EFFECTS OF NITROGEN, PHOSPHORUS, AND CALCIUM ADDITION ON

HERBACEOUS PLANTS IN NORTHERN HARDWOOD ECOSYSTEMS

by

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S.H.Dai. Effects of Nitrogen, Phosphorus, and Calcium Addition on Herbaceous Plants in Northern Hardwood Ecosystems, 67 pages, 3 tables, 5 figures, 2023. Forest Management and Ecology style guide used.

ABSTRACT

The importance of nitrogen (N) availability to herb layers has been well documented, but phosphorus (P) and calcium (Ca) availability may also play key roles in shaping the herb layer. I surveyed the herb layer of eleven northern hardwood forest stands distributed across three sites in a long-term nutrient manipulation experiment in the White Mountains of New Hampshire. Across all stands, N addition resulted in 28% fewer species (p = 0.01) and Ca addition resulted in 29% fewer species (p = 0.09) after 10 years of treatment. Abundance and cover did not respond Ca addition or to N or P addition or their interaction. Of the three herb species found to be ubiquitous in all stands, wild sarsaparilla (*Aralia nudicaulis* L.) N concentrations increased by 15% under N addition (p = 0.004) and decreased by 13% under P addition (p = 0.01). Species richness decline with N addition could be a result of a removal of niches for N-limited conditions.

Keywords: herbs, herbaceous layer, northern hardwoods, northern hardwood ecosystems, N availability, P availability, New Hampshire, community ecology, light availability

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CHAPTER 1: THE ROLE OF NUTRIENTS AND ENVIRONMENTAL FACTORS ON FOREST HERB LAYERS

Importance of the herb layer in temperate forests

The herb layer contributes most of the biodiversity in northern hardwood forests (Gilliam 2007). While more attention has been devoted to trees in forested ecosystems, the herb layer can enhance our understanding of forest ecological processes. For example, the herb layer can indicate forest communities that have existed at previous successional stages, which may be useful for management. For example, aspen-pin cherry thickets with the herb species red baneberry (*Actaea rubra* Willd.) and blue cohosh (*Caulophyllum thalictroides* Michx.) indicate a previously disturbed site, as these two herb species are not typically found in aspen-pin cherry thickets (Stearns 1951). Herb cover and species composition can also indicate soil fertility, with more fertile stands generally supporting more herb species (Small and McCarthy 2005).

Herbaceous plants may compete with regenerating tree seedlings, shaping forest development. In the northern hardwood forests of Michigan, in the absence of herbivory, the presence of an herb-shrub layer reduced the survival and height of planted tree seedlings through light competition (Walters et al. 2016). In northern red oak (*Quercus rubra* L.) stands of Pennsylvania, hay-scented fern (*Dennstaedtia punctilobula* (Michx.) T. Moore) interferes with hardwood regeneration, which can be a concern for forest managers (Happel and Sharpe 2004).

Methods to assess changes in the herb layer

Several metrics can be used to describe herb layer composition. Species richness is the number of unique species seen in a community (Fleishman et al. 2006). Species evenness, the relative abundances of each species in a community, identifies the dominance of species in a

community. Species richness and evenness can be incorporated into diversity indices (e.g. Shannon diversity) to provide a broad understanding of herb diversity.

The indicator species approach characterizes species that are prevalent in different sites or target groups based on the specificity (whether a species is only found in a target group) and fidelity (whether a species is found across all sites) of a species (Borcard et al. 2018, De Cáceres et al. 2010). Indicator herb species were determined based on their abundance relative to soil nutrients, pH, elevation, and other environmental factors in rich-cove forests of the southern Appalachians (Elliott et al. 2014, Jackson et al. 2009, Ulrey 2002), and these same indicator species have been used to further characterize herb composition in forests (Elliott et al. 2014, Jackson et al. 2009).

Another method of quantifying the sensitivity of herb species to environmental factors is through Ellenberg values, which numerically ranks a plant's affinity to environmental conditions (Ellenberg 1992, Hill 1999). Ellenberg values were created for central European vascular plants (mainly in Germany) and some parts of western Europe, but this concept has been applied outside of central Europe. For example, a study in Sweden found that Ellenberg values were useful at predicting soil pH requirements, carbon (C) to nitrogen (N) ratios, and site average N concentration, but were ineffective at predicting other environmental variables (Hedwall et al. 2019). The researchers also concluded Ellenberg values could be useful in the northern boreal Norway spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) forests of Central Europe ,but caution that the predictive power of Ellenberg values decrease in northernmost regions of central Europe (Hedwall et al. 2019). This approach has also been used in the North American Appalachian Mountains to identify species sensitive to changes in N conditions (Walter et al. 2017).

Vernal dam hypothesis

The herb layer can retain nutrients that would otherwise be lost from an ecosystem. At Hubbard Brook Experimental Forest in the White Mountains of New Hampshire, through studying the yellow trout-lily (*Erythronium americanum* Ker.), Muller and Bormann (1978) hypothesized *E. americanum* stores nutrients that would have been leached through snowmelt between the winter and summer seasons, and releases these nutrients through decomposition during the summer. These results were supported by Eickmeier & Schussler (1993) with another spring ephemeral species, Virginia spring beauty (*Claytonia virginica* L.), in Radnor Lake State National Area in Nashville, Tennessee (Eickmeier and Schussler 1993). An experimental test of the vernal dam hypothesis failed to find an difference in N released through decomposition or N uptake rates between herb removal treatments and control plots (Rothstein 2000).

Environmental factors that drive herb diversity in forests

Plants rely on a variety of resources, such as light, water, and nutrients. Slope aspects may play a role in differences in herb cover, as the cooler and wetter conditions of southern facing slopes may support a greater abundance and diversity of herbs. Northeast to south-southeast aspects had higher herb cover than south-southeast to west-southwest aspects at Hubbard Brook Experimental Forest in New Hampshire (Siccama et al. 1970) and in the eastern deciduous forests of Ohio (e.g. Small & McCarthy, 2005). In the mixed hardwood forests of Fernow Experimental Forest in West Virginia, there were no significant differences between species richness, cover, Shannon diversity, and species evenness between south-facing and north-facing aspects (Smith and Stephan 2021). Soil moisture was not found to influence herb cover at Fernow as well (Gilliam 2019).

Light may also play a role in shaping herbaceous communities. Trees intercept a large portion of light that reaches the ground layer of forested ecosystems, and depending on forest canopy cover, light may be a resource that herb plants compete for. Due to this, understory plants are shade-tolerant in nature (Balandier et al. 2022). Light availability can change in a forest due to succession or disturbances; in the case of northeastern hardwood forests, a relatively undisturbed ecotype compared to more fire-prone western US forests, tree fall creates canopy gaps that can increase herb diversity and density. In Hungarian beech forests, the artificial creation of small or large canopy gaps increased species richness and cover due to higher light availability (Gálhidy et al. 2006).

There is debate on whether light heterogeneity or light quantity are the main drivers of species diversity. A study conducted in temperate forests of the northeastern lowlands of Germany found that an increase in light availability, rather than light heterogeneity, increases herb species richness and cover (Dormann et al. 2020). Similar results have been reported in mixed temperate forests of Hungary (Márialigeti et al. 2016). Age may also play a role in determining whether light availability or light heterogeneity shape herb communities, as light heterogeneity is important in mature stands (Bartels and Chen 2010).

Nutrient availability and the herbaceous layer

Nitrogen

Much attention has been devoted to the influence of N on ecological processes due to concerns of N saturation via atmospheric N deposition (Simkin et al. 2016). In the United States, there has been an effort to determine whether increased N or sulfur deposition led to higher, lower, or unchanged probabilities of occurrences for 348 herbaceous species through generalized linear mixed models; the results of this analysis found that 56% of the 348 herb species evaluated

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(e.g. *Uvularia sessilifolia, Actaea rubra,* or *Galium triflorum*) are at risk of decline through increased N deposition (Clark et al. 2019). A worldwide meta-analysis of herbaceous plant communities in N addition studies found fewer species after N addition (Midolo et al. 2019, Soons et al. 2017). However, these worldwide meta-analyses often have few studies conducted in forest ecosystems. For example, 11 of the 189 studies from Soons et al. (2017) were in tropical and wet forests, and none of the studies in the meta-analysis were conducted in temperate forests.

