### **ORIGINAL RESEARCH**



# Treatment effects of nitrogen and phosphorus addition on foliar traits in six northern hardwood tree species

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### Abstract

Foliar traits can reflect fitness responses to environmental changes, such as changes in nutrient availability. Species may respond differently to these changes due to differences in traits and their plasticity. Traits 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 leaf carbon isotope ratio ( $\delta^{13}$ 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 mid- and late-successional forest stands in New Hampshire, USA. We also analyzed the response of tree growth to N and P addition. Nutrient addition shifted 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 in 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 suggest N and P co-limitation. Two species displayed lower foliar P under N addition, and three species displayed lower foliar N under P addition, which also suggests co-limitation. These indications of co-limitation were reflected at the community level. Specific leaf area, LDMC, and  $\delta^{13}$ C 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.

Keywords Trait · Foliage · Nutrient · Limitation · Water-use efficiency

# Introduction

Foliar traits, or measurable characteristics of leaves, can provide insights into plant strategies (Violle et al. 2007; Reich 2014). Most foliar traits align to some degree with the "leaf economics spectrum" (Wright et al. 2004), with values associated with more "acquisitive", fast-growing trees with short-lived leaves (higher specific leaf area (SLA), higher

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foliar nitrogen (N) and phosphorus (P) concentrations, greater photosynthetic capacity) to more "conservative", slow-growing trees with long-lived leaves (lower SLA, lower foliar N and P concentrations, lower photosynthetic capacity; Diaz et al. 2004). The traits originally included in this spectrum were SLA, foliar N, foliar P, leaf lifespan, leaf dark respiration rate, and maximum photosynthetic rate, 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 covary in ways that reflect physiological tradeoffs coordinated within the whole plant: devoting resources into acquiring nutrients and growing quickly versus conserving nutrients and growing more slowly (Reich 2014; Diaz et al. 2016). These traits and their plasticity are an important component of demographic and niche differentiation in plant communities and are key determinants of ecological processes such as succession and response to environmental change (Reich 2014; Lichstein et al 2021).

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Trait variation within species can be influenced by genetics, the environment–light, water, nutrients (Reich 2014)–and genetics x environment interactions (Bradshaw 1965). In the context of nutrient-addition studies, which are commonly used to investigate nutrient limitation, measurements of net primary production or plant biomass typically indicate whether a particular nutrient is limiting, but traits can provide insight into how plants anatomically and physiologically respond to nutrient availability and limitation. For example, increases in nutrient concentrations with nutrient 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 stable carbon isotope ratio (expressed as  $\delta^{13}$ C; Coplen 2011) can provide additional insight on how physiological processes such as photosynthesis and transpiration change in response to nutrient availability. Stable isotope ratios of assimilated C are linearly related to intrinsic wateruse efficiency, or iWUE, the ratio of photosynthesis to stomatal conductance (Pérez-Harguindeguy et al. 2013); higher (less negative)  $\delta^{13}$ C values indicate greater iWUE. Leaves that have lower stomatal densities and stomata that open less often tend will therefore have higher  $\delta^{13}$ C values (Farquhar et al. 1982). Foliar  $\delta^{13}$ C reflects these processes over the lifespan of the leaf, weighted towards the early growing season when leaf mass increases quickly, with some possible contribution from carbohydrates stored the previous year. In this way,  $\delta^{13}$ C is a time-integrated metric reflecting the balance of physiological processes over time, unlike the other traits we analyzed which can directly affect these physiological processes and whose plasticity may be part of an active, functional strategy.

While the leaf economics spectrum was developed to explain plant strategies for acquiring and allocating carbon and nutrients, more recent studies have shown that traits related to water use can fall along this same spectrum (Reich 2014; Prieto et al. 2018). Since high iWUE reflects a conservative resource-use strategy (Prieto et al. 2018), we would expect high  $\delta^{13}$ C to fall along the more conservative end of the leaf economics spectrum. Associations of  $\delta^{13}$ C with the leaf economics spectrum, however, may conflict with other 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 with nutrient addition, increasing nutrient availability could increase water-use efficiency, leading to an enrichment in <sup>13</sup>C with nutrient addition. Indeed, with nutrient addition,  $\delta^{13}$ C nearly always increases (Raven et al. 2004), suggesting that increased photosynthesis generally outpaces any increase in transpiration. Differences in responses to changing 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 changes in nutrient availability. Most of the research in temperate forests has investigated the effects of N addition. Recent studies have shown that northern hardwood species differ in response to N deposition, 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), though differences in site conditions, such as soil texture (Pulito et al. 2015) and nutrient availability (Ostertag 2010), can also influence species' response to nutrient addition. Species can 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 the effects of N on tree growth and traits to better understand the effects of anthropogenic N deposition. Less is known about the effects of P availability on temperate tree growth and leaf functional traits.

