EFFECTS OF NITROGEN AND PHOSPHORUS ADDITION

ON FOLIAR NUTRIENT CONCENTRATIONS

OF SIX NORTHERN HARDWOOD SPECIES

by

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ABSTRACT

D.S. Hong. Effects of Nitrogen and Phosphorus Addition on Foliar Nutrient Concentrations of Six Northern Hardwood Species, 40 pages, 3 tables, 3 figures, 2019. APA style guide used.

Understanding how trees respond to nutrient availability and allocate nutrients is important in addressing nutrient status and limitation in various ecosystems. While nutrient limitation status is well documented in some ecosystems, it is not consistent in northeastern temperate forests. And few studies have compared responses of multiple species to nutrient availability. In this study, we examined the effects of nitrogen and phosphorus addition on foliar N and P across ten forest stands in the White Mountains of New Hampshire. Unsurprisingly, foliar N and P, overall, increased when the respective nutrient was added; the percent increase in foliar P was much higher than that of foliar N. However, they exhibited decreases when the other nutrient was added. Pin cherry had higher foliar N and P concentrations than American beech, red maple, sugar maple, white birch, and yellow birch. With this information on foliar N and P, we hope to improve upon thresholds of N:P ratios previously reported to indicate N versus P limitation.

Key Words: foliar nutrients, nitrogen, phosphorus, nutrient availability, nutrient limitation.

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Chapter 1: Literature Review

Plant productivity is dependent on light, water, and nutrients and can be limited by any of these resources. Liebig's Law of the Minimum (Liebig, 1842) states that plant growth is limited by the resource in the lowest supply relative to plant demand; if one of the essential plant resources is deficient when others are abundant, plant growth will be constrained (Ulrich & Hills, 1973). The more resource-limited an individual is, the more its productivity increases in response to the addition of said resource (Chapin et al, 1987).

Since the beginning of the Industrial Revolution, biogeochemical cycles have been altered via fossil fuel combustion, agriculture practices, and mining (Galloway et al, 1995; Vitousek et al. 1997; Galloway 2004; Vitousek et al. 2010; Peñuelas et al. 2012). Carbon dioxide (CO₂) has increased by 30% in the atmosphere and the rate of nitrogen (N) input into the terrestrial N cycle has doubled from 1.9 Tg N per year in 1900 to 3.8 Tg N per year in 2000, as more atmospheric N is fixed (Smil, 1990 & 1991; Vitousek & Matson, 1993; Ayers et al, 1994; Galloway et al, 1995; Vitousek et al, 1997; Galloway et al, 2003; Sutton et al, 2011). The availability and the mobility of N has increased over large regions of Earth (Vitousek et al, 1997). While N contributes to plant growth and agricultural productivity as it is responsible for the formation of amino acids, proteins, and DNA of plant tissues, excess N has negative impacts on the environment (Vitousek and Howarth, 1991). In the atmosphere, elevated N input from anthropogenic processes can increase the amount of reactive oxides of N, consequently increasing the concentrations of N_2O_2 , a greenhouse gas (Prinn et al, 1990; Albritton et al, 1995), and producing acid rain and photochemical smog (Chamedies et al, 1994). On the ground, increased N availability enhances productivity and biomass accumulation, which can lead to changes in the global cycle of carbon (C), as more organic C is stored within the terrestrial

systems, further altering the rate of increase of CO₂ in the atmosphere and the response of ecosystems to this increase (Peterson & Melillo, 1985; Aber et al, 1989; Schimel, 1995). Moreover, nitrates can leach through soils, resulting in losses of soil cations such as calcium and potassium and increases in soil and water acidity (McNulty & Aber, 1993; Likens et al, 1996; Nixon et al, 1996; Tamme et al, 2009). These negative impacts of excess N in natural systems together contribute to losses of biological diversity (Tilman, 1987 & 1996; Aerts & Berendse, 1988; Huenneke et al, 1990; Aber et al, 1995; Vitousek et al, 1997, Bobbink et al, 2010).

Another consequence of elevated N deposition and alteration of the global N cycle due to human activities is the shift in resource limitation. Nitrogen was once widely limiting in many terrestrial, particularly in temperate and boreal, regions (Gutschick, 1987; Lee et al, 1983; Binkley, 1986; Tilman, 1988; Vitousek et al, 1993; LeBauer & Treseder, 2008) and marine (Howarth, 1988) ecosystems due to the quantity required compared to other necessary nutrients and the cost to acquire and utilize it (Gutschick, 1981; Dickson, 1989; Vitousek & Howarth, 1991). Moreover, inorganic N compounds that are readily available for plants, such as NO_3^- , NO_2^- and NH_4^+ account for less than 5% of the total N in soil (Brady and Weil, 2008). However, limitation by other elements, especially phosphorus (P), has been reported in terrestrial (Walker & Syers, 1976; Vitousek et al, 2010; Cleveland et al, 2013) and freshwater (Schindler, 1977) ecosystems, largely due to the large pool of N in the atmosphere, coupled with increased humaninduced N input (Elser et al, 2009 & 2010; Crowley et al, 2012).

Like N, P is a critical determinant of plant growth and productivity along with other macro- and micro-nutrients (Chapin et al, 2011). Phosphorus is essential for plants as it plays a critical role in respiration and photosynthesis, as well as cell development and storing energy in

the form of ATP, rRNA, and phospholipids (Taiz & Zeiger, 2006). While most of P can be found in P-rich minerals, such as apatite, very little P is readily available for plant uptake because both biological and geochemical processes make P nearly immobile in most soils (Uehara & Gillman, 1981; Cole & Heil, 1981; Cole et al, 1989; Wood et al, 1984). Moreover, excess N can limit available P in forests (Mohren et al, 1986; Tessier & Raynal, 2003; Gress et al, 2007; Braun et al, 2010). N-induced soil acidification can inhibit P mineralization and therefore availability (Compton & Cole, 1998). Nitrogen addition experiments have found a decrease in fine root biomass (Nadelhoffer, 2000) and mycorrhizal activity (Wallenda & Kottke, 1998; Nilsson & Wallander, 2003), which can lead to a decrease in P uptake by plants (Vitousek et al, 2010). Lastly, plants and crops are often harvested and removed from stands, reducing P return to the soil via decomposition, which is also true for N.

The conceptual model by Walker & Syers (1976) on the pattern and regulation of soil P pools and availability during long-term soil and ecosystem development indicates that plant production is generally limited by P on old soils because P availability decreases as primary minerals are depleted over time through weathering, leaching, occlusion by secondary minerals, and the formation of recalcitrant soil organic matter (Uehara & Gillman, 1981; Cole & Heil, 1981; Cole et al, 1989; Wood et al, 1984; Fox, 1991). In contrast, over time, N fixation and deposition should increase the quantity and biological availability of N in the system until available N reaches a stoichiometric balance with P, a rock-derived nutrient (Stevens & Walker, 1970; Walker & Syres, 1976; Gorham et al, 1979). For example, Vitousek & Farrington (1997) found that forest growth in Hawaiian montane rainforests was limited by P on old soils (4.1 million year old), N on young soils (300 year old), and co-limited by N and P on intermediate soils (20,000 year old).

Many studies have investigated limitation by N and P in various ecosystems and sites. For example, N was found to be limiting plant growth in old fields (Pratt, 1984; Tilman, 1986 & 1987), wet grasslands (Vermeer, 1986), and arctic tundra communities (Haag, 1974). Phosphorus was reported to be limiting in Dutch and Australian heathlands (Heddle & Specht, 1975; Aerts & Brendse, 1988), North Carolina bogs (Simms, 1987), and Scottish serpentine grasslands (Carter et al, 1988). In a study conducted in Jamaican montane forest, several species increased stem growth in response to N and P fertilization (Tanner et al, 1990). In a Venezuelan montane forest, N and P together increased stem growth and litterfall, but neither element did so alone (Tanner et al, 1992). But some recent studies have found evidence that limitation has shifted from N to P due to anthropogenic addition of N (Richardson et al, 2004; Menge & Field 2007; Elser et al, 2009; Penuelas et al, 2012; Lu et al; 2013; Hayes et al, 2014).

Nutrient manipulation studies are perhaps the best way to evaluate nutrient limitation (Pardo et al, 2011), but in the absence of nutrient manipulation, foliar N and P concentrations and N:P ratios can be used to indicate plant nutrient status and nutrient limitation to primary productivity in ecosystems (Black, 1968; Grime, 1979; Sterner & Elser, 2002; Gusewell et al, 2005; Agren, 2008). N:P ratios have also been used to establish thresholds of nutrient limitation (Koerselman & Meuleman, 1996; Verhoeven et al, 1996; Aerts & Chapin, 2000; Gusewell & Koerselman, 2002). Because foliar nutrients reflect soil nutrient availability and fertility at the site (Aerts & Chapin, 2000; Hobbie & Gough, 2002; Han et al, 2005; Townsend et al, 2007), they can indicate how individual plants respond to nutrient availability and provide a means to evaluate nutrient deficiency and monitor changes due to natural and anthropogenic disturbances (Mitchell, 1936; Small, 1972; Leaf, 1973; Boerner, 1984; Aerts & Chapin, 2000).

While foliar nutrients, in general, are expected to increase with the addition of that nutrient (Vitousek, 1998; Olander & Vitousek, 2000; Galloway et al, 2008; Lu & Han, 2010; Jones & Power, 2012), they can also exhibit other responses to elevated nutrient availability. Some studies have found increased plant-available N and P concentrations with the addition of the other nutrient (Gusewell et al, 2003; Niinemets & Kull, 2005; Jones & Power, 2012; Lu et al, 2013), and this may be due to the tightly-coupled roles of N and P.

Responses of these foliar nutrient indices can vary due to interspecific differences because often times in multispecies communities, plant species coexist that have different strategies in nutrient acquisition and different nutrient demands (DiTomasso & Aarssen, 1989; Koerselman & Meuleman, 1996). Studies have found varying responses to fertilization by different species within the same site (Davidson et al, 2004; Townsend et al, 2007). Hence, it may be important to look at foliar N, P, and N:P at the species and community level to investigate nutrient limitation (Drenovsky & Richards, 2004; Craine et al, 2008).

As anthropogenic processes continue to alter nutrient cycling and availability in natural ecosystems, it is important to monitor plant responses to these changes. Nutrient manipulation studies can help us better understand and predict potential changes in soil and plant responses to changing nutrient availability and provide some insights into nutrient limitation. Because foliar nutrients generally reflect soil nutrient availability, they can be used to predict nutrient limitation in northern hardwood forests, we hope to improve our understanding of nutrient limitation at our multispecies site.