In the case of herbaceous communities, a long-term N addition experiment at Fernow Experimental Forest in West Virginia resulted in reduced species richness in central Appalachian hardwood forests (Gilliam 2019, Gilliam 2006, 2021, Walter et al. 2017). The N homogeneity hypothesis posits that species adapted to N-limited conditions are outcompeted by species adapted to N-rich conditions due to fewer differences in N content across a site (Gilliam 2006). This hypothesis has been supported by studies done at Fernow, where N fertilized plots eventually experienced species decline after 25 years of fertilization (Gilliam 2019) but not after 17 years of N fertilization (Gilliam 2006). However, other factors may need to be considered in this conclusion; for instance, studies done at Fernow use ammonium sulfate in N fertilized plots, which may have acidified soils. Additionally, the cover of *Rubus* spp., a nitrophilic plant genus, depended on the interaction between light and N fertilization, as *Rubus* spp. would have higher cover in N-fertilized, open canopy plots (Walters et al. 2016).

The herb layer may be the first stratum to respond to N enrichment due to rapid nutrient turnover rates relative to other forest substrates (Elliott et al., 2015; Rawlik et al., 2021). In a Mediterranean-type ecosystem in the Iberian Peninsula, herb N concentrations were higher in N fertilized plots, while tree foliar nutrient concentrations of the same plots had no detectable changes (El-Madany et al. 2021).

Phosphorus

Phosphorus (P) is also an essential macronutrient in plants, contributing to photosynthesis, respiration, enzyme production, and nucleic acid production (Lambers 2022). In temperate forest ecosystems, not as much research has been devoted to P as to N. Disturbances combined with anthropogenic N deposition may shift temperate forests to be more strongly limited by P than N (Rastetter et al. 2013). Experimentally, a long term NxP full-factorial study found evidence of P limitation in foliage (Gonzales et al. 2023, Gonzales and Yanai 2019, Hong et al. 2022) and tree diameter growth (Goswami et al. 2018).

Some experimental studies have assessed how increased P availability influences forest herb communities. Herb cover increased with P addition by five times relative to control plots (Hedwall et al. 2017), and grass and sedge species richness and cover increased with P addition in Finnish forests (Matkala et al. 2020). In European beech (*Fagus sylvatica* L.) and German Norway spruce (*Picea abies* (L.) H. Karst.) stands, herb species richness increased with increasing soil P concentrations (Rieger et al. 2019).

Calcium

Calcium is especially important to consider in the context of acid rain, which displaces base cations from soil cation exchange sites at rates faster than mineral weathering. An experimental addition of Ca silicate to a watershed at Hubbard Brook Experimental Forest in New Hampshire reversed the effects of soil acidification and resulted in increased aboveground productivity (Battles et al. 2014). Higher grass and subshrub species cover was found in a longterm dolomitic lime addition study to acidic soil sites in Germany, but there were no changes in species richness (Thomas et al. 2019). In contrast, a study conducted in spruce forests of eastern Germany found a sharp increase in herb species richness after liming due to the increased presence of disturbance indicator species (Baumann et al. 2021).

Decreased Ca concentrations as a result of acidity may shape herbaceous communities as well. In the Adirondacks, herb species richness decreased with increased acidity along an acid deposition gradient (Zarfos et al. 2019) and fern ground cover decreased with increased soil organic horizon Ca concentrations (Beier et al. 2012). In terms of experimental studies, few studies have examined how Ca addition affects the herb layer. The results of these studies suggest there may not be a detectable difference in herb communities after liming or Ca addition. The inconsistent response of herb communities to Ca addition and a lack of published studies on the effects of Ca addition on hardwood herbaceous communities warrants a more intensive study into this topic.

Conclusion

While the herbaceous layer comprises a small portion of forest biomass, herbs can provide insight to some ecological processes. Most nutrient addition studies have focused on one nutrient's effect on herb diversity, but there are multiple factors that influence herbaceous layer diversity and composition in a forest, including the interactions between nutrients, light availability, and moisture. Understanding the interactions among these factors may assist our understanding of herbaceous layer development.

CHAPTER 2: NUTRIENT TREATMENT EFFECTS ON NORTHERN HARDWOOD HERBACEOUS LAYERS

Introduction

While the herbaceous layer comprises less than 1% of total biomass in northeastern hardwood ecosystems (Muller 2003), it plays an important role in forest function. Up to 90% of a hardwood forest's plant diversity is contained in the herbaceous layer (Gilliam 2007). Additionally, herbaceous plants play key roles in cycling nutrients (Muller 2003), producing litter (Gilliam 2007), and supporting insect communities (Proctor et al. 2012). The sensitivity of the herb layer to disturbances allows for a rapid assessment of site nutrient availability and past history of disturbances, making it a valuable tool for forest management (Small and McCarthy 2005). For instance, changes in nutrient regime may be better detected in herbaceous tissue chemistry than in foliage – a study in Mediterranean savanna ecosystems found significant increases in herbaceous tissue chemistry after two years of fertilization, but no significant changes was observed in foliar nutrients of trees (El-Madany et al. 2021).

One topic of interest in northeastern hardwood forests is how nitrogen (N) availability influences ecosystem functioning. At the Fernow Experimental Forest in the central Appalachian Mountains, N fertilization eliminated herb species adapted to N-limited conditions, reducing forest diversity (Gilliam 2006, Gilliam, Welch, et al. 2016, Walter et al. 2017). In Swedish boreal forests, bryophyte cover increased with increased N application rates, but no increases in total cover were detected (Olsson and Kellner 2006).

Tissue concentrations have been measured to assess the role of herbaceous plants in biogeochemical processes. For two herb species at Fernow, *Viola rotundifolia* (Michx.) and *Rubus allegheniensis* (Porter), N addition increased potassium (K) concentrations, and decreased calcium (Ca) and magnesium (Mg) concentrations. *Viola rotundifolia* did not exhibit the same significant decreases in manganese (Mn), iron (Fe), boron (B), copper (Cu), zinc (Zn), and aluminum (Al) observed in *R. allegheniensis* leaves due to enhanced mobility of micronutrients through N addition (Gilliam et al. 2016). These changes in leaf chemistry may have implications for nutrient cycling, as the herb layer may have higher element concentrations than woody species (Schulze et al. 2009) and decompose quickly (Elliott et al. 2015).

While the effects of N availability have been well documented, the influence of phosphorus (P) and Ca availability on the herb layer have not been as well studied.

In terms of Ca availability, there have been some observational studies documenting herb layers in soil Ca gradients. In northern hardwood forests of the Adirondacks in New York, sites with higher soil Ca had lower fern cover, but the differences were not significant (Beier et al. 2012). Another study in the Adirondacks found increases in species richness along an increasing Ca gradient (Zarfos et al. 2019). A few studies have documented the response of herb layers to liming, which is a Ca carbonate addition. Liming caused sharp increases in bryophyte species characteristic of disturbances were observed in German spruce forests of the Ore Mountains (Baumann et al. 2021) but no detectable differences on species richness of herbs in Central European temperate forests (Thomas et al. 2019). In the case of P, higher soil P availability increased herb layer diversity in European beech (*Fagus sylvatica* L.) or Norway spruce (*Picea abies* (L.) H. Karst) forests in central Europe (Rieger et al. 2019).

It is yet unknown whether the combined effects of N and P addition will produce different results. A worldwide meta-analysis of 189 long-term fertilization experiments in terrestrial and wetland ecosystems found 70% of experiments focused on N addition, with only 16% of experiments on P fertilization, and 14% of experiments on combined N and P

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fertilization (Soons et al. 2017). Across these 189 experiments, N and N+P addition decreased herb species richness, while P addition did not produce a noticeable effect on herb species richness (Soons et al. 2017). However, none of the studies used in the meta-analysis were conducted in temperate forests, which could respond differently to combined N+P addition. For example, an experimental study in southwestern Swedish forests found that N and P addition doubled the number of vascular plant species in the herb layer (Hedwall et al. 2017).

The Multiple Element Limitation in Northeastern Hardwood Ecosystems (MELNHE) study in New Hampshire experimentally tests for N and P nutrient colimitation, and has found effects of N and P addition on soil nutrients (Fisk et al. 2014), increased root growth with N addition (Shan et al. 2022), and P limitation from foliar chemistry (Gonzales et al. 2023, Hong et al. 2022) after long term N and P additions. This study provides the opportunity to understand how N, P, and Ca addition influences herbaceous communities in northern hardwood forests.

