf N concentrations were 4 % lower with P addition (Fig. 1c; main effect of P, $p = 0.05$), one of the few instances of element interactions observed in this study.

Neither N nor P addition had an effect on N resorption proficiency (litter concentration) or efficiency (the proportion resorbed) (Fig. 1c; $p \geq 0.40$). The addition of P, but not N ($p \geq 0.46$), had an effect on both the proficiency and efficiency of P resorption (Fig. 1f). Litter P concentrations were higher where P was added (main effect of P treatment, $p < 0.001$). Compared to the control, litter P concentrations were 130 % higher when only P was added and 69 % higher when N and P were added together (Fig. 1f, Fig. 2; N × P interaction, $p = 0.05$). Trees in plots receiving P additions exhibited 18 % lower P resorption efficiency than those in plots without P (Fig. 1f, Fig. 3; main effect of P, $p = 0.03$).

3.2. N:P ratios

Mean green leaf N:P ratios across all species within a treatment ranged from 16.0 in the P plots to 23.0 in the N plots. The effect of nutrient additions on green leaf N:P ratios reflected the effects on green leaf N and P concentrations: green leaf N:P ratios were 12 % higher with N addition (Fig. 4; main effect of N, $p < 0.001$) and 29 % lower with P addition (Fig. 4; main effect of P, $p < 0.001$).

The mean litter N:P ratio in the control plots was 30.4. Litter N:P was 50 % lower with P addition ($p < 0.001$) but was not affected by N addition ($p = 0.38$; Fig. 2). The N:P resorption efficiency ratio was 34 % higher with P additions (main effect of P, $p < 0.001$), but was not affected by N addition ($p = 0.25$; Fig. 3). In plots with only P added, proportionately more N than P was resorbed (N × P interaction, $p = 0.005$): the N:P resorption efficiency ratio was>1 and significantly higher than in the other three treatments according to Tukey's separation of means.

3.3. Species effects

Compared to the two maple species, beech had higher green leaf N concentrations (Fig. 1b; main effect of species, $p < 0.001$), especially when treated with N (regardless of P treatment; N × P × species interaction, $p = 0.01$), as well as higher N resorption efficiency (Fig. 4; $p = 0.002$).

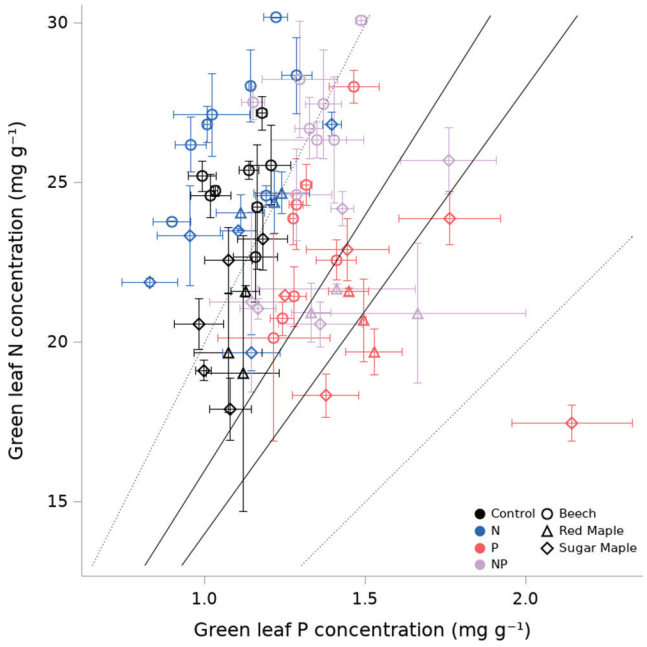

Fig. 2. Effects of factorial N and P additions on green leaf N and P concentrations in eight stands (species mean ± SE, n = 3 trees). Solid lines indicate N:P ratios of 14 and 16 and dotted lines indicate N:P ratios of 10 and 20. Lower N:P ratios suggest limitation by N whereas higher N:P ratios suggest limitation by P. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