Differences in species-level responses to nutrient addition influence the community-level response to these traits. A community-weighted mean (CWM) of a trait, which is calculated by weighting the mean trait value with a relative abundance of species, can 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). Forest community demand for nutrients can also change over time due in part to species turnover during succession, changes in soil nutrient stoichiometry over time following disturbance (Rastetter et al. 2013), and the changes in traits with height (Koch et al. 2004) as dominant trees in even-aged stands grow taller over time. 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 with stand age, and how these responses relate to the alleviation of nutrient limitations provides a more complete perspective of how forests respond to changes in nutrient availability.

We quantified 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- and late-successional forest stands. We explored relationships among five traits-four leaf economics spectrum traits (SLA, LDMC, foliar N, foliar P) and one gasexchange trait ( $\delta^{13}$ C) —to further investigate the role of gasexchange and water use in the leaf economics spectrum. Our research answers the following questions: (1) how do the selected traits relate to each other (2) how do species- and community-level foliar trait values vary with experimental nutrient addition and between mid- and late-successional stands, and (3) do the six hardwood tree species studied exhibit evidence of N or P limitation? The Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) experiment in the White Mountains of New Hampshire serves as an ideal location for this investigation. 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 and hypothesized that these traits would exhibit changes in response to nutrient addition (Table 1), but that species would differ in the magnitude of their response, reflecting differences in plant strategy.

# Materials and methods

# Site description

This study took place in 10 forest stands that are part of the MELNHE study in New Hampshire (Online Resource 1; Yanai et al. 2022). 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 North Woodstock, 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). Mean annual precipitation at Hubbard Brook is around 1,400 mm, evenly distributed throughout the year (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). 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 (Lyons et al 1997); we expected differences in parent material to influence site fertility with Bartlett being the least fertile (e.g., lowest soil base cation saturation), Jeffers Brook most fertile, and Hubbard Brook intermediate. Soils were predominantly well drained or moderately well drained Spodosols developed in glacial till (Vadeboncoeur et al. 2014).

Four of these stands (C8, C9, HBO, JBO) were latesuccessional forests last harvested between 1883 and 1915, while six of these stands were additionally harvested more recently between 1970 and 1990 (Yanai et al. 2022). The mid-successional stands in this study at Bartlett had a mean canopy height of  $15.2 \pm 0.8$  m in 2019, and late-successional stands at Bartlett had a mean canopy height of  $23.0 \pm 0.5$  m (NEON 2022). Forests were dominated by typical northern hardwood species following patterns related to secondary successional forests were dominated by *Fagus grandifolia* 

 Table 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 references to background literature

Trait	Represents	Influenced by Avail- ability of			Expected Changes	References
		Nutrients	Light	Water		
Foliar N	Photosynthetic capacity, com- munity productivity, competitive ability	Х	X	Х	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)	Х	Х		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, posi- tively related to leaf lifespan	Х		Х	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	Х	Х	Х	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	Х	Х	х	Increase with nutrient addition	Cernusak et al. 2013; Raven et al. 2004

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). Stands C4, C6, HBM, and JBM were dominated by a mix of these species.

Each stand had four plots, which consisted of a  $30 \text{ m} \times 30 \text{-m}$  inner measurement area with a 10-m buffer on each side (except for HBM and JBM plots, which had a 20 m×20-m measurement area with a 5-m buffer). Plots were treated annually with N (as  $NH_4NO_3$ , 30 kg ha<sup>-1</sup> yr<sup>-1</sup>), P (as  $NaH_2PO_4$ , 10 kg  $ha^{-1}$  yr<sup>-1</sup>), both N and P, or neither. The N application rate was more than three times the mean atmospheric N deposition rate at Hubbard Brook from 2008 through 2022, which was about 9 kg  $ha^{-1}$  yr<sup>-1</sup> (NADP 2024). 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 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).