Literature Cited

- Aber, J.D., Nadelhoffer, J.K., Steudler, P.A., and Melillo, J.M. (1989) Nitrogen saturation in northern forest ecosystems hypotheses and implications. *BioScience*, 39: 378-386.
- Aber, J.D., A. Magill, S.G. McNulty, R.D. Boone, K.J. Nadelhoffer, M. Downs, and R. Hallett. (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air and Soil Pollution*, 85: 1665–1670.
- Aerts, R. and Berendse, F. (1988) The Effect of Increased Nutrient Availability on Vegetation Dynamics in Wet Heathlands. *Vegetation*, 76(1-2), 63-69.
- Aerts, R. and Chapin, F.S. III. (2000) The mineral nutrition of wild plants revisited: A reevaluation of processes and patterns. *Advances in ecological research*, 30,1-67.
- Ågren, Göran I. (2008) Stoichiometry and Nutrition of Plant Growth in Natural Communities. Annual Review of Ecology, Evolution, and Systematics, 39 (1): 153–70.
- Binkley, D. (1986) Forest Nutrition Management: John Wiley and Sons, New York. 299 pp.
- Black, C.A. (1968) Soil-Plant Relationships. John Wiley & Sons, Chichester, UK.
- Brady, N.C., R.R. Weil, and R.R. Weil. (2008) The Nature and Properties of Soils. *Vol. 13*. Prentice Hall Upper Saddle River, NJ.
- Braun, S., Thomas, V.F.D., Quiring, R., and Flickiger, W. (2010) Does nitrogen deposition increase forest production? The role of phosphorus. *Environ Pollut*, 158: 2043–2052.
- Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, et al. (2010) Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: A Synthesis. *Ecological Applications: A Publication of the Ecological Society* of America, 20 (1): 30–59.
- Boerner, R.E.J. (1984) Foliar nutrient dynamics and nutrient use efficiency of four deciduous tree species in relation to site fertility. *Journal of Applied Ecology*, 21: 1029-1040.
- Carter, S.P, Proctor, J., and Slingsby, D.R. (1988) The effects of fertilization on part of the Keen of Hamar serpentine, *ShetlandTrans. Bot. Soc.* Edinburgh.
- Chapin, F.S., A.J. Bloom, C.B. Field, and R.H. Waring. (1987) Plant Responses to Multiple Environmental Factors. *Bioscience*, 37 (1): 49–57.
- Chapin, F.S. III., Matson, P.A., and Vitousek, P. (2011) Principles of Terrestrial Ecosystem Ecology. New York, NY: *Springer Science & Business Media*.
- Cleveland, C., Townsend, A., and Schmidt, S. (2002) Phosphorus Limitation of Microbial Processes in Moist Tropical Forests: Evidence from Short-term Laboratory Incubations and Field Studies. *Ecosystems*, 5: 680-691.
- Cole, C.V. and R.D. Heil. (1981) Phosphorus effects on terrestrial nitrogen cycling. *Ecol. Bull.* 33: 363–374.
- Cole, C.V., Stewart, J.W.B., Ojima, D.S., Parton, W.J., and Schimel, D.S. (1989) Modelling land use effects of soil organic matter dynamics in the North American Great Plains. *In*: Clarholm M and Bergström L (*Eds*) *Ecology of Arable Land: Perspectives and Challenges* (pp 89–98). Kluwer Academic, Dordrecht.
- Compton, J. and Cole, D.W. (1998) Phosphorus cycling and soil P fractions in Douglas-fir and red alder stands. *For Ecol Manage*, 110: 101–112.
- Craine, J.M., C. Morrow, and Stock, W.D. (2008) Nutrient concentration ratios and co-limitation in South African grasslands. *New Pytologist*, 179: 829-836.
- Crowley, K., McNeil, B., Lovett, G., Canham, C., Driscoll, C., Rustad, L., Denny, E., Hallett, R., Arthur, M., Boggs, J., Goodale, C., Kahl, J., Mcnulty, S., Ollinger, S., Pardo, L.,

Schaberg, P., Stoddard, J., Weand, M., and Weathers, K. (2012) Do Nutrient Limitation Patterns Shift from Nitrogen Toward Phosphorus with Increasing Nitrogen Deposition Across the Northeastern United States? *Ecosystems*, 15. 10.1007/s10021-012-9550-2.

- Davidson, E.A., C.J.R. de Carvalho, I.C.G. Vieira, R.D. Figueiredo, P. Moutinho, F.Y. Ishida, M.T.P. dos Santos, J.B. Guerrero, K. Kalif, and Saba, R.T. (2004) Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecological Applications*, 14: S150-S163.
- Dickson, R.E. (1989) Carbon and Nitrogen Allocation in Trees. *Annales Des Sciences Forestières*.
- DiTommaso, Antonio, and L.W. Aarssen. (1989) Resource Manipulations in Natural Vegetation: A Review. *Vegetation*, 84 (1): 9–29.
- Drenovsky, R.E. and Richards, J.H. (2004) Critical N:P values: predicting nutrient deficiencies in desert shrublands. *Plant and Soil*, 259: 59-69.
- Elser, J.J., T. Andersen, J.S. Baron, A-K Bergström, M. Jansson, M. Kyle, K.R. Nydick, L. Steger, and D.O. Hessen. (2009) Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. *Science*, 326 (5954): 835–37.
- Elser, J.J., W.F. Fagan, A.J. Kerkhoff, N.G. Swenson, and B.J. Enquist. (2010) Biological Stoichiometry of Plant Production: Metabolism, Scaling and Ecological Response to Global Change: Tansley Review. *The New Phytologist*, 186 (3): 593–608.
- Fox, L.E. (1991) Phosphorus chemistry in the tidal Hudson River. *Geochim. Cosmochim. Acta*, 55: 1529-1538.
- Galloway, J.N., W.H. Schlesinger, H. Levy II, A. Michaels, and J.L. Schnoor. (1995) Nitrogen Fixation: Anthropogenic Enhancement-Environmental Response. *Global Biogeochemical Cycles*, 9 (2): 235–52.
- Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Cosby. (2003) The Nitrogen Cascade. *BioScience*, 53(4), 341-356.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. & Vörösmarty, C.J. (2004) Nitrogen cycles: Past, present, and future. *Biogeochemistry*, vol. 70, no. 2, pp. 153-226.
- Gorham E., Vitousek P.M. and Reiners, W.A. (1979) The regulation of chemical budgets over the course of terrestrial ecosystem succession. *Ann. Rev. Ecol. Syst.*, 10: 53–88.
- Gress S.E., Nichols, T.D., Northcraft, C.C., Peterjohn, W.T. (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: what lies beyond nitrogen saturation? *Ecology*, 88: 119–130.
- Grime, J.P. (1979) Plant Strategies and Vegetation Processes. Wiley, Chichester, UK.
- Güsewell, S. and W. Koerselman. (2002) Variation in Nitrogen and Phosphorus Concentrations of Wetland Plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 5 (1): 37–61.
- Güsewell, S. (2005) Responses of wetland graminoids to the relative supply of nitrogen and phosphorus. *Plant Ecology*, 176, 35-55.
- Gutschick, V.P. (1981) Evolved strategies in nitrogen acquisition by plants. Am. Nat., 118: 607-637.
- Gutschick, V.P. (1987) A Functional Biology of Crop Plants. Timber Press, Portland. 230 pp.
- Haag, Richard W. (1974) Nutrient Limitations to Plant Production in Two Tundra Communities. *Canadian Journal of Botany. Journal Canadien de Botanique*, 52 (1): 103–16.

- Han, W., J. Fang, D. Guo, and Y. Zhang. (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist*, 168: 377-385.
- Hayes, P., B.L. Turner, H. Lambers, and Laliberte, E. (2014) Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2million-year dune chronosequence. *Journal of Ecology*, 102: 396-410.
- Heddle, E.M. and Specht, R.L. (1975) Dark Island health (Ninety-Mile Plain, South Australia).
 VIII. The effect of fertilizers on composition and growth, 1950–1972. *Australian Journal* of *Botany*, 23: 151–164.
- Herbert, Darrell A. and James H. Fownes. (1995) Phosphorus Limitation of Forest Leaf Area and Net Primary Production on a Highly Weathered Soil. *Biogeochemistry*, 29 (3): 223–35.
- Hobbie, S.E. & Gough, L. (2002) Foliar and soil nutrients in tundra on glacial landscapes of contrasting ages in northern tundra on glacial landscapes of contrasting ages in northern Alaska. *Oecologia*, 131(3): 453-462.
- Howarth, R.W. (1988) Nutrient limitation of net primary production in marine ecosystems. *Ann. Rev. Ecol. Syst.*, 19: 89-110.
- Huenneke, L.F., Hamburg, S.P., Koide, R., Mooney, H.A., and Vitousek, P.M. (1990) Effects of soil resources on plant invasion and community structure in California serpentine grassland. *Ecology*, 71: 478–491.
- Koerselman, Willem and Arthur F.M. Meuleman. (1996) The Vegetation N:P Ratio: A New Tool to Detect the Nature of Nutrient Limitation. *The Journal of Applied Ecology*, 33 (6): 1441–50.
- Leaf, A.L. (1973) Plant analysis as an aid in fertilizing forests. *In*: Walsh, L.M., Beaton, J.D., *eds.* Soil testing and plant analysis. Madison, WI. *Soil Science Society of America*: 427-454.
- LeBauer, David S. and Kathleen K. Treseder. (2008) Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems Is Globally Distributed. *Ecology*, 89 (2): 371–79.
- Lee, J.A., Harmer, R. and Ignaciuk, R. (1983) Nitrogen as a limiting factor in plant communities. *In*: Lee, J.A., McNeill, S., Rorison, I.H. (*Eds*) Nitrogen as an Ecological Factor (pp 95-112). *Blackwell Scientific*, Oxford.
- Liebig, Justus. (1842) Die Ernährung, Blut- Und Fettbildung Im Thierkörper. Annalen Der Chemie Und Pharmacie, 41 (3): 241–85.
- Likens, G.E., C.T. Driscoll, and D.C. Buso. (1996) Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science*, 272 : 244–246.
- Lu, X., S. Reed, Q. Yu, N. He, Z. Wang, and Han, X. (2013) Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. *Global Change Biology*, 19: 2775-2784.
- McNulty, S.G. and J.D. Aber. (1993) Effects of Chronic Nitrogen Additions on Nitrogen Cycling in a High-Elevation Spruce–fir Stand. Canadian Journal of Forest Research. *Journal Canadien de La Recherche Forestiere*, 23 (7): 1252–63.
- Menge, D.N.L. and Field, C.B. (2007) Simulated global changes alter phosphorus demand in annual grassland. *Global Change Biology*, 13:2582-2591.
- Mitchell, H.L. (1936) Trends in the nitrogen, phosphorus, potassium and calcium content of the leaves of some forest trees during the growing season. *Cornwall-on-the-Hudson*, New York: The Black Rock Forest.
- Mohren, G.M.J., Burg, J., and Burger, F. (1986) Phosphorus deficiency induced by nitrogen input in Douglas fir in The Netherlands.. *Plant and Soil.* 95. 191-200.