In this study, I assessed whether herb communities in ten stands of the MELNHE study changed after 11 years of N and P fertilization. I assess whether N, P, N+P, or Ca addition influence herb species richness, total abundance, diversity, or cover, and whether herb leaf elemental concentrations change with long-term nutrient manipulation.

Methods

Site Description

This study was conducted in 11 experimental stands distributed across the three sites of the MELNHE project in the White Mountains of New Hampshire (Yanai et al., 2022): two young, one mid-aged, and two mature stands at Bartlett Experimental Forest (BEF; 44°03'N, 71°17'W), one mid-aged and one mature stand at Hubbard Brook Experimental Forest (HBEF; 43°56'N, 71°44'W), and one mid-aged and one mature stand at Jeffers Brook (JB; 44°02'N,

71°53'W) (Table 1). The climate is humid continental, with an annual average precipitation of 127 cm and an average temperature of 5.6°C at Bartlett (Adams et al. 2008). Soils are Spodosols formed from glacial drift with thick organic horizons (~5 cm, Vadeboncoeur et al. 2012) and vary in drainage characteristics (Vadeboncoeur et al. 2012, 2014).

Tree species composition is typical of northern hardwood ecosystems (Table 1), with mature stands dominated by sugar maple (*Acer saccharum* Marsh.) and American beech (*Fagus grandifolia* Ehrh.), mid-aged stands dominated by beech, sugar maple, white birch (*Betula papyrifera* Marsh.), and yellow birch (*B. alleghniensis* Britton.), and young forest stands dominated by white birch and pin cherry (*Prunus pensylvanica* L.f) (Table 1).

Four treatment plots were established in each stand and treated annually with N (30 kg N ha^{-1} year⁻¹ as NH₄NO₃), P (10 kg P ha^{-1} year⁻¹ as NaH₂PO₄), N and P (same application rates), or no fertilization (as a control) beginning in 2011. Most stands have a 50 m by 50 m treatment plot comprised of a 30 m by 30 m measurement area with a 10-m buffer on each side; due to space constraints, plots of the mid-aged stands in Jeffers Brook (JBM) and Hubbard Brook (HBM) are 30 m by 30 m, comprised of a measurement area of 20 m by 20 m and a 5-m buffer on each side. Six stands in the MELNHE sites have an additional 50 m by 50 m treatment plot fertilized with a one-time application of Ca (1,150 kg Ca ha^{-1} as CaSiO₃) in 2011, and the mid aged stand at Hubbard Brook (HBM) received the same application rate of Ca in 2015 (Table 1).

Table 1 Characteristics of all stands used in this study. Stands containing a 5th Ca plot are denoted with an asterisk (*). Tree species codes are abbreviated in the USDA Plants Database: FRAM2 - *Fraxinus americana* L., POGR4 - *Populus grandidentata* Michx., FAGR - *Fagus grandifolia* Ehrh., ACRU - *Acer rubrum* L., ACSA - *A. saccharum* Marsh., PRPE2 - *Prunus pensylvanica* L. f., BEPA - *Betula papyrifera* Marsh., BEAL - *B. alleghniensis* Britton.

| Site | Stand | Year cut | Elevation | Aspect | Slope | Basal Area in 2019 (m ² /ha) | | | | | | | | |
|------------------|--------------------|-----------|-----------|--------|-------|---|-------|-------|------|-------|-------|------|-------|--|
| | Stund | i cui cui | (m) | nspeer | (%) | FRAM2 | POGR4 | FAGR | ACRU | ACSA | PRPE2 | BEPA | BEAL | |
| | C1* | 1990 | 570 | SE | 5-20 | 0.04 | 0.00 | 0.24 | 0.03 | 0.00 | 0.26 | 2.45 | 0.00 | |
| | C2 | 1988 | 340 | NE | 15-30 | 0.03 | 0.00 | 1.09 | 2.05 | 0.00 | 0.13 | 0.87 | 0.09 | |
| BEF | C6* | 1975 | 460 | NNW | 13-20 | 0.11 | 0.32 | 3.85 | 9.11 | 1.15 | 0.75 | 7.96 | 7.55 | |
| | C7 | 1890 | 440 | ENE | 5-10 | 1.44 | 0.00 | 20.97 | 0.00 | 13.92 | 0.07 | 0.00 | 1.31 | |
| | C8* | 1883 | 330 | NE | 5-35 | 3.40 | 0.00 | 18.33 | 0.38 | 21.38 | 0.00 | 0.00 | 4.81 | |
| | C9 | 1890 | 440 | NE | 10-35 | 0.00 | 0.00 | 11.87 | 0.00 | 22.15 | 0.00 | 0.00 | 6.42 | |
| | HB-mid (HBM)* | 1970 | 500 | S | 10-25 | 2.88 | 0.00 | 2.27 | 0.09 | 4.30 | 0.00 | 0.51 | 8.55 | |
| HBEF | HB-mature (HBO)* | 1911 | 500 | S | 25-35 | 0.35 | 0.00 | 11.04 | 0.00 | 2.84 | 0.00 | 0.00 | 25.25 | |
| | HBO Ca and Control | 1911 | 500 | SW | 25-35 | 1.51 | 1.09 | 0.58 | 3.32 | 3.59 | 0.08 | 8.26 | 17.30 | |
| Jeffers Brook | JB-mid (JBM)* | ~1975 | 730 | WNW | 25-35 | 0.00 | 0.70 | 0.19 | 0.00 | 4.59 | 0.00 | 7.01 | 12.79 | |
| | JB-mature (JBO)* | 1915 | 730 | WNW | 30-40 | 0.00 | 0.00 | 1.92 | 0.00 | 46.00 | 0.09 | 0.00 | 7.73 | |

Field Methods

In 25 systematically placed 1-m² permanent quadrats in each plot, all vascular plants up to 1 m in height were identified by species and counted. An individual was defined as a single stem growing out of the soil. We noted the presence of additional species outside these 25 quadrats for a more accurate assessment of species richness. Surveys were conducted between June and August in 2010 (pre-treatment) and 2021 (treatment). In 2021, cover by species was assessed by comparing the area of foliar surfaces to an observer's hand (Walters et al. 2015). In 2022, 10 of the stands were resurveyed to evaluate interannual variation from 2021 to 2022.

To characterize light availability, we measured leaf area index from mid-late July 2022 using an LAI-2200 (LI-COR, Lincoln, NE). The average of five LAI measurements were taken from the corner and centers of each treatment plot. Each LAI measurement consisted of an average of three readings taken 2.5 m above the ground.

Soil moisture was measured at the top 5 cm of soil from seven locations of every plot using an ECH20 EC-5 probe (METER, Pullman, WA) in June and July 2014, July and August 2016, and June 2019. Resin-available N (nitrate, NO_3^- , and ammonium, NH_4^+) and P (phosphate, PO_4^-) were measured in plots from 2011-2021 (same methods as Fisk et al., 2014). Net N mineralization rates for all plots were measured in 2009 (Fisk 2022, Fisk et al. 2014). *Laboratory Analyses*

Leaves from the three most ubiquitous herb species (*Trillium erectum* L., *Aralia nudicaulis* L., and *Viburnum lantanoides* Michx.) in our plots were collected in 2010 and 2022 for chemical analysis. Three to four leaves were collected from at least three individuals of each species in each treatment plot. In the case where only two individuals were found in a plot, four leaves were taken from each plant. No leaves were taken if only one individual was present in a

plot. For chemical analyses, all leaves from the same species of each stand and plot were combined, oven-dried at 60°C and ground through a Wiley mill to pass a 40-mesh screen. Nitrogen and carbon concentrations were determined through combustion in a FlashEA 1112 analyzer (Thermo Scientific, Waltham, MA).

Statistical Analyses

Sedges and monocots were primarily found at Hubbard Brook and Jeffers Brook, and contributed to less than 10% of total cover in a plot. These taxonomic groups were omitted from species richness analyses due to a lack of inflorescences for an accurate identification to species, but were kept in analyses of total abundance and cover.

Calcium

We tested the effects of Ca addition on species richness, abundance, total cover, and the effective number of species (the exponential transformation of the Shannon-Weiner diversity index) in 2021. The fixed effects for all models were Ca addition (Ca or no Ca added), stand age (young, mid, or mature), median soil moisture, and site as predictor variables. The random effect for this model was stand. Residuals of species richness, abundance, and cover were normally distributed.