# **Foliar sampling**

Foliage samples were collected from Jeffers Brook (JBM and JBO) and Hubbard Brook (HBO and HBM) between July 29 and August 3, 2021. Samples were collected from Bartlett (C1, C2, C4, C6, C8, and C9) between July 22 and August 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, sampling the following tree species: A. saccharum, A. rubrum, B. alleghaniensis, B. papyrifera, F. grandifolia, and P. pensylvanica (Online Resource 1). P. pensylvanica was sampled only in C1 and C2, as nearly all the 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 because they were not abundant in other study stands. 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 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 chosen leaves (Zukswert 2024) 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 (Online Resource 2). When it was 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 to constant mass at 60 °C. 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  $\delta^{13}$ 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  $\delta^{13}$ C, primarily from stands C2 and C6. Data from these two stands were consequently omitted from  $\delta^{13}C$  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. Concentrations of N and P in foliage are expressed on a unit mass basis in this report, though area-based concentrations

were also analyzed. 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, the mean absolute error of N was 2.4% and of P was 4%; recovery of N and P from all NIST 1515 tissue standards were between 90 and 110% (Zukswert et al. 2024). Duplicates of N were within 9.1% of each other, on average, and duplicates of P were within 3% of each other, on average; all duplicates except three N duplicates were within 20% of each other (Zukswert et al. 2024). NIST 1515 tissue standard  $\delta^{13}$ C values were within 0.09 % $_{0}$  of the certified value on average, within 0.25 % $_{0}$  for all 15 NIST 1515 samples run, and replicates were within 0.12 % $_{0}$  on average, 0.25 % $_{0}$  at most.

### **Tree growth measurements**

All trees  $\geq$  10 cm in diameter at breast height (DBH) were inventoried in the MELNHE study in 2011 (after the first fertilization), 2015, and 2019 (Goswami et al. 2018; Blumenthal et al. in revision). To evaluate the annual relative basal area increment (RBAI) of the trees sampled for foliar traits in 2021 and 2022, we used the DBH measurements in 2011 and 2019 (Fisk et al. 2022). We had DBH measurements in both 2011 and 2019 for 343 of 426 trees or 81% of the trees–the remaining 19% of trees were excluded from the inventory protocol in 2011 (i.e., the diameter in 2011 was less than 10 cm), or were found in the plot buffer and therefore not included in the inventory. Two trees did not have corresponding records in the tree inventory, likely due to data management errors.

RBAI was calculated for each tree as  $(BA_{2019}/BA_{2011})^{1/n}$ —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. This equation describes geometric growth on an annual, not a continuous, basis.

### **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 version 4.2.2 (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 mixedeffects models for RBAI for all species except for *P. pensylvanica*, which did not have enough trees. Fixed effects were N-addition, P-addition, the interaction of N and P, stand age (mid-and late-successional), and site. Stand and plot within stand were included as random intercepts. Plot random intercepts were zero for *F. grandifolia, A. rubrum, A. saccharum*, and *B. papyrifera*, functionally resulting in the testing of N and P at the tree level, rather than plot level, which is a form of pseudoreplication (Hulbert 1984); significant effects were interpreted with caution for these species (Online Resource 3). Analyses were performed in R version 4.2.2 (R Core Team 2022) with the 'Ime4' and 'ImerTest' packages (Bates et al. 2015; Kuznetsova et al. 2017) using the 'Imer' function and Type III sums of squares and Satterthwaite degrees of freedom.

To further explore the effects of N and P fertilization on foliar traits, we ran a linear mixed-effects model for each of five traits (N and P, SLA, LDMC, and  $\delta^{13}$ C) for each of six species, resulting in 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, stand age, 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. Site was considered a fixed effect, rather than a random effect because sites were selected based on differences in bedrock and, presumably, site productivity (see site description). Random effects were stand and plot within the 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 (Online Resource 4). 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  $\delta^{13}$ C for A. rubrum and B. papyrifera (Online Resource 4). Significant differences were characterized by calculating the differences in least-squares means with the 'diffismeans()' function in 'ImerTest', using Satterthwaite degrees of freedom. The one A. rubrum tree in HBO was not included in the A. rubrum model because it was the only A. rubrum tree in that stand. These models were repeated using the subset of trees for which we had DBH measurements and could calculate RBAI (Online Resource 5). This model results only differed in three cases: foliar N did not differ with P addition and foliar P did not differ with N addition in F. grandifolia in the model using the subset of trees with RBAI measurements, and foliar P decreased with N addition for *B. papyrifera* (Online Resource 5). Otherwise, the model results were comparable between both subsets of data in terms of magnitude and significance.

For six of the 30 trait models, stand effects were zero, resulting in the testing of site and stand age at the plot level, rather than at the stand level. Site or stand age was significant in only one of these models ( $\delta^{13}$ C for A. saccharum); this model was interpreted with caution. Similarly, plot effects were zero in six of 30 models, which constitutes pseudoreplication for tests of N, P, and the N x P interaction, 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,  $\delta^{13}$ C for *B. alleghaniensis*, and foliar N for *A. saccharum*; these results were interpreted with caution. Removing markedly high or low values, determined visually, 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. Results were presented with and without the removed values.