- Nadelhoffer, Knute J. (2000) The Potential Effects of Nitrogen Deposition on Fine-Root Production in Forest Ecosystems: REVIEW N Deposition and Fine Roots. *The New Phytologist*, 147 (1): 131–39.
- Nilsson, Lars Ola, and Hakan Wallander. (2003) Production of External Mycelium by Ectomycorrhizal Fungi in a Norway Spruce Forest Was Reduced in Response to Nitrogen Fertilization. *The New Phytologist*, 158 (2): 409–16.
- Nixon, S.W., J.W. Ammerman, L.P. Atkinson, V.M. Berounsky, G. Billen, W.C. Boicourt, W.R. Boynton, et al. (1996) The Fate of Nitrogen and Phosphorus at the Land-Sea Margin of the North Atlantic Ocean. *Biogeochemistry* 35 (1): 141–80.
- Pardo LH, Robin-Abbott, MJ and Driscoll, CT. (2011) Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol Appl*, 21(8):3049–3082
- Peñuelas, J., Sardans, J., Rivas-ubach, A. and Ivan A. Janssens. (2012) The Human-Induced Imbalance between C, N and P in Earth's Life System. *Global Change Biology*, 18 (1): 3–6.
- Peterson, B.J. and Melillo, J.M. (1985) The potential storage of carbon caused by eutrophication of the biosphere. *Tellus*, 37B: 117-127.
- Pratt, P.F. (1984) Nitrogen use and nitrate leaching in irrigated agriculture. *In*: Hauck, R.D. (*Ed*) Nitrogen in Crop Production (pp 319–333) *ASA CSSA SSSA*, Madison, Wisconsin
- Prinn, R., Cunnold, D., Rasmussen, R., Simmonds, P., Alyea, F., Crawford, A., Fraser, P. and Rosen, R. (1990) Atmospheric emissions and trends of nitrous oxide deduced from 10 years of ALE-GAGE data. J Geophys Res, 95: 18369–18385.
- Richardson, S.J., D.A. Peltzer, R.B. Allen, M.S. McGlone, and Parfitt, R.L. (2004) Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia*, 139(2): 267-276.
- Schimel, D.S., Parton, W.J., Cole, C.V., Ojima, D.S. and Kittel, T.G.F. (1990) Grassland biogeochemistry: Links to atmospheric processes. *Climatic Change*, 17: 13-25.
- Schimel, David S. (1995) Terrestrial Biogeochemical Cycles: Global Estimates with Remote Sensing. *Remote Sensing of Environment*, 51 (1): 49–56.
- Schindler, D.W. (1977) Evolution of phosphorus limitation in lakes. Science, 195: 260-262.
- Simms, E.L. (1987) The effects of nitrogen and phosphorus addition on the growth, reproduction, and nutrient dynamics of two ericaceous shrubs. *Oecologia*, 71:541–547.
- Small, E. (1972). Photosynthetic rates in relation to nitrogen recycling as an adaptation to nutrient deficiency in peat bog plants. *Canadian Journal of Botany*, 50, 2227-2233.
- Smil, V. (1990) Nitrogen and phosphorus. *In*: B.L. Turner et al., *eds.*, The Earth as Transformed by Human Action, *Cambridge University Press*, New York, pp. 423-436.
- Smil, Vaclav. (1991) Population Growth and Nitrogen: An Exploration of a Critical Existential Link. *Population and Development Review*, 17 (4): 569–601.
- Sterner, Robert & Elser, J.J. (2002) Ecological Stoichiometry: The Biology of Elements From Molecules to The Biosphere.
- Stevens, P.R. and Walker, T.W. (1970) The chronosequence concept and soil formation. *Quart. Rev. Biol.*, 45: 333–350.
- Sutton, M., Oenema, O., Erisman, J., Liep, A., Grinsven, H., and Winiwarter, W. (2011) Too much of a good thing. *Nature*, 472, 159–161.
- Taiz, L. and Zeiger, E. (2012) Plant physiology. 5th Edition, *Sinauer Associates, Inc.*, Sunderland.

- Tamme T., Reinik M., and M. Roasto. (2009) Nitrates and Nitrites in Vegetables: Occurrence and Health Risks. *In*: Watson, R.R., Preedy, V.R., *editors*. Bioactive Foods Promoting Health: Fruits and Vegetables. *Academic Press*; Salt Lake City, UT, USA. pp. 307–321.
- Tanner, E.V.J., V. Kapos, S. Freskos, J.R. Healey, and A.M. Theobald. (1990) Nitrogen and Phosphorus Fertilization of Jamaican Montane Forest Trees. *Journal of Tropical Ecology*, 6 (2): 231–38.
- Tanner, E.V.J., V. Kapos, and W. Franco. (1992) Nitrogen and Phosphorus Fertilization Effects on Venezuelan Montane Forest Trunk Growth and Litterfall. *Ecology*, 73 (1): 78–86.
- Tessier, J.T. and Raynal, D.J. (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology*, 40, 523–534.
- Tilman, D. (1986) Nitrogen-limited growth in plants from different successional stages. *Ecology*, 67: 555-563
- Tilman, D. (1987) Secondary Succession and the Pattern of Plant Dominance Along Experimental Nitrogen Gradients. *Ecological Monographs*, 57 (3): 189–214.
- Tilman, D. (1988) Plant Strategies and the Dynamics and Structure of Plant Communities. *Princeton University Press*, Princeton. 360 pp.
- Tilman, D., D. Wedin, and J. Knops. (1996) Productivity and Sustainability Influenced by Biodiversity in Grassland Ecosystems. *Nature*, 379 (6567): 718–20.
- Townsend, A.R., C.C. Cleveland, G.P. Asner, and Bustamante, M.M.C. (2007) Controls over foliar N:P ratios in tropical rain forests. *Ecology*, 88: 107-118.
- Uehara, G. and Gillman, G.P. (1981) The Mineralogy, chemistry and physics of tropical soils with variable charge clays. *Westview Press*: Boulder, Colorado.
- Ulrich, A. and Hills, F.J. (1973) Plant analysis as an aid in fertilizing sugar crops, part 1. sugar beets. Pages 271-288 *in* L.M. Walsh and J.D. Beaton, *eds*. Soil testing and plant analysis. *Soil Science Society of America*, Madison, WI.
- Verhoeven, J.T., W. Koerselman, and A.F. Meuleman. (1996) Nitrogen- or Phosphorus-Limited Growth in Herbaceous, Wet Vegetation: Relations with Atmospheric Inputs and Management Regimes. *Trends in Ecology & Evolution*, 11 (12): 494–97.
- Vermeer, H.J.G. (1986) The effect of nutrients on shoot biomass and species composition of wetland and hayfield communities. *Acta Oecologia*, 7, 31–41.
- Vitousek, P.M. and Howarth, R.W. (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, 13: 87-115.
- Vitousek, P.M. and P.A. Matson. (1993) Agriculture, the Global Nitrogen Cycle, and Trace Gas Flux. In Biogeochemistry of Global Change: Radiatively Active Trace Gases Selected Papers from the Tenth International Symposium on Environmental Biogeochemistry, San Francisco, August 19–24, 1991, edited by Ronald S. Oremland, 193–208. Boston, MA: Springer US.
- Vitousek, P.M. and Farrington, H. (1997) Nutrient Limitation and Soil Development: Experimental Test of a Biogeochemical Theory. *Biogeochemistry*, 37(1), 63-75.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. (1997) Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. Ecological Applications: A Publication of the Ecological Society of America 7 (3): 737–50.

- Vitousek, P.M., Porder, S., Houlton, B.Z., and Chadwick, O.A. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20:5–15.
- Walker, T.W. and Syers, J.K. (1976) The fate of phosphorus during pedogenesis. *Geoderma* 15: 1-19.
- Wallenda, Thomas, and Ingrid Kottke. (1998) Nitrogen Deposition and Ectomycorrhizas. The New Phytologist, 139 (1): 169–87.
- Wood, T., F.H. Bormann, and G.K. Voight. (1984) Phosphorus cycling in a northern hardwood forest: biological and chemical control. *Science*, 223: 391-394.

Chapter 2: Effects of nitrogen and phosphorus addition on foliar nutrient concentrations of six northern hardwood species

Introduction

Nitrogen limitation of primary productivity and other biological processes is common in terrestrial ecosystems, particularly in temperate (Mitchell & Chandler, 1939; Miller, 1981) and boreal regions (Agren, 1983; van Cleve & Zasada, 1976; van Cleve et al, 1983; Bonan, 1990) and in marine ecosystems (Howarth, 1988) due to the large quantity required compared to other essential nutrients and the cost to acquire and utilize N (Gutschik, 1981 & 1987; Lee et al, 1983; Binkley, 1986; Kimmins, 1987; Tilman, 1988; Tamm, 1991; Vitousek and Howarth, 1991; Vitousek et al, 1993). However, since the beginning of the Industrial Revolution, the N cycle has been altered via fertilizer production and application and fossil fuel combustion (Galloway et al, 1995; Vitousek et al, 1997; Galloway, 2004; Vitousek et al, 2010; Peñuelas et al. 2012), which together doubled N input into the terrestrial N cycle between 1900 and 2000 (Vitousek & Matson, 1993; Galloway et al, 1995; Sutton et al, 2011). With more N in the soil, plant production can become less limited by N availability (Ayers et al, 1994; Vitousek et al, 1997; Galloway et al, 2003).

With this shift in N availability, phosphorus (P) is expected to become more often limiting as increased N supply enhances primary production, and thus, demand for both N and P (Vitousk et al, 2010). Economic theory suggests that plants will allocate resources so as to remain co-limited by multiple resources (Bloom et al, 1985), and the Multiple Element Limitation model (MEL) predicted a shift from N to P limitation in secondary succession as northern hardwood forests recover from harvesting disturbances, until recycling of N and P

becomes stoichiometrically balanced (Rastetter et al, 2013). Fertilization studies in Hawaiian montane rainforests showed that primary successional ecosystems were primarily N-limited, whereas later successional ecosystems were P-limited (Vitousek, 2004; Vitousek & Farrington, 1997). This pattern reflects the progressive depletion of primary mineral P by weathering as soils develop (Peltzer et al, 2010; Menge et al, 2012).

The Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) project (Fisk et al, 2014) was established to study N and P acquisition and limitation through a series of nutrient manipulations in northern hardwood forests. There have been conflicting reports of nutrient limitation in this forest type, with some studies finding a greater growth response to P and others finding a greater response to N (Vadeboncoeur, 2010). In the MELNHE sites, fine root growth responses to nutrient addition suggested P limitation in mid-aged forests and N limitation in mature forests (Naples & Fisk 2010). However, aboveground growth responses to factorial N and P addition suggested P limitation in both mid-aged and mature stands, but N limitation in the youngest stands (Goswami et al, 2018). The differences in species composition in stands of different ages may explain some of these differences in responses to nutrient addition.

In this study, we report foliar nutrient concentrations 4 to 6 years after the initiation of N and P fertilization in ten stands in the MELNHE study, focusing on the six most abundant species. We predicted that tree species, stand age and site quality would influence responses of foliar nutrient concentrations to N and P addition, but that overall the concentration of each nutrient would increase in response to the addition of that nutrient. We also tested for interactive effects of N and P addition on foliar nutrients perhaps suggestive of unbalanced inorganic nutrition or NxP colimitation (Elser et al, 2007; Harpole et al, 2011; Rastetter et al, 2013).

Methods

Site Description

This study took place in ten stands in the White Mountains of central New Hampshire, USA: six at the Bartlett Experimental Forest, two at the Hubbard Brook Experimental Forest, and two at Jeffers Brook (Table 1). The sites are on different bedrock types and the stands vary in age and species composition (Bae et al. 2015; Table 1). All soils are primarily well drained acid Spodosols of sandy-loam texture developed in glacial drift, deposited approximately 14,000 years ago (Vadeboncoeur et al. 2014). The climate is humid continental, with annual temperature and precipitation averaging 4.4°C and 1300 mm at Bartlett Experimental Forest, 5.7°C and 1400 mm at Hubbard Brook Experimental Forest (Bailey et al. 2003), and 6.3°C and 990 mm at Jeffers Brook (Wake et al, 2014). Nitrogen deposition in this region exceeded 8 kg N ha⁻¹ yr⁻¹ for much of the 1980s and 1990s but declined sharply in the early 21st century to about 2-4 kg N ha⁻¹ yr⁻¹ (NADP, 2014).

Species composition in the forest stands is typical of the northern hardwoods forest type; we studied American beech (*Fagus grandifolia*), pin cherry (*Prunus pensylvanica*), red maple (*Acer rubrum*), sugar maple (*A. saccharum*), white birch (*Betula papyrifera*) and yellow birch (*B. alleghaniensis*) (Table 1). Species composition varied by stand, mainly due to successional changes in dominance. All the stands originated following intensive forest harvest with the age of the stands at the time of sampling ranging from 25 to over 100 years (Table 1).

Experimental plots in these stands were established as a part of the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) project (Fisk et al. 2014). All stands in Bartlett Experimental Forest and the mature stands at Hubbard Brook Experimental Forest and Jeffers Brook contained four 50 x 50 m plots that have been treated annually since 2011 with either N (30 kg N/ha/yr as NH₄NO₃), P (10 kg P/ha/yr as NaH₂PO₄), or N+P (at the same rates), and an untreated control. Each plot included a 10 m wide buffer to avoid edge effects and sampling was conducted within the interior 30 x 30 m. In the mid-aged stands at Hubbard Brook Experimental Forest and Jeffers Brook, the four plots were 30 x 30 m with a 5 m buffer and received the same treatment. In a few cases, trees were sampled from the buffer because there were not enough trees in the measurement area.

Sample Collection

Pre-treatment foliage was collected in 2008, 2009 and 2010, and post-treatment foliage was collected in 2014, 2015 and 2016, in the last week of July and first week of August. Foliage was collected using a 12-gauge shotgun except in pre-treatment collection in the two young stands at Bartlett Experimental Forest, which were sampled with a pole pruner as the trees were still small in stature. Leaves were usually collected from 3 trees of each species in each plot from sun-exposed portions of the middle to upper canopy. There were 28 plot-species combinations in which 4 trees were sampled pre-treatment, often due to different trees being sampled in different years. Post-treatment there were 4 species-plot combinations represented by only 1 tree and 13 represented by only 2 trees, ostensibly because there were too few trees of that species in the plot.

Sample Processing

At least 10 leaves per tree were selected for analysis, avoiding those that showed evidence of disease or damage from buckshot or herbivory. Leaves were oven-dried at 60°C to

constant mass and ground in a Wiley mill to pass through a 40- mesh screen. Carbon and N concentrations were determined through combustion in a CN elemental analyzer (FlashEA 1112 analyzer, Thermo Scientific). Concentrations of P were determined by dry ashing ~0.25 g of ground sample at 470°C in a muffle furnace and digesting on a hot-plate with 5 or 10 mL of 6N HNO₃ (Siccama et al. 1994). Concentrations of Ca, K, Mg, Mn, and Sr were also determined on the dry-ashed samples (Appendix). The digests were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 5300 DV, Perkin-Elmer). In all analyses, one blank, two replicates of standard material (NIST 1515 or arginine), and one duplicate sample were processed with each group of 30-40 samples. An in-house quality control followed by a blank was run after every 10 samples, and the machine was recalibrated if >5% drift was observed in the in-house standards.

For the standard reference material, recovery of N was within 5% of the certified value for 14 of 17 samples (with a mean of -1%), and within 10% for the remaining 3 samples (with a mean of -7%). For P, recovery was within 5% of the certified value for 28 of 34 samples (with a mean of 1.5%), within 10% for 3 samples (with a mean of 6.6%), and within 15% for the remaining 3 samples (with a mean of 10.8%). Replicate standards differed by \leq 4% for each pair (with a mean of 3.6%) and \leq 4% for each pair of duplicate samples (with a mean of 3.1%). Duplicate samples were averaged for subsequent analyses.

Data Analysis

Foliar nutrient concentrations were averaged by species within each plot because only 36% of the trees sampled pretreatment could be paired with post-treatment samples; many trees in the young stands were <10 cm dbh and not tagged. We tested for systematic interannual

differences in pre-treatment and post-treatment foliar N and P concentrations because these would contribute a bias; not every tree was sampled in every year. We used a linear mixedeffects model (nlme package in R; Pinheiro et al. 2016) and a nested analysis of variance (ANOVA) with year and species as fixed effects and plot (nested within stand) as random effect. The concentrations did not differ systematically by year for either pre-treatment foliar N (F=1.73, p = 0.19) or P (F=0.90, p = 0.34) or post-treatment foliar N (F=3.61, p = 0.06) or P (F=0.64, p = 0.42).

We tested for treatment effects on post-treatment foliar N and P concentrations and foliar N:P, using a linear mixed-effects model and a nested analysis of covariance (ANCOVA) with pre-treatment foliar concentrations as a covariate. Interactions between treatments (N and P addition) were included, and species and stand age were included as fixed effects. Stands, nested within forest site (BEF, HBEF or JB), were included as random effects in a factorial, split-split design, where the first split is at the treatment plot level and the second is at the species level within a plot. Forest stand was the unit of replication (n = 10). This factorial approach compares response variables in plots with N addition (i.e. N and N+P plots) to those with no N addition (i.e. control and P plots); similarly, we compare plots with P addition to those with no P addition. We also ran separate analyses for each species using the same approach as above, but without species in the model.

We used post-hoc Tukey comparisons of least-squares means to test the differences among our treatments for cases in which the main effects were significant across all analyses. All statistical tests and graphs were performed using R (Version 1.1.463).

Results

Foliar nitrogen

As expected, N addition increased foliar N concentrations, by 11% on average across ten stands and six species (F=52.61, p < 0.01 for the main effect of N addition in ANCOVA; Table 2 & 3; Figure 1). Surprisingly, foliar N concentrations were lower with P addition, by 3% (F=4.39, p < 0.03). The effects of N and P additions together on foliar N were the same as the predicted main effects of N and P addition (i.e. N x P interaction was not significant for foliar N; F=0.18, p = 0.67).

Foliar N concentrations varied by species (F=11.47, p < 0.01; Table 2; Figure 1). Pin cherry had 14% higher foliar N concentration than American beech (p < 0.01; Table 3), 20% higher than red maple (p < 0.01), 13% higher than sugar maple (p < 0.02), 18% higher than white birch (p < 0.01), and 14% higher than yellow birch (p < 0.01). Another notable difference was that American beech had 6% higher foliar N than white birch (p = 0.11).

We also looked at the response of each species to treatment. Foliar N of pin cherry, sugar maple, and white birch responded only to N addition. In comparison to trees in plots receiving no N, adding N increased foliar N concentrations of pin cherry by 18% (F=16.49, p < 0.01; Table 2 & 3; Figure 1), those of sugar maple by 11% (F=43.30, p < 0.01), and those of white birch by 12% (F=12.90, p < 0.01).

Foliar N of red maple and yellow birch responded to P as well as to N addition. Red maple had 13% higher foliar N in plots with N addition than in plots without N (F=33.91, p < 0.01 for the main effect of N addition in ANCOVA; Table 2 & 3; Figure 1), and had 5% lower

foliar N in plots with P addition than in plots without P (F=12.46, p < 0.01). Yellow birch had 8% higher foliar N concentrations with N addition (F=11.34, p < 0.01), but 4% lower with P addition (F=3.39, p < 0.08). For red maple, adding N and P together resulted in lower foliar N than the predicted main effects of N and P addition (F=12.32, p < 0.01 for N x P interaction). No other species showed a significant interactive effect of N plus P addition on foliar N.

American beech was the only species that differed in foliar N concentration across age classes (F=6.09, p < 0.05; Table 2 & 3; Figure 1), with trees in young stands having 14% higher foliar N than those in mature stands (p < 0.04). Overall, foliar N of American beech responded to N addition with 10% higher concentrations (F=24.24, p < 0.01 for the main effect of N addition in ANCOVA), but did not respond to P addition (F=0.85, p = 0.36).

Foliar phosphorus

Community-wide foliar P concentrations, on average, were 42% higher with P addition (F=164.33, p < 0.01 for the main effect of P addition in ANCOVA; Table 2 & 3; Figure 2) and 7% lower with N addition (F=7.06, p < 0.01). Foliar P was lower when N and P were added together in comparison to the main effects of N and P addition (F=1.92, p < 0.16 for N x P interaction): addition of N and P together showed 39% higher foliar P than N addition alone (p < 0.001), but 9% lower than P addition alone (p = 0.03).