Nitrogen and Phosphorus

Species richness, abundance, and the effective number of species were analyzed as response variables in in analyses of covariance (ANCOVAs), with LAI, soil moisture, resinavailable N, resin-available P, and pretreatment community metrics as covariates. The predictor variables for all models were N addition (N or N+P plots), P addition (P or N+P plots), stand age (young, mid, or mature) and site, along with the two-way interaction between N and P addition, with stand as a random effect. All models had homogeneity of variance, tested through a Levene test.

Herb cover was analyzed using ANOVA with predictor variables of N addition, P addition, the interaction between N and P addition, and stand age, with LAI, soil moisture, resin N, and resin P as covariates and stand as a random effect.

Assessing the Effects of N, P, and Ca on Environmental Factors

We analyzed the environmental measurements used as covariates, namely LAI, soil moisture, resin P, and resin N, were analyzed in ANOVA. The fixed effects were N addition, P addition, their interaction, stand age, and site. Stand was a random effect.

Herb Community Composition

Herb community data were converted into a dissimilarity matrix through Bray-Curtis ordination, which assesses differences in herb species composition based on the number of species shared across plots and the total numbers of individuals counted at each site. The dissimilarity values of each species across all plots were then plotted onto a two-dimensional non-metric multidimensional scaling (NMDS) plot. A permutational multivariate analysis of variance (PERMANOVA) on the dissimilarity matrix used in the NMDS plot was used to statistically test whether treatment (N, P, N+P, or no fertilization), survey year, site, and age class leads to distinct herbaceous communities. The stress values were 0.15 in 2010, and 0.17 for 2021.

To identify herb species characteristic of sites (BEF, HBEF, or JB), N addition, P addition, and stand age class (young, mid-aged, or mature), we used an indicator species analysis through the *indicspecies* package in R (De Cáceres and Legendre 2009).

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To group species indicative of N addition, we used 2009 soil N mineralization rates for 2010 data and 2021 resin-available NH_4^+ and NO_3^- for post-treatment (2021) data. To group species indicative of P addition, we used 2009 bicarbonate extractable P for 2010 and resin-available PO_4^- for 2021. P-values were adjusted to account for the multiple permutations used in the indicator species analyses (Benjamini and Hochberg 1995).

Herb C and N Content

To assess differences in N leaf concentrations for *Trillium erectum, Aralia nudicaulis,* and *Viburnum lantanoides*, we used ANCOVA tests for each species with pretreatment leaf N concentrations as a covariate. The predictor variables were N addition, P addition, the two-way interaction between N and P addition, and stand age and the random effects were stand nested within site.

Residuals for all models were normally distributed, based on Shapiro-Wilk tests. When significant effects were found in the ANCOVA tests ($\alpha = 0.05$), a Tukey's post-hoc test was conducted. All analyses were conducted in R 4.1.0 using the *vegan* package.

Results

Response of Herb Species Richness, Abundance, Cover, and Diversity to Nutrient Addition

Ten years following wollastonite addition, there were 29% fewer species in Ca plots than their associated control plots (p = 0.0001 for the main effect of Ca) across 7 northern hardwood stands (Figure 1). There were no pretreatment measurements taken in the Ca plots. Leaf area index was not a significant covariate in explaining species richness differences in Ca and control plots (p = 0.94). No soil moisture and resin strip data were collected at Ca plots.

Ten years of N addition resulted in 28% fewer species compared to plots receiving no N addition across 11 northern hardwood stands (p = 0.01 for the main effect of N addition) (Figure

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2). Phosphorus addition did not produce detectable differences in species richness (p = 0.96), and there was no interaction between N and P addition (p = 0.84). Pre-treatment species richness in 2010 was positively correlated with post-treatment species richness in 2021 (p < 0.001) and LAI was negatively correlated with post-treatment species richness (p < 0.01).

The effective number of species was 7% lower under N addition (p = 0.46) and 13% lower under P addition (p = 0.13), but in NP plots, the effective number of species was 18% lower than controls, resulting in a significant interaction of N and P addition (p = 0.04). Neither covariate, the pretreatment effective number of species (p = 0.13) nor LAI (p = 0.11) was a significant predictor of the effective number of species in 2021.

Total herb abundance was not sensitive to N addition (p = 0.09), P addition (p = 0.18), the interaction of N and P addition (p = 0.77), or Ca addition (p = 0.29). Cover was insensitive to N (p = 0.27), P (p = 0.39), the interactive effects of N and P addition (p = 0.29), and Ca addition (p = 0.30). Pretreatment abundance (p < 0.01), soil moisture (p = 0.02), and LAI (p < 0.001) were significant covariates for predicting post-treatment abundance.



Figure 1 Species richness of Ca vs control plots in 2021. The black line is a 1:1 line; stands with lower species richness in Ca plots lie below this line. See Table 1 for defined abbreviations.



Figure 2 Species richness of plots in 2021 compared to 2010 (pretreatment). The dashed line indicates a 1:1 line; plots that lost species lie below this line, and those that gained species lie above this line.

Stand Age

For the seven stands with Ca and control plots, mature stands had 77% more species than mid-aged stands, and 47% more species than young stands (p = 0.03). Total abundance was 18 times higher in mature stands than in mid-aged or young stands (p < 0.001). Cover (p = 0.14) and effective number of species (p = 0.82) did not vary across stand ages.

For all eleven stands receiving N×P addition, mature, mid-aged, and young stands did not have detectable differences in species richness (p = 0.61), abundance (p = 0.70), effective number of species (p = 0.12), or cover (p = 0.63).

Effects of N, P, and Ca addition on environmental measurements

Leaf area index did not vary consistently with N addition (p = 0.19) or P addition (p = 0.31) but declined with stand age (p = 0.01); young stands had a 9% higher average LAI than mid-aged stands and 21% higher LAI than mature stands. Leaf area index did not vary consistently across sites (p = 0.49).

Soil moisture was 1% lower (in units of volts) under N addition (p = 0.40) and 2% lower under P addition (p = 0.22). NP plots were 2% volts higher than controls, resulting in a significant interaction of N and P addition (p = 0.04) (Figure 3). Jeffers Brook was 13% wetter than Hubbard Brook and 16% wetter than Bartlett (p < 0.001). Soil moisture did not vary consistently with stand age (p = 0.21).

Resin-available N was increased four-fold in N addition plots (p < 0.001) and decreased by 14% in P addition plots (p = 0.08), consistent with earlier reports in the MELNHE study (M. C. Fisk et al., 2014). Resin-available N did not vary consistently with stand age (p = 0.99) or site (p = 0.54). Resin-available P was increased six-fold in P addition plots (p < 0.001) and decreased by 58% in N addition plots (p = 0.09). Resin-available N did not vary consistently with stand age (p = 0.12) or site (p = 0.21).



Figure 3 Average median soil moisture from all plots of all surveyed stands. Each point represents the average median soil measurement of a treatment plot taken from two sampling periods in 2014, one sampling period in 2016, and one sampling period in 2017.

Herbaceous Community Composition

In this study, 82 herb species were encountered. Herb communities were compared over an 11-year (2010 to 2021) and 1-year (2021 to 2022) period. There were 33% fewer herbaceous species after 11 years of nutrient additions (p = 0.001 for the main effect of year in the PERMANOVA). Herb composition was different among sites (p = 0.001) and stands (p =0.001). Herb communities were also different across age classes (p = 0.001) (Figure 4). Community composition did not change significantly between 2021 and 2022, (p = 0.29), suggesting that the difference between 2020 and 2021 reflected long-term change and not interannual variability. Young stands were more dissimilar between 2010 and 2021 than older stands (p = 0.001 for the interaction between sampling year and stand age).

The most abundant species varied by year and stand (Table 2). A full table of all species present at each stand can be seen in Appendix A.