Linear mixed-effects models with the same fixed effects described above were run for each trait at the community level using CWMs of each trait. CWMs for each plot (CWM<sub>Total</sub>) 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, the interaction of N and 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.

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<sub>Inter</sub>) and the contribution of intraspecific variability to CWM<sub>Total</sub> (CWM<sub>Intra</sub>; Lepš et al. 2011). CWM<sub>Inter</sub> was calculated the same way as CWM<sub>Total</sub> 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<sub>Inter</sub> attributed to differences in species abundance across plots. CWM<sub>Intra</sub> was calculated by subtracting CWM<sub>Inter</sub> from CWM<sub>Total</sub> (Lepš et al. 2011).

Results from all linear mixed-effects models run were considered statistically significant if  $p < \alpha = 0.05$  and marginally significant if  $p < \alpha = 0.10$ . We interpreted significant and marginally significant results. In all, we repeated five models excluding extreme values, determined visually, and reported the results of these models with and without these values.

# Results

### **Relationships among foliar traits**

Foliar N, foliar P, SLA, and LDMC strongly co-varied, all contributing most strongly to the first principal component axis (PC1), which explained 36% of variance in the data (Fig. 1). In particular, foliar N and P were highly positively correlated with each other, and foliar N, P, and SLA were negatively correlated with LDMC (Fig. 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 (Fig. 1). Carbon



**Fig. 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**). Ellipses show a 95% confidence region. 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%

isotope ratio was not strongly correlated with PC1, but was slightly, positively correlated with LDMC and negatively correlated with foliar N and P (Fig. 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 (Fig. 1A). *Betula papyrifera* and *B. alleghaniensis* were located between *P. pensylvanica* and the other species (Fig. 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 (Fig. 1B).

# Tree growth response to N and P addition and stand age

Only *Betula* species showed a significant response to nutrient treatments. The mean annual relative basal area increment (RBAI, expressed in % per year) was 41% higher with N addition for *B. alleghaniensis* (RBAI = 3.64% per year with N addition, 2.58% per year without N addition; p < 0.01) and 23% higher with N addition for *B. papyrifera* (3.98% per year with N addition, 3.23% per year without N addition; p = 0.03; Fig. 2, Online Resource 3). RBAI was 202% higher on average in mid-successional stands than late-successional stands for *A. saccharum* (254% without outlier; p < 0.01, p = 0.05 without outlier), 192% higher



**Fig. 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- and late-successional forest stands. Tree species include *Acer rubrum* (ACRU), *Acer sac*-

charum (ACSA3), Betula alleghaniensis (BEAL2), Betula papyrifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)

on average in mid-successional stands for *F. grandifolia* (RBAI=5.67% per year in mid-successional stands, 1.94% per year in late-successional stands; p = 0.04), and 117% higher in mid-successional stands for *B. alleghaniensis* (RBAI=4.25% per year in mid-successional stands; p = 0.05, Fig. 2, Online Resource 3).

### 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 nearly every species ( $p \le 0.04$ ; Figs. 3 and 4, Online Resource 4); the only exception was A. *rubrum*, for which we did not detect an increase of N with N addition (p = 0.44; Online Resource 4). For F. grandifolia, P. pensylvanica, A. *rubrum*, and B. alleghaniensis, foliar N was lower with P addition (p = 0.09, 0.07, 0.046, and 0.01, respectively; Online Resource 4); foliar N did not consistently vary with P addition for A. saccharum or B. papyrifera ( $p \ge 0.20$ ; Fig. 3, Online Resource 4). For F. grandifolia, P. pensylvanica, and B. alleghaniensis, foliar P was lower with N addition ( $p \le 0.045$ ; Fig. 5, Online Resource 4). Foliar P did not consistently vary with N addition for A. rubrum or B. papyrifera  $(p \ge 0.12)$  and was actually slightly higher with N addition for A. saccharum (p = 0.02; Fig. 4, Online Resource 4). For two species (A. saccharum and B. alleghaniensis), the interaction between N and P was significant (p = 0.02 and 0.049, respectively; Online Resource 4); 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 (Fig. 4, Online Resource 4). We did not observe consistent effects of stand age or site on either foliar N or P. Analyses with area-based concentrations of foliar N and P, instead of



**Fig. 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- and late-successional stands. Tree spe-

cies include Acer rubrum (ACRU), Acer saccharum (ACSA3), Betula alleghaniensis (BEAL2), Betula papyrifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)



**Fig.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- and late-successional stands. Tree spe-

cies include Acer rubrum (ACRU), Acer saccharum (ACSA3), Betula alleghaniensis (BEAL2), Betula papyrifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)

mass-based concentrations, gave similar results, but with fewer statistically significant findings (Online Resource 6).