Overall, foliar P varied by species (F=7.79, p < 0.01; Table 2 & 3; Figure 2). Pin cherry had 40% higher foliar P than American beech (p < 0.01), 27% more than red maple (p < 0.01), 24% more than sugar maple (p < 0.01), 25% more than white birch (p < 0.01), and 14% more

than yellow birch (p = 0.02). The only other significant species differences were for yellow birch, which had 21% higher foliar P than American beech (p < 0.01) and 17% higher than red maple (p = 0.08).

Looking at each species's response to treatment, foliar P of American beech, sugar maple and white birch responded to P addition but were not affected by N addition. In comparison to plots without P addition, foliar P of American beech was 22% higher with P addition (F=83.55, p < 0.01 for the main effect of P addition in ANCOVA; Table 2 & 3; Figure 2). Similarly, sugar maple foliar P was 38% higher (F=27.59, p < 0.01) and white birch foliar P was 41% higher (F=66.25, p < 0.01) with P addition.

White birch did not respond to N addition alone but adding N and P together showed lower foliar P than the predicted main effects of N and P addition (F=2.37, p < 0.15 for N x P interaction; Table 2 & 3; Figure 2): white birch trees that were treated with both N and P showed 50% higher foliar P than those treated with just N (p < 0.001) and 9% higher than those treated with just P (p = 0.40). Foliar P concentrations of white birch in young stands were 11% lower than those in mid-aged stands (F=5.45, p = 0.14).

Foliar P of pin cherry, red maple and yellow birch was higher with P addition but lower with N addition. With P addition, foliar P concentrations were 81% higher for pin cherry (F=53.23, p < 0.01 for the main effect of P addition; Table 2 & 3; Figure 2), 27% higher for red maple (F=34.22, p < 0.01), and 49% higher for yellow birch (F=104.31, p < 0.01). With N addition, foliar P concentrations were 18% lower for pin cherry (F=5.34, p < 0.05), but 10% higher for red maple (F=7.14, p < 0.05), and 10% higher for yellow birch (F=7.37, p < 0.01). Adding N and P together resulted in lower foliar P for pin cherry compared to the main

effects of N and P addition (F=3.13, p < 0.10 for N x P interaction); pin cherry trees that were treated with both N and P showed 64% higher foliar P than those treated with just N (p < 0.02), but 23% lower than those treated with just P (p < 0.06).

Foliar N:P

Community-wide average foliar N:P responded to both N addition (F=43.92, p < 0.01 for the main effect of N addition in ANCOVA; Table 2 & 3; Figure 3) and P addition (F=249.67, p < 0.01). Foliar N:P of trees that received N addition was, on average, 16% lower (p < 0.01) than those that did not receive N addition, whereas for those that received P addition, it was 30% higher (p < 0.01) than those without P addition. Adding N and P together did not have a significant interactive effect on foliar N:P (F=0.17, p = 0.67; Figure 1).

Species differed significantly in their foliar N:P (F=4.90, p < 0.01; Table 2 & 3; Figure 3). The most notable contrasts were for yellow birch, which had 13% lower N:P than American beech (p < 0.01) and 11% lower than sugar maple (p = 0.06). Foliar N:P also varied by stand age (F=3.47, p = 0.11), with trees in young stands showing 10% higher N:P than those in mid-aged stands (p = 0.10).

Foliar N:P of five species (i.e., excluding red maple) responded similarly to nutrient additions, decreasing in the N addition plots and increasing in the P addition plots. Specifically, for American beech, foliar N:P was 13% lower with N addition (F=38.28, p < 0.01 for the main effect of N addition in ANCOVA; Table 2 & 3; Figure 3) and 20% higher with P addition (F=130.64, p < 0.01). For white birch, foliar N:P was 13% lower with N addition (F=7.81, p =

0.01) and 31% higher with P addition (F=71.84, p < 0.01). For pin cherry, foliar N:P was 30% lower with N addition (F=6.02, p = 0.03) and 48% higher with P addition (F=39.73, p < 0.001). For sugar maple, foliar N:P was 19% lower with N addition (F=11.40, p < 0.01) and 27% higher with P addition (F=52.33, p < 0.001). For yellow birch, foliar N:P was 13% lower with N addition (F=13.19, p < 0.01) and 33% higher with P addition (F=119.23, p < 0.01).

In contrast, foliar N:P of red maple responded only to P addition (F=46.43, p < 0.001; Table 2 & 3; Figure 3), and was 24% higher with P addition (p < 0.001). No significant interactive effects of N+P addition on foliar N:P were observed for any species.

Of the five species, American beech (F=3.56, p = 0.10; Table 2 & 3; Figure 3) and white birch (F=4.37, p = 0.17) alone varied in foliar N:P by stand age. American beech trees in young stands had 14% higher foliar N:P than those in mid-aged stands (p = 0.10) and white birch trees in young stands had 12% higher foliar N:P than those in mid-aged stands (p = 0.17).

Discussion

Decades of anthropogenic N deposition in the northeastern United States might be expected to alter biogeochemical cycling (Berendse et al, 1993; Aber et al, 1995; Vitousek et al, 1997; Stevens et al, 2018), resulting in changes in N and P acquisition by trees. Temperate forests are thought to be primarily N-limited (McGroddy et al, 2004; Reich and Oleksyn, 2004), but in the MELNHE sites, aboveground growth between 2011 and 2015 showed evidence of P limitation in mid-aged and mature stands and of N limitation in young stands (Goswami et al, 2018). Similarly, my findings in this nutrient-manipulation study indicate that northern hardwood forests respond to both N and P addition with adjustments in foliar chemistry, and that species differ in their response to N and P addition and by stand age.

Foliar N:P in untreated plots was high in comparison to many other forests, ranging from 18.7±0.7 for red maple to 23.5±0.8 for American beech (Table 3; Figure 3). While higher foliar N:P is indicative of P-limitation and vice versa, these foliar N:P ratios were above the ratios believed to correspond to P limitation: 17.7 in understory vegetation in the Catskill region, NY (Tessier & Raynal, 2003), 14.7 in a beech forest in Europe (Ljungstrom & Nihlgard, 1995), and 13.8 in a montane forest in Hawaii (Herbert & Fownes, 1995).

It was not surprising that adding N increased foliar N (Table 3; Figure 1) and adding P increased foliar P (Table 3; Figure 2) across all stands and species. High foliar nutrient concentrations reflect increased uptake of available soil nutrients (Mugasha et al, 1999; Binkley & Fisher, 2013; Sariyildiz & Anderson, 2005) and additions of N and P have been shown to increase plant and soil concentrations of the respective nutrient (Olander and Vitousek 2000; Galloway et al. 2008; Lu and Han 2010; Jones and Power 2012). The relative effects of P addition on foliar P were much greater than for those of N addition and foliar N; this was true as a percentage of post-treatment concentration, across stands and by species (Figure 1 & 2). One explanation of this observation is that our rate of P addition was high relative to N, with an N:P of 3, much lower than the ratios of these nutrients in foliage. The relatively higher P addition was chosen because P is strongly and often irreversibly sorbed in acid soils like those in our study area (Wood et al, 1984; Compton & Cole, 1998).

Community-wide average foliar N and P concentrations were significantly suppressed by the addition of the other nutrient (Table 3; Figure 1 & 2). This response may be attributed to a dilution of effect associated with increased leaf production when the demand for the other

nutrient is met (Vitousek, 1984; Haases & Rose, 1995; Ostertag, 2010); however, we have not observed significant responses of leaf litter production to the treatments (unpublished data). In contrast to the community-wide responses, foliar P of red maple and yellow birch actually increased with N addition (Table 3; Figure 2). Addition of N could lead to stimulation of P-mineralizing enzymes (Marklein & Houlton, 2012; Lu et al, 2013).

Species differ in foliar nutrient concentrations, which may be due to the differences in species's life-history strategy (Niinemets & Kull, 2005; Hagen-Thorn et al, 2006; Wood et al, 2011; Reed et al, 2012). Pin cherry, an extreme pioneer species, had the highest foliar N and P concentrations compared to other species. Pin cherry is an "exploitative" species that grows rapidly (Fahey et al, 1998) and has very high tissue nutrient concentrations (Mou et al, 1993) when resources are abundant.

Diameter growth of trees increased in response to P addition in mature and mid-aged stands in our study sites between 2011 and 2015, indicating P limitation (Goswami et al, 2018). We expected these tree growth responses would be correlated with pre-treatment foliar N:P ratios, with greater responses for stands with higher N:P ratios. It was surprising that we found no clear relationship between aboveground growth responses and foliar N:P for any species (Figure 4). This analysis could be done at a community level since the growth response reported by Goswami et al. (2018) was significant at the community level but not for many of the individual species.

Literature Cited

- Aber, J.D., A. Magill, S.G. McNulty, R.D. Boone, K.J. Nadelhoffer, M. Downs and R. Hallett. (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air and Soil Pollution*, 85: 1665-1670.
- Agren, G.I. (1983) Nitrogen productivity of some conifers. Can. J. For. Res., 12: 494-500.
- Ayers, R.U., W.H. Schlesinger and R.H. Socolow. (1994) Human impacts on the carbon and nitrogen cycles. *In: Industrial Ecology and Global Change* (Eds) by R.H. Socolow et al., pp. 121-155, *Cambridge University Press*, New York.
- Bae, K., T.J. Fahey, R.D. Yanai, et al. (2015) Soil nitrogen availability affects belowground carbon allocation and soil respiration in northern hardwood forests of New Hampshire. *Ecosystems*, 18: 1179–1191.
- Bailey, A., J.W. Hornbeck, J.L. Campbell and C. Eagar. (2003) Hydrometeorological database for Hubbard Brook Experimental Forest: 1955–2000. Volume 305. USDA Forest Service, Northeastern Research Station, Delaware, Ohio, USA.
- Berendse, F., R. Aerts and R. Bobbink. (1993) Atmospheric nitrogen deposition and its impact on terrestrial ecosystems. Pages 104–121 in C. C. Vos and P. Opdam, editors. Landscape ecology of a stressed environment. Chapman and Hall, London, UK.
- Binkley, D. (1986) Forest Nutrition Management. John Wiley and Sons, New York. 299 pp.
- Binkley, D. and R.F. Fisher. (2013) Ecology and management of forest soils. *4th ed*. Hoboken, NJ: *Wiley-Blackwell*. 362 p.
- Bloom A.J., F.S. Chapin and H.A. Mooney. (1985) Nutrient limitation in plants an economic analogy. *Ann. Rev. Ecol. Syst.*, 16: 363-392.
- Bonan, G.B. (1990) Carbon and nitrogen cycling in North American boreal forests. II. Biogeographic patterns. *Can. J. Forest Res.*, 20: 1077-1088.
- Compton, J. and D.W. Cole. (1998) Phosphorus cycling and soil P fractions in Douglas-fir and red alder stands. *For Ecol Manage*, 110: 101–112.
- Elser, J.J., M.E.S. Bracken, E.E. Cleland, D.S. Gruner, W.S. Harpole, H. Hillebrand, et al. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.*, 10: 1135–1142.
- Fahey, T.J., J.J. Battles and G.F. Wilson. (1998) Responses of early successional northern hardwood forests to changes in nutrient availability. *Ecol. Monogr.*, 68: 183–212.
- Fisk, M.C., T.J. Ratliff, S. Goswami and R.D. Yanai. (2014) Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. *Biogeochemistry*, 118: 195-204.
- Galloway, J.N., W.H. Schlesinger, H. Levy II, A. Michaels and J.L. Schnoor. (1995) Nitrogen Fixation: Anthropogenic Enhancement-Environmental Response. *Global Biogeochemical Cycles*, 9(2): 235–52.
- Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling and B.J. Cosby. (2003) The Nitrogen Cascade. *BioScience*, 53(4), 341-356.
- Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter,

A.R. Townsend and C.J. Vörösmarty. (2004) Nitrogen cycles: Past, present, and future. *Biogeochemistry*, 70(2): 153-226.