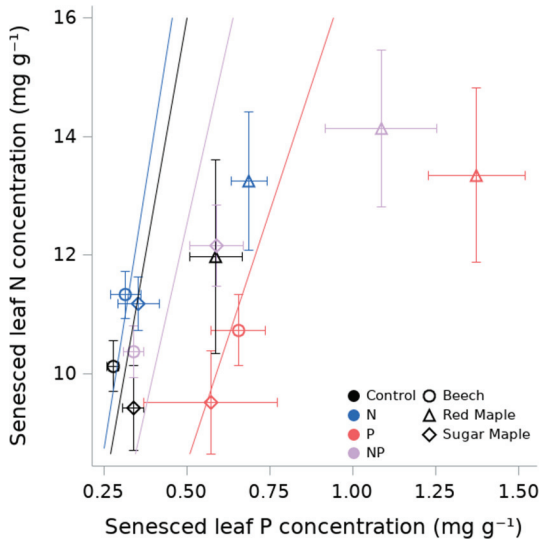

Fig. 3. Effects of factorial N and P additions on litter N and P concentrations in all stands (species means ± SE, n = 8 stands). Solid lines indicate mean N:P ratios for all species in each treatment.

The three species did not differ consistently in green leaf P concentrations (main effect of P, $p = 0.18$), but sugar maple responded more (41 % increase) to P addition than beech (19 %) or red maple (16 %) (Fig. 1e, Fig. 4; P × species interaction, $p = 0.03$).

For litter, red maple had the highest P concentrations (Fig. 2; main effect of species, $p < 0.001$) and the largest increase in P concentrations with P addition (P × species, $p = 0.03$). Red maple also had the lowest P resorption efficiency (Fig. 3; main effect of species, $p < 0.001$). Beech had higher P resorption efficiency than the maples in all but the P plots

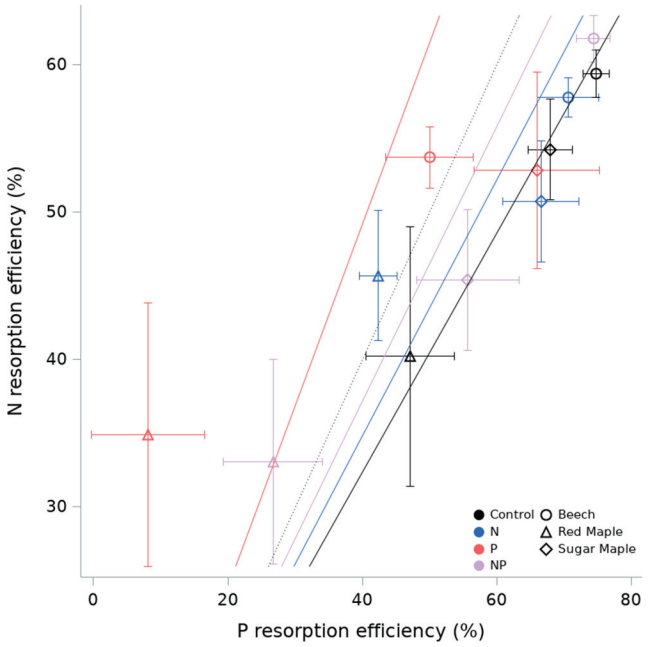

Fig. 4. Effects of factorial N and P additions on N and P resorption efficiency in all stands (species means ± SE, n = 8 stands). The dotted 1:1 line indicates equal resorption of N and P. The colored lines correspond to mean N:P resorption ratios for each treatment.

(Fig. 1e, Fig. 3; N × P × species, $p = 0.02$). Species did not differ consistently in litter N concentrations ($p = 0.08$), though red maple tended to exhibit higher litter N concentrations than sugar maple or beech.

Species did not differ consistently in green leaf N:P ratios (Fig. 4; $p = 0.16$). High litter P concentrations in red maple resulted in a low average litter N:P ratio of 16.5, compared to 27.7 in sugar maple and 31.9 in beech (Fig. 2; main effect of species, $p < 0.001$). Similarly, low P resorption efficiency in red maple led to the highest N:P resorption efficiency ratios (Fig. 3; main effect of species, $p < 0.001$). The addition of P affected the resorption ratio of red maple more than other species; the increase in N:P resorption ratios with P addition was 67 % in red maple, 21 % in beech, and only 8 % in sugar maple (Fig. 3; P × species interaction, $p = 0.01$).

3.4. Age and site effects

Green leaf N (Fig. 1a) was the only metric that differed significantly with stand age, with concentrations in successional stands 7 % higher, on average, than those in mature stands (main effect of age, $p = 0.004$). At JB in particular, green leaf N concentrations in the successional stand were 11 % higher than those in the mature stand (age × site interaction, $p = 0.05$).