Consistent with effects on individual species, community-weighted foliar N increased by  $2.7 \pm 0.6 \text{ mg g}^{-1}$  with N addition (p < 0.01) and decreased by  $1.1 \pm 0.6 \text{ mg g}^{-1}$  with P addition (p = 0.06; Fig. 5, Online Resource 4). Community-weighted foliar P was  $0.73 \pm 0.05 \text{ mg g}^{-1}$  higher with P addition than without (p < 0.01), and was  $0.30 \pm 0.07 \text{ mg g}^{-1}$ lower with N + P addition than P addition alone (t = 4.36, p < 0.01), but did not differ between the control and N addition treatments (difference in means =  $0.01 \pm 0.07 \text{ mg g}^{-1}$ , t = 0.11, p = 092). These patterns were also detected for CWM<sub>Intra</sub> ( $p \le 0.02$ ) but not CWM<sub>Inter</sub> ( $p \ge 0.18$ ; Online Resource 4).

LDMC was influenced by nutrient addition at the species level, but SLA was not (Fig. 6, Fig. 7). LDMC was  $29 \pm 11 \text{ mg g}^{-1}$  lower on average with P addition for A.

saccharum (p=0.02), and  $28 \pm 7 \text{ mg g}^{-1}$  lower for *P. pensylvanica* after removing an outlier (p=0.03; Fig. 6, Online Resource 4). Neither community-weighted LDMC nor SLA differed among plots with N or P addition ( $p \ge 0.49$ , Online Resource 4).

Enrichment of <sup>13</sup>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 (p=0.07) and by 0.39±0.22 ‰ for *A.* saccharum (p=0.09; Fig. 8, Online Resource 4). CWM  $\delta^{13}$ C did not differ from controls with N or P addition ( $p \ge 0.13$ ; Fig. 5, Online Resource 4). CWM<sub>Intra</sub>  $\delta^{13}$ C was 0.28±0.14 ‰ higher with N addition (p=0.053 for the main effect of N; Fig. 5, Online Resource 4), but CWM<sub>Inter</sub>  $\delta^{13}$ C did not differ (p=-0.57; Online Resource 4), suggesting that these differences in  $\delta^{13}$ C were driven by differences within species. CWM<sub>Inter</sub>  $\delta^{13}$ C was 0.09±0.04 ‰ greater with P addition (p=0.046 for the main effect



**Fig. 5** Community-weighted means of foliar N (a), P, (b), leaf dry matter content (LDMC, c), specific leaf area (SLA, d), and carbon-isotope ratio ( $\delta^{13}$ C, e) in forest plots receiving N addition (blue), P addition (red), N and P addition (purple), or no nutrients (control, gray)

of P in ANOVA), but CWM<sub>Intra</sub> was not (p = 0.63; Online Resource 4), suggesting that the differences in  $\delta^{13}$ C were due to plot differences in species abundance, not within-species variability.

# Effects of stand age and site

Physical traits of foliage (SLA and LDMC) differed by stand age, with  $28 \pm 12$  mg g<sup>-1</sup> higher LDMC on average

in late-successional than mid-successional stands for *F.* grandifolia (p=0.06) and  $28.9 \pm 9.7 \text{ mg g}^{-1}$  higher LDMC on average in late-successional stands for *B. alleghaniensis* (p=0.03; Fig. 6, Online Resource 4). LDMC was also higher at Hubbard Brook and Jeffers Brook than at Bartlett for *F. grandifolia* and *B. alleghaniensis* (p=0.09 and 0.07 for *F. grandifolia* and *B. alleghaniensis*; Fig. 6, Online Resource 4). SLA was  $3.78 \pm 1.28 \text{ m}^2 \text{ kg}^{-1}$  lower in late-successional than mid-successional stands for *A. saccharum* (p=0.047)



**Fig.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 late-successional stands. Tree spe-

cies include Acer rubrum (ACRU), Acer saccharum (ACSA3), Betula alleghaniensis (BEAL2), Betula papyrifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)

and was  $2.62 \pm 1.02 \text{ m}^2 \text{ kg}^{-1}$  lower in late-successional than mid-successional stands for *B. alleghaniensis* (p = 0.03), but did not differ with stand age for *F. grandifolia* (p = 0.54; Fig. 7, Online Resource 4).