The group factors were stand age class, N addition plots (N, N+P plots), P addition plots (P or N+P plots), and site, while the quantitative environmental measurements were soil moisture, soil N, and soil P. The indicator species analyses identified species characteristic of site, stand, and stand age, but not N or P addition (Table 3). Twenty one of the 86 species encountered were indicative of Jeffers Brook, while four species were indicative of Hubbard Brook (Table 3). Hobblebush (*Viburnum lantanoides*) was found to be indicative of mature aged stands while stiff clubmoss (*Spinulum annotinum*) was found to be indicative of young stands (Table 3). No indicator species were found between the Ca addition and their associated control plots.

| | | C1 | | | C2 | | | C6 | | | C7 | | | C8 | | | С9 | | HI | BCa | | HBM | | | нво | | | JBM | | | JBO | |
|--------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Species | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 | 2010 | 2021 | 2022 |
| Dryopteris intermedia | 0 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0.04 | 0 | 0 | 0 | 0 | 0 | 0.05 | 0 | 0 | 0 | 0.44 | 0.48 | 0 | 3.08 | 3.42 | 0 | 2.54 | 3.25 | 0.4 | 2.83 | 3.31 | 1.62 | 8.65 | 5.65 |
| Tiarella cordifolia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.46 | 2.56 | 1.99 | 3.35 | 8.34 | 7.21 |
| Oxalis montana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.76 | 1.16 | 0 | 0 | 0 | 0 | 0 | 0.43 | 1.18 | 0.98 | 1.17 | 4.47 | 4.24 | 2.21 |
| Huperzia lucidula | 0.04 | 0 | 0 | 0.39 | 0 | 0 | 0 | 0.67 | 0.32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.9 | 2.23 | 0.51 | 0.86 | 0.43 | 0.25 | 0.29 | 0.08 | 0.71 | 0.13 | 0.12 | 2.52 | 2.35 | 0 |
| Maianthemum canadense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.17 | 1.29 | 0 | 0 | 0 | 0.32 | 0.02 | 0 | 3.14 | 0.83 | 2.01 | 4.86 | 0.48 | 0.02 |
| Viburnum lantanoides | 0.25 | 0.02 | 0.01 | 0.03 | 0 | 0.01 | 0.04 | 0.17 | 0.04 | 1.08 | 0.14 | 0.22 | 0.79 | 0.69 | 0.08 | 0.37 | 0.59 | 0.54 | 0.08 | 0.4 | 0 | 0.11 | 0.08 | 1.07 | 1.01 | 1.09 | 0.15 | 0.2 | 0.27 | 0.82 | 1.5 | 0.6 |
| Taxus canadensis | 0 | 0 | 0 | 0 | 0.03 | 0 | 0 | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 1.23 | 3.59 | 4.05 | 3.37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Uvularia sessilifolia | 0 | 0.12 | 0.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0.12 | 0 | 0.25 | 0.02 | 0 | 0 | 0 | 0.72 | 1.16 | 0 | 1.48 | 2.57 | 0 | 0.29 | 0.23 | 0 | 0.21 | 0.52 | 0 | 0.07 | 0.33 |

Table 2 Nine most abundant species (stems per 100 m² of quadrats) of species in the study by stand and date.

| | | S | Site | Age | | | | | | | | |
|------------------------------|--------------------|-------------|-------------------------------|--------------------|-------------|-----------------------|--------------------|-------------|-------------------------|--------------------|-------------|--|
| Hubba | rd Brook | | Jeffers | Brook | | Y | Young | | Mature | | | |
| Species | Indicator Value | p- value | Species | Indicator Value | p- value | Species | Indicator Value | p- value | Species | Indicator Value | p- value | |
| Aster acuminatus | 0.38 | 0.01 | Actaea rubra | 0.47 | 0.001 | Spinulum annotinum | 0.46 | 0.11 | Viburnum lantanoides | 0.51 | 0.11 | |
| Dennstaedtis punctilobula | 0.33 | 0.03 | Amelanchier sp. | 0.38 | 0.001 | | | | | | | |
| Huperzia lucidula | 0.35 | 0.03 | Arisaema triphyllum | 0.41 | 0.002 | | | | | | | |
| Trillium undulatum | 0.47 | 0 | Clintonia borealis | 0.71 | 0.001 | | | | | | | |
| | | | Dryopteris carthusiana | 0.42 | 0.001 | | | | | | | |
| | | | Dryopteris intermedia | 0.48 | 0.001 | | | | | | | |
| | | | Lactuca floridana | 0.43 | 0.001 | | | | | | | |
| | | | Lysimachia borealis | 0.44 | 0.001 | | | | | | | |
| | | | Maianthemum racemosum | 0.49 | 0.001 | | | | | | | |
| | | | Maianthemum canadense | 0.66 | 0.001 | | | | | | | |
| | | | Oxalis montana | 0.56 | 0.001 | | | | | | | |
| | | | Phegopteris connectilis | 0.45 | 0.001 | | | | | | | |
| | | | Polystichum acrostichoides | 0.33 | 0.041 | | | | | | | |
| | | | Rubus sp. | 0.27 | 0.011 | | | | | | | |
| | | | Solidago flexicaulis | 0.37 | 0.002 | | | | | | | |
| | | | Streptopus lanceolatus | 0.55 | 0.001 | | | | | | | |
| | | | Streptopus roseus | 0.49 | 0.001 | | | | | | | |
| | | | Tiarella cordifolia | 0.6 | 0.001 | | | | | | | |
| | | | Trillium erectum | 0.45 | 0.002 | | | | | | | |
| | | | Urtica dioica | 0.43 | 0.001 | | | | | | | |
| | | | Viola sp. | 0.28 | 0.041 | | | | | | | |

Table 3 Indicator species of site and stand ages observed in this study. P-values were adjusted using the Bonferroni method.



Figure 4 Non-metric multidimensional scaling (NMDS) plot of pretreatment and post treatment herbaceous community composition, with each point representing a treatment plot (control, N, P, or NP). More separation between points indicates dissimilarity between communities.

Regional Differences Among Plant Communities

For control and Ca addition plots of the seven stands receiving Ca addition, Jeffers Brook had 70% more species than Hubbard Brook, and twice the number of species than Bartlett Experimental Forest (p < 0.001). Abundance at Jeffers Brook was 2.3 times higher than Hubbard Brook, and 10 times higher than Bartlett (p < 0.001). Cover at Jeffers Brook was twice that of Hubbard Brook, and 20-times higher than Bartlett (p = 0.01). The effective number of species was not consistently affected by Ca addition (p = 0.09).

For stands N×P addition, sites did not differ in species richness (p = 0.31), abundance (p = 0.21), effective number of species (p = 0.57), or cover (p = 0.09).

Effects of N and P Addition on Leaf C and N Content

The three herb species that were found in all 11 stands were analyzed for tissue chemistry. Leaf N concentrations increased by 15% for wild sarsaparilla (*Aralia nudicaulis*) with N addition (p = 0.004) and decreased by 13% with P addition (p = 0.01) (Figure 5). Red trillium leaf N did not vary consistently with N addition (p = 0.13) or P addition (p = 0.14) and there was no significant interaction between N and P addition (p = 0.56). Hobblebush leaf N also did not vary consistently with N addition (p = 0.26) or P addition (p = 0.61) and there was no significant interaction between N and P addition (p = 0.82). Pretreatment leaf concentrations were significant in explaining variation in post-treatment leaf concentrations in all three models.



Figure 5 Leaf N concentrations of wild sarsaparilla (*Aralia nudicaulis*), with points colored by treatments of each stand. Error bars are not present due to all samples being composited before laboratory analyses.

Discussion

Nutrient Effects on Herb Community Metrics

Nitrogen addition decreased species richness, which is consistent with other N fertilization experiments in grasslands (Harpole et al. 2016) and forests (Gilliam et al. 2016) and a worldwide meta-analyses of N fertilization experiments (McDonnell et al. 2020, Simkin et al. 2016). In the case of Jeffers Brook, where herb layers are more dense, increased N availability may increase the competitive ability of certain nitrophilous species, eliminating herb species that are unable to compete (Gilliam 2006). Certain herbs, such as perennials, herbs with N-fixing symbioses, or native herbs could be more susceptible to extinction with N addition (Suding et al. 2005). This concept may not apply to sites with sparse herb layers, such as Bartlett or Hubbard Brook. Instead, environmental factors, such as light, may play more of a role in shaping herb communities (Fahey et al. 1998).

Phosphorus addition did not produce a detectable effect on species richness, which is consistent with a study of European beech and Norway spruce forests in middle and northern Germany (Rieger et al. 2019).