For differences among sites, green leaf N (Fig. 1b) and P (Fig. 1e) concentrations were significantly higher at JB than at HB or BEF ($p \leq 0.005$). Green leaf N:P ratios were lowest at JB, averaging 18.3, and highest at HB, averaging 20.1 (main effect of site, $p = 0.02$). Trees at the three sites did not respond to N addition with the same magnitude; that is, the increase in green leaf N:P ratios with N addition was 18 % at HB, 15 % at BEF, but only 1 % at JB (N treatment × site interaction, $p = 0.04$). Resorption proficiency and efficiency of P (Fig. 1e), but not N (Fig. 1b; $p \geq 0.14$), differed among sites. Overall, resorption of P was more proficient ($p = 0.055$) and efficient ($p = 0.004$) at JB, the site with the highest N availability. The response of P resorption efficiency to treatment varied among the sites (Fig. 1e). The effect of P addition on P resorption efficiency was largest at BEF; with P addition, P resorption efficiency was 57 % lower at BEF, 7 % lower at JB, and 5 % lower at HB (Fig. 1e; P treatment × site interaction, $p = 0.02$). Consistent with the high P resorption at JB, litter N:P ratios were highest at JB, averaging 31.5 ($p = 0.04$). In comparison, litter N:P ratios were only 22.2 at HB and 22.4 at BEF.

4. Discussion

Northern hardwood forests have been presumed to be N limited, especially on recently deglaciated landscapes (Walker and Syers, 1976; Du et al., 2020). Greater responses to N addition than P addition have been observed in previous fertilization studies in the northeastern US (Vadeboncoeur, 2010), but we observed several signs that these eight stands are limited primarily by P rather than limited by N alone or co-limited by N and P. First, the N:P ratios of foliage in the untreated plots of these successional and mature stands (Fig. 2) were generally in the range presumed to indicate P limitation (20.8 ± 0.6; Koerselman and Meuleman, 1996; Tessier and Raynal, 2003). Litter N:P ratios and N:P resorption ratios in untreated plots also indicated greater P conservation than N conservation. Global estimates of average resorption efficiency are typically very similar for N and P, such that the N:P resorption ratio is close to 1. For example, Aerts (1996) estimated mean global resorption efficiencies of 50 % for N and 52 % for P. Mean resorption efficiencies in our control plots were 51 % for N and 66 % for P (Fig. 4). Not only was P resorption efficiency in the present study higher than the global average, but the N:P resorption ratio was<1, indicating P limitation. Among the treatments, it was only with P addition that the average N:P resorption ratio increased above 1, a sign of greater N resorption than P resorption.

Consistent with P limitation, responses of foliar P concentrations and resorption to P addition were stronger than responses of foliar N concentrations and resorption to N addition. There was a larger proportional increase in green leaf P concentrations following P addition than in green leaf N concentrations following N addition (Fig. 1); this, in turn, resulted in a greater impact of P addition on green leaf N:P ratios (Fig. 2). Similarly, the addition of P, but not N, affected litter N:P and N:P resorption ratios. Phosphorus addition strongly suppressed P resorption efficiency and proficiency (though the effect on P resorption proficiency was weaker when N was added along with P; Fig. 3), which could reflect an alleviation of P limitation. In contrast, N addition had little effect on N uptake (i.e., foliar concentrations, Fig. 2) or conservation (i.e., resorption, Fig. 1) which further suggests that N is not the primary limiting nutrient in these stands.

Since resorption bears an energetic cost, trees may be more likely to reach complete resorption, defined as the maximum nutrient withdrawal from senescing leaves (Killingbeck, 1996), of a nutrient that is limiting. According to the proposed thresholds (Killingbeck, 1996), litter N concentrations did not achieve complete resorption in our stands (concentrations were > 1 %). In contrast, P resorption was complete (concentrations < 0.05 %) in the control and N plots and intermediate (<0.08 %) in the P and NP plots (as defined by Killingbeck, 1996). Incomplete N resorption but complete P resorption is another sign of P limitation; the relief of P limitation may be demonstrated by the shift to intermediate P resorption with P addition.

A small but significant decrease in green leaf N concentrations following P addition was one of our only observations of a multiple element response. A decrease in a non-limiting nutrient following addition of a limiting nutrient is a sign of single element (i.e., P) limitation (Shaver and Chapin, 1980; Bracken et al., 2015); indeed, tree diameter growth in these stands was significantly increased by P additions, but N and P together did not cause a greater synergistic response, and N alone resulted in no detectable increase in growth (Goswami et al., 2018). The lack of additional element interactions in N and P concentrations in these stands points to limitation by P alone rather than co-limitation by N and P (Iversen et al., 2010; Bracken et al., 2015).