- Galloway J.N., A.R. Townsend, J.W. Erisman, M. Bekunda, Z. Cai, J.R. Freney, L.A. Martinelli, S.P. Seitzinger and M.A. Sutton. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, 320(5878): 889-892.
- Goswami, S., M.C. Fisk, M.A. Vadeboncoeur, et al. (2018) Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology*, 99: 438–449.
- Gutschick, V.P. (1981) Evolved strategies in nitrogen acquisition by plants. Am. Nat., 118: 607-637.
- Gutschick, V.P. (1987) A functional biology of crop plants. Timber Press, Portland. 230 pp.
- Haase, D.L. and R. Rose. (1995) Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *For. Sci*, 41: 54–66.
- Hagen-Thorn, A., I. Varnagiryte, B. Nihlgard and K. Armolaitis. (2006) Autumn nutrient resorption and losses in four deciduous forest tree species. *For. Ecol. Manag.*, 228: 33– 39.
- Harpole, W.S., J.T. Ngai, E.E. Cleland, E.W. Seabloom, E.T. Borer, M.E.S. Bracken, et al. (2011) Nutrient co-limitation of primary producer communities. *Ecol. Lett.*, 14: 852–862.
- Herbert, D.A. and J.H. Fownes. (1995) Phosphorus limitation of forest leaf area and net primary production on a highly weathered soil. *Biogeochemistry*, 29(3): 223–35.
- Howarth, R.W. (1988) Nutrient limitation of net primary production in marine ecosystems. *Ann. Rev. Ecol. Syst.*, 19: 89-110.
- Jones, A.G. and S.A. Power. (2012) Field-scale evaluation of effects of nitrogen deposition on the functioning of heathland ecosystems. *Journal of Ecology*, 100: 331-342.
- Kimmins, J.P. (1987) Forest Ecology. Macmillian Publishing, New York. 531 pp.
- Lee, J.A., R. Harmer and R. Ignaciuk. (1983) Nitrogen as a limiting factor in plant communities. In: Lee, J.A., McNeill, S., Rorison, I.H. (Eds) Nitrogen as an Ecological Factor (pp 95-112). Blackwell Scientific, Oxford.
- Ljungström, M. and B. Nihlgård. (1995) Effects of lime and phosphate additions on nutrient status and growth of beech (Fagus sylvatica L.) seedlings. *Forest Ecology and Management*, 74: 133-148
- Lü, X.T. and X.G. Han. (2010) Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. *Plant Soil*, 327: 481–491.
- Lu, X., S. Reed, Q. Yu, N. He, Z. Wang and X, Han. (2013) Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. *Global Change Biology*, 19: 2775-2784.
- Marklein, A.R. and B.Z. Houlton. (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, 193: 696-704.
- McGroddy, M.E., T. Daufresne and L.O. Hedin. (2004). Scaling of C:N:P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. *Ecology*, 85: 2390–2401.

- Menge, D.N.L., L.O. Hedin and S.W. Pacala. (2012) Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. *PLoS One*, 7: e42045.
- Miller, H.G. (1981) Forest fertilization: some guiding concepts. Forestry, 54: 157-167.
- Mitchell, H.L. and R.F. Chandler. (1939) The nitrogen nutrition and growth of certain deciduous trees of Northeastern United States. *Black Rock Forest Bull.*, 11: 1–94.
- Mou, P., T.J. Fahey and J.W. Hughes. (1993) Effects of soil disturbance on vegetation recovery and nutrient accumulation following whole-tree harvest of a northern hardwood ecosystem. *Journal of Applied Ecology*, 12: 661–675.
- Mugasha, A.G., D.J. Pluth and S.E. Macdonald. (1999) Effects of fertilization on seasonal patterns of foliar mass and nutrients of tamarack and black spruce on undrained and drained minerotrophic peatland sites. *For Ecol Manage.*, 116(1-3): 13–31.
- Naples, B.K. and M.C. Fisk. (2010) Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry*, 97: 109-121.
- Niinemets, Ü. and K. Kull. (2005) Co-limitation of plant primary productivity by nitrogen and phosphorus in a species-rich wooded meadow on calcareous soils. *Act ecol.* 28: 345-356.
- Olander, L.P. and P.M. Vitousek. (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, 49: 175-190.
- Ostertag, R. (2010) Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. *Plant Soil*, 334: 85-98.
- Peltzer, D.A., D.A. Wardle, V.J. Allison, W.T. Baisden, R.D. Bardgett, O.A. Chadwick, L.M. Condron, R.L. Parfitt, S. Porder, S.J. Richardson, B.L. Turner, P.M. Vitousek, J. Walker and L.R. Walker. (2010) Understanding ecosystem retrogression. *Ecological Monographs*, 80: 509-529.
- Peñuelas, J., J. Sardans, A. Rivas-ubach and I.A. Janssens. (2012) The Human-induced imbalance between C, N and P in earth's life system. *Global Change Biology*, 18(1): 3-6.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar and R Core Team (2016) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-127.
- Rastetter, E.B., R.D. Yanai, R.Q. Thomas, M.A. Vadeboncoeur, T.J. Fahey, M.C. Fisk, B.L. Kwiatkowski and S.P. Hamburg. (2013) Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. *Ecological Applications*, 23: 621-642.
- Reed, S.C., A.R. Townsend, E.A. Davidson and C.C. Cleveland. (2012) Stoichiometric patterns in foliar nutrient resorption across multiple scales. *New Phytologist*, 198: 173-180.
- Reich, P.B. and J. Oleksyn. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl. Acad. Sci. U.S.A.*, 101: 11001–11006.
- Sariyildiz, T. and J.M. Anderson. (2005) Variation in the chemical composition of green leaves and leaf litters from three deciduous tree species growing on different soil types. *For Ecol Manage.*, 210(1-3): 303-319.
- Siccama, T.G., S.P. Hamburg, M.A. Arthur, R.D. Yanai, F.H. Bormann and G.E. Likens. (1994) Corrections to the allometric equations and plant tissue chemistry for the Hubbard Brook Experimental Forest. *Ecology*, 75: 246-248.

- Stevens, C.J., T.I. David and J. Storkey. (2018) Atmospheric nitrogen deposition in terrestrial ecosystems: Its impact on plant communities and consequences across trophic levels. *Functional Ecology*, 32(7): 1757-1769.
- Sutton, M., O. Oenema, J. Erisman, A. Liep, H. Grinsven and W. Winiwarter. (2011) Too much of a good thing. *Nature*, 472: 159–161.
- Tamm, C.O. (1991) Nitrogen in terrestrial ecosystems. Questions of productivity, vegetational changes, and ecosystem stability. *Ecological studies* 81, Springer-Verlag, Berlin, Heidelberg. 116 p.
- Tessier, J.T. and D.J. Raynal. (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology*, 40: 523–534.
- Tilman, D. (1988) Plant strategies and the dynamics and structure of plant communities. *Princeton University Press*, Princeton. 360 pp.
- Vadeboncoeur, M.A. (2010) Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. *Canadian Journal of Forest Research*, 40(9): 1766-1780.
- Vadeboncoeur, M.A., S.P. Hamburg, R.D. Yanai and J. D. Blum. (2014) Rates of sustainable forest harvest depend on rotation length and weathering of soil minerals. *Forest Ecology and Management*, 318: 194-205.
- Van Cleve, K. and J. Zasada. (1976) Response of 70-yr-old white spruce to thinning and fertilization in interior Alaska. *Can. J. For. Res.*, 6: 145-152.
- Van Cleve, K., L. Oliver, R. Schlentner, L.A. Viereck and C.T. Dyrness. (1983) Productivity and nutrient cycling in taiga forest ecosystems. *Can. J. For. Res.*, 12: 747-766.
- Vitousek, P.M. (1984) Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology*, 65: 285–298.
- Vitousek, P.M. and R.W. Howarth. (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry*, 13: 87-115.
- Vitousek, P.M. and P.A. Matson. (1993) Agriculture, the global nitrogen cycle, and trace gas flux. *In Biogeochemistry of Global Change*: Radiatively Active Trace Gases Selected Papers from the Tenth International Symposium on Environmental Biogeochemistry, San Francisco, August 19–24, 1991, edited by Ronald S. Oremland, 193–208. Boston, MA: Springer US.
- Vitousek, P.M., L.R. Walker, L.D. Whiteaker and P.A. Matson. (1993) Nutrient limitation to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry*, 23: 197-215.
- Vitousek, P.M. and H. Farrington. (1997) Nutrient Limitation and Soil Development: Experimental Test of a Biogeochemical Theory. *Biogeochemistry*, 37(1), 63-75.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger and D.G. Tilman. (1997) Human alteration of the global nitrogen cycle:

sources and consequences. *Ecological Applications*: A Publication of the Ecological Society of America 7 (3): 737–50.

- Vitousek, P.M. (2004) Nutrient cycling and limitation: Hawaii as a model system; Molina MJ, Morel FMM, Morgan JJ, Tilman D, editors. Princeton, NJ: *Princeton University Press*. 221 p.
- Vitousek, P.M., S. Porder, B.Z. Houlton and O.A. Chadwick. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20: 5–15.
- Wake, C., E. Burakowski, P. Wilkinson, K. Hayhoe, A. Stoner, C. Keeley and J. Labranche. (2014) Climate change in southern New Hampshire: past, present, and future. *Carbon Solutions New England*. Durham, NH.
- Wood, T., F.H. Bormann and G.K. Voight. (1984) Phosphorus cycling in a northern hardwood forest: biological and chemical control. *Science*, 223: 391-394.
- Wood, T.E., D. Lawrence and J.A. Wells. (2011) Inter-specific variation in foliar nutrients and resorption of nine canopy-tree species in a secondary neotropical rain forest. *Biotropica*, 43: 544–551.