We observed fewer herb species in Ca addition plots, which was surprising as soil Ca was positively correlated with species richness in the Adirondack Mountains of New York (Zarfos et al. 2019). A study in the boreal forests of Finland found 30% more herb species at locations with high soil Ca (Närhi et al. 2011). More herb species were also found in regions with high Ca in hardwood forests of the Adirondack Mountains of New York, although there was not a strong association between species richness and soil Ca concentrations (Beier et al. 2012). The lower number of species in Ca plots may be due to the lower representation of stands in Ca addition plots relative to stands receiving NxP addition, or reflective of exchangeable Ca differences. The lack of a difference in species richness between Ca and control plots may also be due to the limited dispersal of herb layer plants in general, which would prevent some species from colonizing more suitable conditions (Hughes and Fahey 1991). From a methodological standpoint, the Ca plots were established after fertilization treatments began and were not randomly assigned. This would increase the risk of Ca stands being different from stands receiving N×P fertilization, despite our efforts to keep all plots as similar as possible.

We found total herb cover to be higher in mature stands than mid age stands, consistent with findings from nine sites in the southern Appalachian Mountains, where cover of spring flora was higher in mature, primary forest stands than younger, secondary forest stands (Duffy and

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Meier 1992). In contrast, at Fernow, 50 year-old stands had higher herb cover than 110 year-old stands, which was not explained by LAI or canopy cover (Smith and Stephan 2021).

Herb communities were apparently stable over a one-year period (2021-2022). No significant interannual variation in the herb layer was also observed in other studies of this ecotype (Gilliam 2006, Hughes and Fahey 1991), which could be a product of the low dispersal capabilities and longevity of herbs found in northern hardwood forests (Neufeld and Young 2014), the vast majority of which are perennials.

Herb chemical concentrations

Wild sarsaparilla (*Aralia nudicaulis*) increased in leaf N concentration with N addition, and decreased in leaf N concentrations with P addition, which together may imply N-limitation. Decreased P concentrations of *A. nudicaulis*, but not increased N concentrations, were detected after 6 years of N fertilization in Canadian boreal forests (Jung et al., 2018). The increased N concentrations of *A. nudicaulis* in this study may be due to an extended fertilization duration relative to this study.

Environmental factors and herb community composition

Total abundance was higher in wetter plots, consistent with findings in European beech forests (Gálhidy et al. 2006), Appalachian hardwood forests (Smith and Stephan 2021) and Hubbard Brook Experimental Forest in New Hampshire (Siccama et al. 1970). Species richness and the effective number of species were not sensitive to large-scale measurements soil moisture in this study, as in other studies that measured soil moisture at in other studies (Gilliam 2019, Zarfos et al. 2019). While our study measured soil moisture at a 30 m scale, the herb layer may respond to fine scale variation in soil moisture. A study conducted in Hungarian European beech (*Fagus sylvatica* L.) dominated stands found increased total herb cover and species richness in response to soil water content measurements done at a scale of 5 m (Gálhidy et al. 2006) *Summary*

This is the first documentation of changes in herb communities under N and P addition in a temperate forest ecosystem. Our results support findings from Fernow Experimental Forest and meta-analyses concerning the role of nutrient addition on herb layer composition. Fewer herb species due to N addition could be the result of nitrophilous herbs outcompeting non-nitrophilous herbs in regions with denser herb layers, but light availability may play a larger role in shaping sparse herb layers. Calcium addition plots had fewer species than their associated controls, potentially due to limited dispersal of herb layer species in this ecotype or calciphilous plants outcompeting non-calciphilous herb layer species.

Chapter 3: Conclusions and Reflection

Overview

Chapter 1 presents a case for why herbaceous plants are important in northern hardwood ecosystems and demonstrates how understudied this stratum is in ecological research. The effects of N addition on herb layers have been well documented, while the effects of P and Ca addition on herb layers have not been as well studied, despite the importance of these two elements in ecosystem functioning. I also examine whether resource heterogeneity or resource availability shape herb communities in stands of different ages.

Chapter 2 of my thesis assesses whether N, P, or Ca addition have changed herb layer composition over a decadal time period. Calcium addition decreased species richness by 28%, N addition decreased species richness by 29%, and there was a decrease in diversity when N and P were added together relative to that predicted by the main effects of N and P addition. *Limitations of the study*

It would be useful to know the extent of soil acidification from N fertilization in the MELNHE study. Application rates of 50 kg N ha⁻¹ yr⁻¹ acidified soils at Harvard Forest as early as 12 years after N treatments began (Evans et al. 2008), and it seems likely that our application rate of 30 kg ha⁻¹ yr⁻¹ would eventually produce an acidifying effect. Forest floor pH in 2012 did not vary consistently in N addition plots to other treatment plots (Fisk et al. 2014); additional measurements are planned for summer 2023. Notably there was not a detectable effect of N addition on exchangeable base cations measured in 2017 (Walsh 2022).

Opportunities for future study

No differences in herb community composition were detected between 2021 and 2022. A resurvey after a longer span of time could provide insights on whether herb community composition changes further because of long-term N and P addition.

Quadrat-level environmental measurements, such as light transmittance, soil moisture, and soil N and P content, could shed light on small-scale variation in species richness and cover. These additional measurements could lead into a study of whether resource heterogeneity or availability shape herbaceous communities (Bartels and Chen 2010, Gilliam 2019).

More accurate stem maps are being developed for the MELNHE stands, which could be used with quadrat placements to understand whether surrounding tree composition influences herb layers.

Novel contributions of my study

This is the first documentation of herb communities under N and P addition in a temperate forest ecosystem. Comparing herb communities from the same quadrats used in 2010 and 2021 made it possible to control for significant pre-treatment variation among plots, and the comparison of measurements from 2021 and 2022 us to assess interannual variability in hardwood herb layers.

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Appendices

Appendix 1 List of all herbaceous species observed, by sampling year and site, divided by taxonomic group (lycopods, woody angiosperms, herbaceous angiosperms, ferns, and graminoids). Nomenclature follows the USDA Plants Database (<u>https://plants.usda.gov/</u>). BEF = Bartlett Experimental Forest, HB = Hubbard Brook Experimental Forest, JB = Jeffers Brook.

| Constan Name | Year | | | | | | | | | | | |
|--------------------------------|----------------|-------------|-------------|--|--|--|--|--|--|--|--|--|
| Species Name | 2010 | 2021 | 2022 | | | | | | | | | |
| | Lycopods | | | | | | | | | | | |
| Lycopodium obscurum | BEF, HB | BEF, HB | | | | | | | | | | |
| Lycopodium clavatum | BEF, JB | | | | | | | | | | | |
| Spinulum annotinum | BEF | | BEF | | | | | | | | | |
| Huperzia lucidula | BEF, HB, JB | BEF, HB, JB | BEF, HB, JB | | | | | | | | | |
| Woody angiosperms | | | | | | | | | | | | |
| Aralia nudicaulis | BEF, HB, JB | BEF, HB, JB | BEF, HB, JB | | | | | | | | | |
| Sambucus racemosa | JB | | | | | | | | | | | |
| Taxus canadensis | BEF | BEF | BEF | | | | | | | | | |
| Viburnum lantanoides | BEF, HB, JB | BEF, HB, JB | BEF, HB, JB | | | | | | | | | |
| Viburnum sp. | BEF | | | | | | | | | | | |
| | Ferns | | | | | | | | | | | |
| Dennstaedtis punctilobula | | BEF, HB | BEF | | | | | | | | | |
| Dryopteris carthusiana | JB | | | | | | | | | | | |
| Dryopteris intermedia | BEF, JB | HB, JB | BEF, HB, JB | | | | | | | | | |
| Parathelypteris noveboracensis | | JB | BEF, HB, JB | | | | | | | | | |
| Osmunda cinnamomea | | HB, JB | | | | | | | | | | |
| Athyrium filix-femina | | JB | | | | | | | | | | |
| Herbac | eous angiosper | rms | | | | | | | | | | |
| Actaea rubra | JB | JB | JB | | | | | | | | | |
| Allium tricoccum | JB | | | | | | | | | | | |
| Amelanchier sp. | JB | | | | | | | | | | | |
| Arisaema triphyllum | | JB | HB, JB | | | | | | | | | |