Finally, trees in the plots with lower P availability (i.e., those not receiving P additions) exhibited a more conservative P use strategy

through lower green leaf P concentrations (Figs. 1 and 2), elevated P resorption efficiencies (Figs. 1 and 4), and less variation in green leaf P concentrations (Fig. 1) and P resorption efficiencies (Fig. 4). These modes of maximizing P use efficiency are likely to be observed when P is limiting (Aerts and Chapin, 2000).

Our finding of widespread P limitation in the successional and mature stands of all three sites of the MELNHE experiment four years post-treatment is consistent with reports of foliar N and P resorption in stands of all three age classes at BEF in the three years prior to treatment (See et al., 2015) and in young stands at BEF three years post-treatment (Gonzales and Yanai, 2019); these studies, like ours, noted foliar N:P ratios indicative of P limitation. It is possible that P limitation in temperate forests of the northeastern US developed following decades of atmospheric N deposition in the latter half of the 20th century. Even though anthropogenic N deposition in this region has declined in this century, the foliar N:P ratios we observed remain in the putative P-limited range. We cannot determine whether these types of forests were P-limited prior to the Industrial Revolution.

A positive feedback loop sustains P limitation. In earlier observations in these stands, P resorption was greater with higher soil N availability pretreatment (See et al., 2015) and with N addition after treatment (Gonzales and Yanai, 2019). That high N availability could constrain P cycling via increased P conservation and litter inputs with high N:P may be a mechanism for maintaining stoichiometric balance that ultimately reinforces high N availability relative to P availability. This pattern was also observed across site-level differences in nutrient availability. At JB, the most N- and P-rich site, P resorption proficiency and efficiency were significantly higher than at the other two sites (Fig. 1); this response would not be predicted by the theory of a single element 'concentration control' on resorption (i.e., that resorption efficiency will be higher on more nutrient-poor sites), but it is consistent with previous observations of higher P resorption in areas with high N availability (See et al., 2015). High P resorption at JB subsequently led to litter of higher N:P, further suggesting that P recycling is constrained by a feedback loop driven by high N availability.

Species composition influenced nutrient cycling dynamics in this study. The three studied species included two congeners that differ in understory tolerance (red maple - intermediate; sugar maple - very tolerant), as well as the very tolerant American beech. That red maple exhibited significantly lower P resorption than sugar maple or beech (Figs. 3 and 4) may reflect successional differences in nutrient use strategy, regardless of limitation status. Further, low P resorption means higher P concentrations in the senesced leaves of red maple, which may ultimately benefit species that can respond to higher P availability, such as sugar maple, which exhibited a 41 % increase in foliar P concentrations following P addition (Fig. 4).

Species differences in nutrient cycling may also relate to differences in mycorrhizal associations (Craig et al., 2018). Zhao et al. (2020) reported greater resorption of both N and P in temperate deciduous trees with ectomycorrhizal than those with arbuscular mycorrhizal associations, suggesting that foliar resorption patterns were related to mycorrhizal nutrient economies. In our study, green leaf N concentrations and N resorption efficiency were higher in the ectomycorrhizal-associated beech than the arbuscular mycorrhizal-associated maples. Similarly, red maple in a younger stand at BEF exhibited significantly lower N concentrations than beech, yellow birch (*Betula alleghaniensis;* ectomycorrhizal), or white birch (*Betula papyrifera*; ectomycorrhizal), but the AM-associated pin cherry (*Prunus pennsylvanica)* exhibited the highest foliar N concentrations, likely due to its successional status as a fast-growing pioneer species (Gonzales and Yanai 2019). Information on foliar nutrient resorption from additional species and stands in the MELNHE study may further clarify the degree of influence of mycorrhizal associations and successional differences on resorption dynamics.

In summary, we found multiple indicators of stand-level P limitation in addition to main effects of species type, successional stage, and local nutrient availability on foliar nutrient concentrations and resorption. Evidence that these temperate forest stands in the northeastern United States are P-limited included the N:P ratios in the control plots, the efficiency and relative proportion of N and P resorbed, the magnitude of response in foliar nutrient concentrations and resorption to N versus P addition, the general lack of multi-element interactions, and indications of a conservative P use strategy in the control and N plots. Results from this and previous studies in these stands also suggest that high N availability may perpetuate P limitation by constraining P cycling.

CRediT authorship contribution statement

Kara E. Gonzales: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Ruth D. Yanai:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Timothy J. Fahey:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Melany C. Fisk:** Conceptualization, Methodology, Investigation, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data will be published as a package in EDI.

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