Community-weighted LDMC was  $51.5 \pm 9.4 \text{ mg g}^{-1}$ higher on average in late-successional stands than in midsuccessional stands (p < 0.01), but SLA did not differ with stand age (p = 0.88, Fig. 5, Online Resource 4). Stand age was statistically significant for both CWM<sub>Inter</sub> and CWM<sub>Intra</sub> LDMC, meaning that the higher LDMC in late-successional stands was due both to species composition differences between mid-and late-successional stands and within-species variability ( $p \le 0.01$ ; Online Resource 4). CWM<sub>Intra</sub> LDMC was higher at Hubbard Brook than at Jeffers Brook and Bartlett Experimental Forest (p = 0.02; Fig. 5, Online Resource 4). CWM<sub>Inter</sub> SLA was  $1.48 \pm 0.41 \text{ m}^2 \text{ kg}^{-1}$  higher in late-successional stands for SLA, reflecting species differences (p = 0.01; Online Resource 4).

Enrichment of <sup>13</sup>C was greater in late-successional stands than mid-successional for *A. saccharum*, indicated by a mean  $\delta^{13}$ C value that was  $0.84 \pm 0.26 \%$  higher (p < 0.01; Fig. 8, Online Resource 4), but did not differ with stand age in any other species. While CWM<sub>Total</sub>  $\delta^{13}$ C did not differ with stand age, CWM<sub>Inter</sub>  $\delta^{13}$ C was  $0.53 \pm 0.21\%$  lower in late-successional stands than in mid-successional stands (p = 0.048), but CWM<sub>Intra</sub>  $\delta^{13}$ C was  $0.56 \pm 0.22\%$  higher in late-successional stands (p = 0.06; Online Resource 4).

Community-weighted foliar N was  $2.0 \pm 0.9$  mg g<sup>-1</sup> higher in mid-successional stands than in late-successional stands (p=0.07, Fig. 5, Online Resource 4). Foliar N did not differ by age in *F. grandifolia*, *A. saccharum*, or *B. alleghaniensis*, the three species found in both stand age classes



**Fig. 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- and late-successional stands. Tree species include *Acer rubrum* (ACRU), *Acer* 

rifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)

saccharum (ACSA3), Betula alleghaniensis (BEAL2), Betula papy-

 $(p \ge 0.22, \text{Fig. 3}, \text{Online Resource 4})$ . Community-weighted foliar P differed by site (p = 0.04; Fig. 5, Online Resource 4), being  $0.25 \pm 0.08 \text{ mg g}^{-1}$  higher at Hubbard Brook than at Bartlett Experimental Forest and  $0.31 \pm 0.10 \text{ mg g}^{-1}$  higher at Hubbard Brook than at Jeffers Brook. No other traits differed by site.

# Discussion

# Trait relationships and plant strategy

We evaluated the response of foliar traits in six northern hardwood tree species to low-level, long-term N and P addition to improve our understanding of the plasticity of these traits in response to changing nutrient limitations. The trait responses indicated an adherence to the leaf economics spectrum for four of the five traits, while  $\delta^{13}$ C was weakly correlated with this spectrum overall. Four of these traits showed a shift to a more acquisitive resource strategy under nutrient addition; while  $\delta^{13}$ C increased, indicating that any increases in transpiration were less than proportional to increases in photosynthesis.

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, as visualized in the first PCA axis. Other traitbased studies have commonly found that the first PCA axis reflects the leaf economics spectrum (Wright et al. 2004; Zukswert and Prescott 2017; Hecking et al. 2022). Carbon isotope ratio ( $\delta^{13}$ C) did not correlate strongly with this axis, but fell on the same side of the leaf economics spectrum



**Fig.8** Foliar stable carbon isotope ratio ( $\delta^{13}$ 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- and late-successional stands. Tree species include *Acer rubrum* (ACRU), *Acer sac*-

axis as LDMC, suggesting that high  $\delta^{13}$ C, indicating high iWUE, was weakly associated with a more conservative overall strategy. 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) have found stronger associations in this direction, perhaps because they are from more waterlimited environments, where conservation of water is of greater importance than in the humid temperate forests we studied.  $\delta^{13}$ 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, where a path analysis suggested that the effects of rainfall on SLA consequently influenced  $\delta^{13}C$ (i.e., greater rainfall lead to higher SLA and lower  $\delta^{13}$ C; Schulze et al. 2006).