Chapter 3: Conclusions

In these forests where P appears to be the most limiting nutrient, foliar N and P responses to nutrient addition suggested that foliar P was more sensitive to P addition than was foliar N to N addition. Similar to the findings in a previous study in one of the young MELNHE stands (Gonzales & Yanai, 2019), foliar N and P were lower with the addition of the other nutrient. Because trees are expected to respond to the addition of a limiting nutrient with increased growth, the concentrations of other nutrients may be diluted due to higher production of leaves (Vitousek, 1984; Haase & Rose, 1995; Ostertag, 2010). We have the ability to investigate this theory as we have information on annual litter production. Species differed in their foliar N and P responses to nutrient addition, suggesting a need for further research to look into their physiologies and how each species adapts to changes in nutrient availability.

Site	Stand	Lat.	Long.	Year Cut	Age Class	Studied Species			
BEF	C1	44°02'N	71°19'W	1990	Young	American beech, pin cherry, and white birch			
	C2	44°04'N	71°16′W	1988	Young	American beech, pin cherry, red maple, white birch, and yellow birch			
	C4	44°03'N	71°16′W	1978	Mid-Aged	American beech, pin cherry, red maple, white birch, and yellow birch			
	C6	44°02'N	71°16′W	1975	Mid-Aged	American beech, pin cherry, red maple, white birch, and yellow birch			
	C8	44°03'N	71°18′W	1883	Mature	American beech, sugar maple, and yellow birch			
	С9	44°03'N	71°17'W	~1890	Mature	American beech, sugar maple, and yellow birch			
HBEF	HBM	43°56′N	71°44'W	1970	Mid-Aged	American beech, red maple, white birch, and yellow birch			
	нво	43°56′N	71°44'W	~1900	Mature	American beech, sugar maple, and yellow birch			
JB	JBM	44°02'N	71°53'W	~1975	Mid-Aged	American beech, pin cherry, sugar maple, white birch, and yellow birch			
	JBO	44°02'N	71°53'W	~1900	Mature	American beech, sugar maple, and yellow birch			

 Table 1. Stand descriptions for the Bartlett Experimental Forest (BEF), Hubbard Brook

 Experimental Forest (HBEF), and Jeffers Brook (JB).

Table 2. Community-level (all species) and species-level results of mixed effects model showing the main effects of nutrient addition and stand age on foliar N concentrations, foliar P concentrations, and foliar N:P, using pre-treatment foliar indices as covariates. Numerator and denominator degrees of freedom are reported and separated by a comma.

		Foliar N		Foliar P		Foliar N:P	
Predictor	df	F	F	Р	Р	F	Р
Community-level							
Pre-treatment	1,135	266.1	<0.01	137.6	<0.01	105.4	<0.01
N	1,135	52.61	<0.001	7.07	<0.01	43.92	<0.001
Р	1,135	4.40	0.03	164.3	<0.001	249.6	<0.01
Species	5,135	11.48	<0.001	7.80	<0.001	4.90	<0.001
Age	2,5	1.02	0.42	1.19	0.37	3.47	0.11
N x P	1,135	0.18	0.66	1.93	0.16	0.17	0.67
American beech							
Pre-treatment	1,26	7.29	0.01	4.41	0.04	12.24	<0.01
N	1,26	24.24	<0.001	0.04	0.85	38.28	<0.001
Р	1,26	0.85	0.36	83.55	<0.001	130.6	<0.001
Age	2,5	6.09	0.04	0.99	0.43	3.56	0.10
N x P	1,26	1.29	0.26	1.75	0.19	1.74	0.19
Pin cherry							
Pre-treatment	1,11	3.03	0.10	2.82	0.12	10.50	<0.01
N	1,11	16.49	<0.01	5.34	0.04	6.02	0.03
Р	1,11	1.47	0.25	53.23	<0.001	39.73	<0.001
Age	1,2	0.71	0.48	0.18	0.71	1.67	0.32
N x P	1,11	0.56	0.46	3.13	0.10	0.10	0.75
Red maple							
Pre-treatment	1,6	3.78	0.09	9.65	0.02	0.00	0.99
N	1,6	33.91	<0.01	7.14	0.03	0.09	0.76
Р	1,6	12.46	0.01	34.22	<0.01	46.43	<0.001
Age	1,1	4.15	0.29	3.21	0.32	0.82	0.52
N x P	1,6	12.32	0.01	0.18	0.68	1.29	0.29
Sugar maple							
Pre-treatment	1,11	0.03	0.86	0.18	0.68	0.23	0.63
N	1,11	43.30	<0.001	1.53	0.24	11.40	<0.01
Р	1,11	0.00	0.95	27.59	<0.001	52.33	<0.001
Age	1,1	1.70	0.41	4.19	0.28	1.12	0.48
N x P	1,11	0.78	0.39	1.68	0.22	0.32	0.58
White birch							
Pre-treatment	1,14	22.42	<0.001	23.86	<0.001	21.17	<0.001
N	1,14	12.90	<0.01	0.20	0.66	7.81	0.01
Р	1,14	1.39	0.25	66.25	<0.001	71.84	<0.001
Age	1,2	2.09	0.28	5.45	0.14	4.37	0.17
N x P	1,14	0.02	0.88	2.37	0.14	1.34	0.26
Yellow birch							
Pre-treatment	1,23	30.97	<0.001	3.94	0.05	22.04	<0.001
Ν	1,23	11.34	<0.01	7.37	0.01	13.19	<0.01
Р	1,23	3.39	0.07	104.3	<0.001	119.2	<0.001
Age	2,4	2.24	0.22	0.20	0.82	2.33	0.21
N x P	1,23	0.38	0.54	1.71	0.20	0.01	0.90

Note: Significant P values are highlighted in boldface type.

	Pre	-treatment Fo	liage	Post-treatment Foliage			
Treatment	N (mg/g)	P (mg/g)	N:P	N (mg/g)	P (mg/g)	N:P	
American beech							
CONTROL	24.0 ±0.6	1.08 ±0.03	22.4 ±0.7	25.3 ±0.4	1.09 ±0.03	23.5 ±0.8	
Ν	23.8 ±0.6	1.15 ±0.05	21.0 ±0.9	27.2 ±0.6	1.07 ±0.04	25.8 ±1.0	
Р	23.4 ±0.6	1.07 ±0.03	21.9 ±0.5	24.1 ±1.1	1.29 ±0.03	18.8 ±0.9	
NP	24.0 ±0.5	1.11 ±0.03	21.6 ±0.6	27.4 ±0.5	1.33 ±0.03	20.8 ±0.7	
Pin cherry							
CONTROL	28.3 ±2.5	1.49 ±0.10	19.0 ±1.2	32.5 ±3.0	1.47 ±0.09	22.4 ±2.5	
Ν	26.2 ±1.8	1.35 ±0.10	19.5 ±0.9	35.2 ±3.4	1.31 ±0.06	27.0 ±3.1	
Р	27.7 ±1.1	1.47 ±0.06	18.9 ±0.6	29.6 ±2.3	2.87 ±0.28	10.4 ±0.3	
NP	26.8 ±1.1	1.39 ±0.04	19.3 ±0.5	35.0 ±2.8	2.17 ±0.08	16.1 ±1.2	
Red maple							
CONTROL	18.4 ±1.1	1.09 ±0.08	16.9 ±0.3	19.5 ±0.8	1.05 ±0.06	18.7 ±0.7	
Ν	19.0 ±0.8	1.15 ±0.04	16.5 ±0.6	23.7 ±0.7	1.17 ±0.04	20.3 ±0.5	
Р	19.8 ±1.5	1.12 ±0.08	17.8 ±0.04	19.7 ±1.9	1.33 ±0.12	14.8 ±0.1	
NP	18.9 ±1.7	1.20 ±0.08	15.8 ±0.9	20.9 ±0.3	1.42 ±0.09	14.8 ±0.8	
Sugar maple							
CONTROL	19.9 ±1.0	1.10 ±0.06	18.2 ±1.1	20.7 ±1.0	1.06 ±0.04	19.5 ±0.8	
Ν	19.1 ±1.4	1.20 ±0.11	16.5 ±1.7	23.0 ±1.2	1.10 ±0.09	21.3 ±1.5	
Р	19.5 ±0.8	1.09 ±0.06	18.1 ±1.1	20.8 ±1.3	1.60 ±0.16	13.6 ±1.5	
NP	18.6 ±0.5	1.08 ±0.03	17.2 ±0.5	22.6 ±1.0	1.37 ±0.11	16.7 ±0.8	
White birch							
CONTROL	23.5 ±1.0	1.09 ±0.06	21.7 ±1.1	23.6 ±1.2	1.08 ±0.07	22.4 ±2.1	
Ν	23.7 ±0.7	1.08 ±0.06	22.0 ±0.6	26.5 ±0.6	1.04 ±0.08	26.3 ±1.9	
Р	23.9 ±1.0	1.07 ±0.04	22.4 ±0.7	23.2 ±1.5	1.39 ±0.07	16.7 ±0.9	
NP	24.1 ±1.3	1.16 ±0.03	20.8 ±0.9	25.9 ±1.0	1.59 ±0.08	16.4 ±0.7	
Yellow birch							
CONTROL	24.6 ±1.4	1.30 ±0.06	19.3 ±1.3	25.6 ±1.1	1.35 ±0.08	19.6 ±1.6	
Ν	25.5 ±0.6	1.33 ±0.04	19.3 ±0.7	27.9 ±0.8	1.29 ±0.05	21.9 ±1.1	
Р	24.3 ±1.0	1.26 ±0.05	19.2 ±0.4	23.9 ±0.7	2.00 ±0.12	12.3 ±0.8	
NP	25.2 ±0.7	1.24 ±0.03	20.3 ±0.6	26.9 ±0.7	1.74 ±0.05	15.6 ±0.6	

Table 3. Mean and standard error of foliar N, P, and N:P by treatment across species in pre-treatment and post-treatment.



Figure 1. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar N concentrations of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch. Points represent means of two to four trees per plot; stands are indicated by labels. The dashed black line indicates 1:1, hence increases in foliar N lie above the line whereas the plots that fall below the line had lower foliar N after treatment.