| Aster sp. | BEF | | |
|----------------------------|-------------|-------------|-------------|
| Caulophyllum thalictroides | | | JB |
| Chimaphila maculata | HB | | |
| Clintonia borealis | JB | HB, JB | HB, JB |
| Cornus alternifolia | JB | | |
| Cypripedium acaule | BEF | | |
| Epifagus virginiana | | BEF | BEF |
| Epipactis helleborine | | JB | BEF, HB, JB |
| Erythronium americanum | JB | | |
| Eurybia sp | | JB | |
| Galium triflorum | | JB | JB |
| Gaultheria procumbens | BEF | | |
| Goodyera pubescens | | BEF | |
| Lactuca floridana | JB | | |
| Lysimachia borealis | HB, JB | | |
| Maianthemum racemosum | BEF, HB, JB | BEF, HB, JB | BEF, HB, JB |
| Maianthemum canadense | HB, JB | HB, JB | HB, JB |
| Medeola virginiana | BEF, HB, JB | BEF, HB, JB | BEF, HB |
| Mitchella repens | BEF, HB | BEF, HB | HB |
| Monotropa uniflora | JB | BEF, HB, JB | BEF, HB, JB |
| Nabalus altissimus | | JB | JB |
| Oclemena acuminata | HB, JB | HB | HB |
| Onoclea sensibilis | JB | | |
| Oxalis montana | JB | HB, JB | HB, JB |
| Phegopteris connectilis | JB | HB, JB | HB, JB |
| Phegopteris hexagonoptera | BEF, HB, JB | | |
| Polygonatum pubescens | | BEF | BEF |
| Polystichum acrostichoides | JB | JB | JB |
| | | | |
| Prenanthes alba | | JB | |

| | | 1 | |
|----------------------------|-------------|-------------|-------------|
| Pyrola eclipta | | JB | |
| Ribes lacustre | | JB | JB |
| Rubus idaeus | | JB | |
| Rubus pubescens | | JB | JB |
| Rubus sp. | JB | JB | |
| Solidago flexicaulis | JB | | |
| Streptopus lanceolatus | HB, JB | HB, JB | HB, JB |
| Symphyotrichum cordifolium | JB | | |
| Tiarella cordifolia | JB | JB | JB |
| Trientalis borealis | | HB, JB | BEF, HB, JB |
| Trillium erectum | BEF, HB, JB | BEF, HB | HB |
| Trillium sp. | BEF | BEF | BEF, HB |
| Trillium undulatum | BEF, HB, JB | BEF, HB | HB |
| Urtica dioica | JB | JB | JB |
| Uvularia sessilifolia | BEF, HB, JB | BEF, HB, JB | BEF, HB, JB |
| Viola pubescens | HB, JB | | |
| Viola rotundifolia | BEF | BEF, HB, JB | BEF, HB, JB |
| Viola sp. | JB | HB, JB | HB, JB |
| (| Graminoids | | |
| Carex intumescens | | JB | JB |
| Carex plantaginea | JB | | |
| Carex platyphylla | | JB | |

Appendix 2 Summary of p-values, directionality, and effect sizes $(in \eta_p^2)$ of all NxP ANCOVA models with response variables of species richness, abundance, effective number of species, and cover. A colon indicates interaction terms. Significant p-values at a = 0.05 are bolded.

| | Response variable | | | | | | | | | | | |
|----------------------|---|---------------------------------|--------------------------------|---|--|--|--|--|--|--|--|--|
| Fixed effects | Species Richness | Abundance | Effective Number of Species | Cover | | | | | | | | |
| Nitrogen | $p = 0.01 \\ \eta_p{}^2 = 0.34$ | p = 0.09 $\eta_p^2 = 0.03$ | p = 0.46 $\eta_p^2 = 0.30$ | $\begin{array}{l} p = 0.85 \\ \eta_{p}{}^{2} < 0.001 \end{array}$ | | | | | | | | |
| Phosphorus | p = 0.96 $\eta_p^2 = 0.01$ | p = 0.18 $\eta_p^2 = 0.09$ | p = 0.13 $\eta_p^2 = 0.10$ | p = 0.50 $\eta_p^2 = 0.06$ | | | | | | | | |
| Nitrogen:Phosphorus | $\begin{array}{l} p = 0.84 \\ {\eta_p}^2 < 0.001 \end{array}$ | p = 0.77 $\eta_p^2 = 0.76$ | $p = 0.03 \\ \eta_p^2 < 0.001$ | $\begin{array}{l} p = 0.68 \\ \eta_{p}{}^{2} < 0.001 \end{array}$ | | | | | | | | |
| Site | p = 0.31 $\eta_p^2 = 0.38$ | p = 0.21 $\eta_p^2 = 0.24$ | p = 0.57 $\eta_p^2 = 0.59$ | p = 0.08 $\eta_p^2 = 0.73$ | | | | | | | | |
| Stand Age | p = 0.61 $\eta_p^2 = 0.93$ | p = 0.71 $\eta_p^2 = 0.76$ | p = 0.12 $\eta_p^2 = 0.70$ | p = 0.55 $\eta_p^2 = 0.51$ | | | | | | | | |
| Covariates | | Res | ponse variable | | | | | | | | | |
| Mean LAI | $p = 0.002 \\ \eta_p{}^2 = 0.40$ | p = 0.34 $\eta_p^2 = 0.10$ | p = 0.23 $\eta_p^2 = 0.30$ | p = 0.26 $\eta_p^2 = 0.06$ | | | | | | | | |
| Median Soil Moisture | p = 0.61 $\eta_p^2 = 0.02$ | $p = 0.01 \\ \eta_p{}^2 = 0.10$ | p = 0.17 $\eta_p^2 = 0.03$ | p = 0.50 $\eta_p^2 < 0.001$ | | | | | | | | |
| Resin N | p = 0.34 $\eta_p^2 = 0.01$ | p = 0.09 $\eta_p^2 < 0.001$ | p = 0.17 $\eta_p^2 = 0.02$ | p = 0.54 $\eta_p^2 = 0.03$ | | | | | | | | |
| Resin P | p = 0.60 $\eta_p^2 = 0.02$ | p = 0.74 $\eta_p^2 = 0.01$ | p = 0.95 $\eta_p^2 = 0.04$ | p = 0.83 $\eta_p^2 = 0.01$ | | | | | | | | |

| | Response variable | | | |
|---------------|--|--|--------------------------------|-----------------------------------|
| Fixed effects | Species Richness | Total Stem Density | Effective Number of Species | Cover |
| Calcium | p = 0.01 $\eta_p^2 = 0.57$ | p = 0.29 $\eta_p^2 = 0.03$ | p = 0.05 $\eta_p^2 = 0.64$ | p = 0.30 $\eta_{p}^{2} = 0.13$ |
| Site | $\begin{array}{c} p = 0.05 \\ \eta_{p}{}^{2} = 0.0.75 \end{array}$ | $\begin{array}{l} p < 0.001 \\ \eta_{p}{}^{2} = 0.94 \end{array}$ | p = 0.74 $\eta_p^2 = 0.43$ | p = 0.02 $\eta_{p^2} = 0.71$ |
| Stand Age | p = 0.51 $\eta_p^2 = 0.69$ | $\begin{array}{l} p < 0.001 \\ \eta_{p}{}^{2} = \textbf{0.90} \end{array}$ | p = 0.82 $\eta_p^2 = 0.59$ | p = 0.14 $\eta_{p}^{2} = 0.78$ |
| Covariates | | | | • |
| Mean LAI | p = 0.005 $\eta_p^2 = 0.17$ | $\frac{p < 0.001}{\eta_p^2 = 0.77}$ | p < 0.001 $\eta_p^2 = 0.79$ | p = 0.36 $\eta_{p^2} = 0.14$ |

Appendix 3 Summary of p-values, directionality, and effect sizes (in η_p^2) from Ca ANOVA models.

Appendix 4 Summary of Average Species Richness, Total Stem Density, Average Effective Number of Species, and Cover for all Nitrogen and Phosphorus ANCOVA models

| Treatment | Average Species Richness (± SE) | Average Total Abundance (± SE) (# stems/m ² ground) | Average Effective Number of Species (± SE) | Average Cover $(\pm SE)$ $(m^2 plant/m^2$ ground) |
|--------------------------|---------------------------------------|--|--|---|
| Control | 7.20 ± 0.69 | 5.36 ± 1.55 | 5.73 ± 0.44 | 15.43 ± 5.96 |
| Nitrogen | 5.14 ± 0.75 | 2.40 ± 1.67 | 6.09 ± 0.49 | 16.41 ± 6.35 |
| Phosphorus | 7.28 ± 0.82 | 8.26 ± 1.92 | 5.79 ± 0.50 | 24.16 ± 6.79 |
| Nitrogen + Phosphorus | 4.98 ± 0.65 | 4.45 ± 1.48 | 4.70 ± 0.41 | 16.17 ± 5.62 |

Appendix 5 Average community metrics (species richness, total abundance, effective number of species, and cover) in the seven stands with Ca addition and control plots.

| Treatment | Average Species Richness (± SE) | Average Total Stem Density (± SE) (# stems/m ² ground) | Average Effective Number of Species (± SE) | Average Cover (± SE) (m ² stems/m ² ground) |
|-----------|---------------------------------------|---|--|--|
| Control | 12.13 ± 1.89 | 9.67 ± 1.60 | 5.40 ± 1.28 | 21.58 ± 6.51 |
| Calcium | 8.58 ± 1.89 | 7.54 ± 1.60 | 4.29 ± 1.28 | 19.14 ± 6.51 |

Appendix 6 Species accumulation curves comparing the number of species encountered as the number of plots surveyed increases in a) 2010 and b) 2021. Shaded grey bands represent the 95% confidence interval of the number of species encountered.