The low correlation of  $\delta^{13}C$  with the PC1 axis, stronger correlation of  $\delta^{13}C$  with the PC2 axis, and increases of

charum (ACSA3), Betula alleghaniensis (BEAL2), Betula papyrifera (BEPA), Fagus grandifolia (FAGR), and Prunus pensylvanica (PRPE2)

 $\delta^{13}$ C with N addition could reflect the fact that, unlike the other traits, nutrient addition does not shift  $\delta^{13}C$  towards the acquisitive end of the leaf economics spectrum. The response of  $\delta^{13}$ C to nutrient addition we observed has been widely reported and is more in line with a tradeoff between nutrient-use efficiency and water-use efficiency (Cramer et al. 2009), which complicates the interpretation of trait values following a single axis that represents plant strategies in response to carbon, nutrient, and water use and acquisition (e.g., Reich 2014). At a fundamental level, the tradeoff in nutrient-use and water-use efficiency can be explained by biochemical processes. For example, under increased water availability, plants have higher nutrient-use efficiency (i.e., more biomass per unit nutrient, Aerts and Chapin 2000), but also more open stomata and increased transpiration rates, and thereby lower water-use efficiency (Raven et al. 2004). Conversely, water-use efficiency increases with N addition due to a greater influence of N on photosynthetic rates than stomatal conductance (Ripullone et al. 2004), reflecting the key role of proteins such as RuBisCO in controlling photosynthesis rates. Ultimately, if photosynthesis is far more often limited by light and nutrient availability than by water, there is little advantage to strategies that actively increase water-use efficiency.

The distribution of leaf trait centroids associated with treatments showed that the control plots had the highest LDMC values and that the nutrient addition treatments tended to have higher foliar N and P, though the magnitude of these differences was small (Fig. 1B). This result suggests a shift in trait values from conservative to more acquisitive following nutrient addition, particularly for traits associated 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). Beyond the effect of iWUE on  $\delta^{13}$ C of plant tissues, additional variation can be introduced by post-photosynthetic fractionation, which may vary among trees with differing allocation to respiration versus the synthesis of compounds via various fractionating biochemical pathways, though such differences among species are poorly characterized (Badeck et al. 2005; Brüggemann et al. 2011; Ma et al. 2023).

The PCA illustrated how species differed from each other in terms of foliar traits, suggesting that, based on their positions along the PC1 axis, A. saccharum, F. grandifolia, and A. rubrum were conservative species, followed by B. alleghaniensis and B. papyrifera, with P. pensylvanica being acquisitive (Fig. 1A). This spectrum from relatively conservative to more 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 is often classified as intermediate in tolerance (Burns and Honkala 1990); notably, this species is exceptionally plastic (Abrams 1998), and our evaluation may provide information about its behavior in one part of its range.

# **Traits and nutrient limitation**

While our tree growth results indicated primarily N limitation, our trait 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 the 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 differential responses to N and P addition at the community level. Trait responses to nutrient addition within species seem to drive trait differences at the community level, which could reflect a scaling up of the influence of nutrient addition on biochemical and physiological processes.

For B. alleghaniensis and B. papyrifera, we detected N limitation based on a response of RBAI to N addition. Results for *B. papyrifera*, however, may be accentuated by 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 in temperate forests in general (Vitousek and Howarth 1991; Finzi 2009; Vadeboncoeur 2010). After decades of N deposition in this area (Likens et al. 2021), foliar and soil N availability at Hubbard Brook, among other areas of the northeastern United States, have now been decreasing over time. This decline in N availability is perhaps as a result of declining N deposition in recent decades and increased carbon flux to soils, promoting N limitation (Groffman et al. 2018; Mason et al. 2022). The first analysis of RBAI in MELNHE from 2011 to 2015 using all inventoried trees showed P limitation (Goswami et al. 2018); these results were dominated by small trees. More recent measurements (2019) show a greater response to N and P addition together than to either alone, suggesting N and P co-limitation (Blumenthal et al. in revision). Our current analyses of a subset of these trees provide evidence of N limitation, but also potential co-limitation of N and P, as indicated by foliar N and P concentrations, as discussed below.

Foliar N and P concentrations in our stands demonstrated evidence of N and P co-limitation at both 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, *B. 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 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* (Online Resource 5).

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 an expected 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 and Houlton 2012). Indeed, in these same forest stands prior to fertilization, phosphatase production increased with soil N availability (Ratliff and Fisk 2016). In this way, both Acer species could be co-limited by N and P, and perhaps more so than the other species, i.e., N addition might have stimulated a greater production of phosphatases by their roots and mycorrhizae. While most of the tree genera in our study plots are ectomycorrhizal, Acer (and Prunus) trees form relationships exclusively with arbuscular mycorrhizae (Brundrett and Tedersoo 2020), and this mycorrhizal type has been associated with increased acid phosphatase activity relative to ectomycorrhizal trees (Ma et al. 2021), which lends further support for this interpretation. Ectomycorrhizal fungi, in contrast, are far more taxonomically and functionally diverse (Agerer 2001), include taxa that produce proteolytic enzymes to acquire N (Pelletier and Zak 2017; Wang et al. 2020), and have been found to decline in abundance with N fertilization (Morrison et al. 2016; Bashian-Victoroff et al. 2025).