Figure 2. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar P concentrations of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch. Points represent means of two to four trees per plot; stands are indicated by labels. The dashed black line indicates 1:1, hence increases in foliar P lie above the line whereas the plots that all below the line had lower foliar P after treatment.



Figure 3. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar N:P of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch. Points represent means of two to four trees per plot; stands are indicated by labels. The dashed black line indicates 1:1, hence increases in foliar N:P lie above the line whereas the plots that fall below the line had lower foliar N:P after treatment.

Appendix: Foliar Ca, K, and Mg responses to nutrient addition.

		Foliar Ca		Foliar K		Foliar Mg	
Predictor	Df	F	Р	F	Р	F	Р
Community-level							
Pre-treatment	1,135	113.22	<0.01	246.40	<0.01	334.36	<0.01
Ν	1,135	14.15	<0.001	0.34	0.56	9.06	<0.01
Р	1,135	0.92	0.33	0.03	0.87	0.01	0.91
Species	5,135	3.30	<0.01	3.99	<0.01	8.28	<0.001
Age	2,5	0.07	0.93	0.73	0.52	1.79	0.25
N x P	1,135	1.22	0.27	0.03	0.86	0.003	0.95
American beech							
Pre-treatment	1,26	16.92	<0.001	9.22	<0.01	3.38	0.07
Ν	1,26	4.66	0.04	0.09	0.77	0.18	0.67
Р	1,26	0.34	0.56	0.35	0.56	0.19	0.67
Age	2,5	0.73	0.52	0.66	0.55	0.35	0.71
N x P	1,26	1.22	0.27	2.85	0.10	0.00	0.99
Pin cherry							
Pre-treatment	1,11	3.83	0.07	0.66	0.43	2.56	0.13
Ν	1,11	5.34	0.04	0.78	0.39	0.22	0.64
Р	1,11	0.04	0.85	0.13	0.72	0.08	0.78
Age	1,2	1.02	0.41	0.01	0.93	0.36	0.60
N x P	1,11	0.95	0.35	0.00	0.96	0.58	0.46
Red maple							
Pre-treatment	1,6	0.83	0.39	0.69	0.43	7.19	0.03
Ν	1,6	2.75	0.14	1.70	0.24	3.51	0.11
Р	1,6	0.01	0.92	0.01	0.93	0.16	0.70
Age	1,1	1.71	0.41	9.88	0.19	0.42	0.63
N x P	1,6	0.00	0.95	0.11	0.75	0.55	0.48
Sugar maple							
Pre-treatment	1,11	9.14	0.01	1.10	0.31	0.99	0.34
Ν	1,11	5.68	0.03	10.75	<0.01	0.33	0.57
Р	1,11	1.03	0.33	2.82	0.12	0.09	0.77
Age	1,1	3.65	0.30	0.01	0.92	1.10	0.48
N x P	1,11	0.91	0.36	0.11	0.74	0.24	0.63
White birch							
Pre-treatment	1,14	17.35	<0.001	10.44	<0.01	15.88	<0.01
Ν	1,14	1.76	0.20	2.14	0.16	0.90	0.35
Р	1,14	3.71	0.07	0.24	0.63	0.59	0.45
Age	1,2	0.01	0.92	3.25	0.21	0.01	0.93
N x P	1,14	0.28	0.60	0.00	0.97	1.29	0.27
Yellow birch							
Pre-treatment	1,23	14.13	<0.01	54.62	<0.001	9.99	<0.001
Ν	1,23	7.47	0.01	1.78	0.19	8.84	<0.01
Р	1,23	0.00	0.98	1.31	0.26	0.05	0.81
Age	2,4	3.80	0.12	8.11	0.03	3.60	0.12
N x P	1,23	0.39	0.54	0.95	0.33	0.28	0.60

Table A.1. Community-level (all species) and individual species-level results of mixed effects model showing the main effects of nutrient addition and stand age on foliar Ca, K, and Mg concentrations.

Note: Significant P values are highlighted in boldface type.

Figure A.1. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar Ca of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch across all stands under nutrient additions. The dashed black line indicates 1:1, hence the points that lie above the line indicate increases in foliar N whereas the points that fall below the line indicate decreases.

Figure A.2. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar K of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch across all stands under nutrient additions. The dashed black line indicates 1:1, hence the points that lie above the line indicate increases in foliar N whereas the points that fall below the line indicate decreases.

Figure A.3. Comparison of pre-treatment (x-axis) and post-treatment (y-axis) foliar Mg of a) American beech, b) pin cherry, c) red maple, d) sugar maple, e) white birch, and f) yellow birch across all stands under nutrient additions. The dashed black line indicates 1:1, hence the points that lie above the line indicate increases in foliar N whereas the points that fall below the line indicate decreases.

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EDUCATION

M.S., ESC Environmental Monitoring, SUNY College of Environmental Science and Forestry, Syracuse, NY, (expected in December 2019)

B.S., Environmental Science, Trinity College, Hartford, CT, 2015

ACADEMIC HONORS AND AWARDS

ESF Career Fellowship Conference Grant (\$500), SUNY-ESF, 2019

Fall 2018 Alumni Association Grant (\$1,000), SUNY-ESF, 2018

Graduate Student Association's Excellence in Teaching Award, SUNY-ESF, 2018

Honors in Environmental Science, Trinity College, 2015

Environmental Activism Award, Trinity College, 2015

RELEVANT TEACHING EXPERIENCE

General Chemistry Laboratory TA, SUNY-ESF, Spring 2018 ~ Fall 2019

- Previewed the experiments prior to the students to ensure success, ran laboratory experiments and graded lab reports while holding office hours. ~20 students in each section and I had two sections per semester.

Introduction to Soils TA, SUNY-ESF, 2017

- Ran field trips and laboratory experiments and graded lab reports while holding office hours. Twenty-three students in the section.

Organic Chemistry Laboratory TA, Trinity College, Spring 2014 & 2015

- Ran laboratory experiments and graded lab exercises while holding help sessions. ~20 students in the section.

General Chemistry Tutor, Trinity College, Fall 2012

- Had weekly sessions with students in need and prepared practice problems.

RELEVANT COURSEWORK

Environmental Policy & Governance, SUNY-ESF, Spring 2018

Regression Analysis, SUNY-ESF, Fall 2017 Quantitative Methods & Models in R, SUNY-ESF, Fall 2017 Plant Ecology & Global Change, SUNY-ESF, Spring 2017 Analysis of Variance, SUNY-ESF, Spring 2017 Writing for Scientific Publication, SUNY-ESF, Spring 2017 Forest Ecology, SUNY-ESF, Fall 2016 Introduction to Soils, SUNY-ESF, Fall 2016 Biogeochemistry, Syracuse University, Fall 2016 Organic Chemistry I & II, Trinity College, Fall 2012 & Spring 2013 Environmental Chemistry, Trinity College, Fall 2011 & Spring 2012

PRESENTATIONS

- Hong, D.S. and R.D. Yanai. Foliar nutrient concentrations and resorptions of northern hardwood species in an NxP manipulation study. 2019 Ecological Society of America Annual Meeting, August 2019. Poster.
- Hong, D.S., Fahey, T.J., Fisk, M.C., Johnston, M.T., and R.D. Yanai. The timing of senescence affects foliar P resorption. 56th Annual Hubbard Brook Cooperators Meeting, July 2018. *Oral.*
- Hong, D.S. and R.D. Yanai. Fall litter variation in nutrient concentration of six northern hardwood species. New York Society of American Foresters 2019 Annual Meeting, January 2019. Poster.
- Hong, D.S. and R.D. Yanai. Foliar analysis of four northern hardwood species indicates nutrient limitation. LTER 2018 All Scientists' Meeting, October 2018. *Poster*.
- Hong, D.S. and R.D. Yanai. Foliar N and P resorption by species and treatment. 55th Annual Hubbard Brook Cooperators Meeting, July 2018. *Oral*.
- Hong D.S., and R.D. Yanai. Foliar analysis of five northern hardwood species indicates nutrient limitation. Spotlight on Research, SUNY-ESF, April 2018. *Poster*.
- Hong, D.S., K.E. Phelps and R.D. Yanai. Vector analysis shows nutrient status of five northern hardwood species in response to nutrient additions. New York Society of American Foresters 2018 Annual Meeting, January 2018. *Poster*.

- Hong, D.S. and R.D. Yanai. American beech and sugar maple regeneration responses to soil chemistry and nutrient additions in northern hardwood temperate forests. 2017 Forest Ecosystem Monitoring Cooperative Conference, University of Vermont, December 2017. *Poster*.
- Hong, D.S. and R.D. Yanai. American beech and sugar maple regeneration responses to soil chemistry and nutrient additions in northern hardwood temperate forests. Rochester Academy of Sciences Fall Scientific Paper Session, St. John Fisher College, November 2017. *Poster*.
- Hong D.S., A.D. Wild and R.D. Yanai. Battle of the babies: Beech interference with maple regeneration. 54th Annual Hubbard Brook Cooperators Meeting, July 2017. *Oral.*
- Hong D.S., A.D. Wild and R.D. Yanai. Battle of the babies: Beech interference with maple regeneration. Spotlight on Research, SUNY-ESF, April 2017. *Poster*.
- Lasser, G.A., D.S. Hong, Y. Yang, K.E. Phelps, G. Pu and R.D. Yanai. Effect of nutrients on foliar characteristics of pin cherry, American beech, yellow birch and white birch. Spotlight on Research, SUNY-ESF, April 2017. *Poster*.
- Hong D.S., A.D. Wild and R.D. Yanai. Beech interference with maple regeneration: future change in forest composition. 53rd Annual Hubbard Brook Cooperators Meeting, July 2016. *Oral*.
- Hong, D.S. and J.R. Gourley. Determination of the baseline concentration of mercury and percentage of organic material in sub-alpine forest soil before clear-cutting within the White Mountain National Forest. Trinity College 28th Annual Science Research Symposium. April 2015. Poster.
- Hong, D.S., Barajas, L. and J.R. Gourley. The effect of clear cutting on sub-alpine forest soil mercury (Hg) and organic material within the White Mountain National Forest, New Hampshire. Trinity College 27th Annual Science Research Symposium. May 2014. *Poster*.
- Hong. D.S. Beslity, J.O, and J.R. Gourley. The effect of clear cutting on sub-alpine forest soil nutrients and trace metals within the White Mountain National Forest, New Hampshire. Trinity College 26th Annual Science Research Symposium. May 2013. *Poster*.
- Hong, D.S., Beslity, J.O. and J.R. Gourley. Accumulation of mercury in Mill Pond Park of the Park River watershed, Newington, CT. Geological Society of America Northeastern Section – 48th Annual Meeting. March 2013. *Poster*.