Appendix 7 NMDS plots for a) 2010 and b) 2021, with environmental vectors for N addition, P addition, site, age, and LAI Arrows represent the amount of variation accounted for by LAI, site, and stand age.

a)





Curriculum Vitae

Scott Dai

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EDUCATION

- 2021–2023 M.S. in Forest Resources Management (concentration Ecology & Evolution) SUNY ESF
- 2019 **B.S. in Biology** (concentration Ecology & Evolution), *magna cum laude* The George Washington University

FELLOWSHIPS, GRANTS, & AWARDS

Edna Bailey Sussman Trust Special Merit Award | SUNY ESF
 Luther Rice Undergraduate Research Fellowship | The George Washington University
 Wilbur V. Harlan Research Fellowship | GW Department of Biology

RESEARCH EXPERIENCE

2021–2023 SUNY-ESF | Syracuse, NY

Master's Student | Advisor: Dr. Ruth Yanai

- Developed and implemented an independent two-year project on the effects of nitrogen, phosphorus, and calcium addition influence northern hardwood herbaceous layers.
- Led two summer field crews in a ten-week research internship program researching the effects of nutrient colimitation on northern hardwood ecosystems, organizing fieldwork for fertilization, four intern projects, root biomass collection, soil respiration, and tree inventories.
- Research project assistant for Dr. Ruth Yanai for projects involving uncertainty in carbon accounting and root biomass.
- Compiled and organized datasets for publication to the Environmental Data Initiative (EDI) data repository.
- Organized and planned fall fieldwork for leaf litter collection, tree core extraction, and root biomass sampling.

2020–2021 Archbold Biological Station | Buck Island Ranch, FL

Agroecology Research Intern | Advisors: Dr. Elizabeth Boughton & Dr. Gregory Sonnier

- Research intern for an agroecology program researching the influence of fire, cattle grazing, and nutrient cycling on subtropical ranch management.
- Designed a seed dispersal research project assessing the role of agricultural drainage ditches on seed transportation.
- Assisted in field projects involving legacy soil phosphorus, net primary productivity, wetland vegetation surveys, and soil respiration.

2019 Organization of Tropical Studies (OTS) | South Africa Undergraduate Researcher | Dr. Laurence Kruger, Dr. Lisa Nupen, & Dr. Bernard Coetzee

- Field researcher and research assistant in a study abroad course with OTS South Africa, with emphasis on the roles of fire and herbivory in subtropical savannas.
- Co-developed research studies on faunal biodiversity in rhino middens and riparian invasive species monitoring.
- Assisted in faculty field projects concerning biodiversity after fire disturbances, soil-plant interactions along elevational gradients, and ant communities of vineyard ecosystems.

2017–2019 The George Washington University | Washington, DC

Undergraduate Research Assistant | Advisor: Dr. Keryn Gedan

- Researcher and field assistant for an applied coastal ecology lab researching the influence of sea level rise on salt marsh-forest ecotones and coastal agricultural fields.
- Designed and proposed a funded independent research project on the influence of salinity on weed functional traits.
- Conducted a literature review on seed dormancy and assisted in a greenhouse experiment that assessed the effects of elevated salinity and nutrient regimes on seed germination.
- Assisted in field projects involving salt marsh and forest monitoring plots, deer herbivory, and soil seedbank analysis.

2017 Organization of Tropical Studies (OTS) | Costa Rica

Undergraduate Researcher | Advisor: Dr. Scott Walter

- Field researcher and research assistant in a summer abroad course with OTS Costa Rica, with emphasis on the role of disturbances in tropical rainforests.
- Assisted in a field study that applied the intermediate disturbance hypothesis on spider populations.
- Designed a behavioral ecology research project on the response of acacia ants to physical disturbances.

TEACHING EXPERIENCE

2023

| 2023 | Forest Management and Wild | life SUNY ESF |
|------|-----------------------------|-----------------------|
| | Graduate Teaching Assistant | Dr. Andy Vander Yacht |

- Aided in the development of a new course for SUNY ESF; created a gradebook for assignments, quizzes, and projects, allocated undergraduate to group projects, and provided input on course content.
- Evaluated and graded wildlife management plan projects for 4 undergraduate groups.
- Facilitated weekly debates on topics concerning wildlife habitat management.

Natural Resources Ecology | SUNY ESF

Graduate Teaching Assistant | Dr. John Drake

- Assisted in the planning and implementation of five outdoor field labs on genetically modified willow trees, energy consumption, forest structure, sugarbush, and human demography.
- Graded two lab assignments on energy use and human demography.
- Provided feedback on reflection assignments about climate change, competitive exclusion, and conservation vs. preservation.

2022 Introduction to Forest Soils | SUNY ESF Graduate Teaching Assistant | Dr. Russell Briggs

| | Led 5 field labs on soil horizon identification, topographical influences on soil drainage properties, and soil best management practices. Supervised and led two indoor soils labs on soil texture and soil chemical properties. Graded and provided feedback on ten group reports on soil mapping and soil classification. |
|------|--|
| 2020 | Outdoor Instructor The Outdoor School Taught and led grade 5-9 outdoor lessons in edible plants, water quality, reptile adaptations, and other outdoor skills. |
| 2018 | University Physics I The George Washington University Undergraduate Learning Assistant Dr. Gerald Feldman Co-facilitated an introductory activity-based general physics class of over 100 undergraduates. Assisted students with physics problems, exam preparation, and labs, and scheduled weekly meetings with professors to address course concerns and evaluate student performance. |
| 2017 | Step 1: Introduction to Inquiry-Based Learning The George Washington University Teaching Assistant Dr. Meghan Hollibaugh-Baker & Dr. Alicia Bitler Implemented and provided feedback on student lesson plan creation, classroom management, and organization, while also assisting professors in lesson planning and teaching. |
| 2016 | Breakthrough Fort Worth Summer Teaching Fellow Ms. Nicole Masole Created and taught a chemistry summer class for 8th grade students negatively affected by the achievement gap in education. |

MANUSCRIPTS

Dai, Sonnier, & Boughton. Seed rain through agricultural drainage ditches. In prep.

SCIENTIFIC PRESENTATIONS

- 2023 **Scott Dai.** What's up with herbs? Northern hardwood herbaceous layers after 11 years of NxP addition. *Oral and poster presentation at the NYSAF 2022 Annual Meeting on 26 January 2023 in Syracuse, New York.*
- 2022 **Scott Dai.** Long-term NxP fertilization effects on herbaceous layer communities in ten northern hardwood forest stands. *Poster presentation at ESA 2022 on 15 August 2022 in Montreal, Québec.*
- 2022 **Scott Dai.** What's up with herbs? Checking in on the herbaceous layer after 11 years of NxP addition. *Oral presentation at the Hubbard Brook Ecosystem 59th Annual Cooperators' Meeting on 14 July 2022 at the University of New Hampshire.*

| 2021 | Scott Dai. Herbaceous and Vivacious: How do ground layer plants respond to N by P fertilization? <i>Oral presentation at the Hubbard Brook Ecosystem 58th Annual Cooperators' Meeting on 7 July 2021 in Bartlett, New Hampshire.</i> |
|------|--|
| 2019 | Scott Dai , Tim Kirsten, Emily Rehmann, and Jessica Rehmann. Resource hotspots: Rhino middens support higher arthropod and bird biodiversity. <i>Oral presentation at the 2019 OTS Capstone Projects Seminar on 2 May 2019 at Skukuza, South Africa</i> . |

2018Scott Dai. Functional Traits of Weeds in Saline Agricultural Fields. Poster presentation at the
2018 Harlan Poster Session on 28 September 2018 at The George Washington University.