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 treatments. We observed the same relationships in CWM<sub>Intra</sub> (but not CWM<sub>Inter</sub>) for both nutrients, verifying that these responses 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 late-successional 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 had initially predicted that we would see a decrease in LDMC with the addition of a limiting nutrient (Online Resource 1). We observed decreases in LDMC with P addition (but not N addition) for P. pensylvanica and A. saccharum. These results 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 reflects a more acquisitive strategy and greater photosynthetic rate. In observational studies, plants with lower LDMC have been observed in areas with high soil P (Cui et al. 2022). In fertilization studies, LDMC has been observed to decrease with P addition in some cases, such as in 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 correlations could be due to biochemical relationships 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), which are involved in carbon assimilation. The observed change in LDMC with P addition and not N, therefore, could be due to differences in N and P biochemistry within leaves, rather than the degree of nutrient limitation, 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*, which could suggest N

limitation in those species if it indicates an increase in production with N addition. *Betula papyrifera* also demonstrated an increase in RBAI with N addition, perhaps reflecting an increase in biochemical capacity for photosynthesis, which would decrease the inter-cellular concentrations of CO<sub>2</sub> fixed during photosynthesis for the same stomatal conductance (Raven et al. 2004).  $\delta^{13}$ C has also been shown to increase with tree height (Vadeboncoeur et al. 2020); thus, an increase in tree growth and in  $\delta^{13}$ C with N addition could in part reflect an increase in tree height. Higher CWM<sub>Inter</sub>  $\delta^{13}$ C with P addition, but lack of detectable change in CWM<sub>Intra</sub>  $\delta^{13}$ C or CWM<sub>Total</sub>  $\delta^{13}$ 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.

# Traits and stand age

Stand age differences were observed for SLA, LDMC, foliar N, and  $\delta^{13}$ C. These effects were most clear and consistent for LDMC. The late-successional 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 CWM<sub>intra</sub> LDMC, higher SLA, and lower foliar N in latesuccessional 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 late-successional stands (Fig. 3, Fig. 7). Community-weighted  $\delta^{13}$ C did not differ with stand age, but CWM<sub>Inter</sub> of  $\delta^{13}$ C was lower in late-successional stands while  $CWM_{Intra}$  was higher. The lower  $CWM_{Inter}$  of  $\delta^{13}C$ in late-successional stands is likely due to the absence of *P. pensylvanica*, which had the highest  $\delta^{13}$ C of all species studied (Fig. 8). At the species level, lower SLA in late-successional stands for B. alleghaniensis and A. sac*charum*, and higher  $\delta^{13}$ C in late-successional 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). Because trees in the latesuccessional stands are taller, on average than those in the mid-successional stands (NEON 2022), differences in tree height could in part explain differences in traits with stand age. If the 8 m of mean canopy height difference between the mid- and late-successional stands were to explain the stand age effect on  $\delta^{13}$ C (e.g. 0.84 ‰ for sugar maple), that would require an effect of 0.1% per meter. This is within the range of variation reported for temperate angiosperms (McDowell et al. 2011; Vadeboncoeur et al 2020), though reported effects can vary widely among sites and species (Vadeboncoeur et al 2020).

### Conclusion

Relationships among leaf economics traits and  $\delta^{13}$ C, a gasexchange trait associated with water-use efficiency, are less consistent with the hypothesis that these traits lie along a single resource-use axis and are more consistent with a tradeoff between nutrient-use efficiency and water-use efficiency. 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-with the exception of  $\delta^{13}$ C. Community-level differences in trait values with nutrient addition were driven less by differences in species composition and more by changes within species with nutrient addition. Our results support hypotheses that N and P are co-limiting within these northern hardwood forest communities, although they provide more direct evidence for N limitation of tree growth in *Betula* species. The effects of changes in N and P on traits can differ due to their different and multiple roles in plant biology, complicating comparisons between these two limiting nutrients. This study provides insights to further explore relating to species differences in response to nutrient addition and how these differences contribute to community-level changes.

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Author contribution statement JZ and RD had the idea, JZ and MV collected the data, and JZ analyzed the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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**Code availability** R code and supporting files are available on GitHub at the following link: https://github.com/jennamz48/Zukswert\_etal\_MELNHE\_foliar\_traits\_NP.

#### Declarations

Conflict of interest There are no conflicts of interest to disclose.

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