

INDEPENDENT AND INTERACTIVE EFFECTS OF NITROGEN AND PHOSPHORUS ADDITION
ON FOREST SOIL RESPIRATION

by

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ABSTRACT

T.A. Mann. Independent and Interactive Effects of Nitrogen and Phosphorus Addition on Forest Soil Respiration. 60 pages, 6 tables, 3 figures, 2021. APA style guide used.

The suppressive effect of nitrogen (N) addition on soil respiration is well documented, but the extent to which it may be moderated by stand age or the availability of soil phosphorus (P) is not well understood. We measured soil respiration across three stand age classes in a full-factorial NxP fertilization experiment spanning ten years in the White Mountain National Forest in New Hampshire, USA. Soil respiration was consistently reduced in N-treated plots (8-15%), but no P effect was detected. However, in the summer and fall of 2012-2014, the magnitude of the N effect was reduced by P addition. Mid-successional stands (26-41 years old at the time of the first nutrient addition) consistently had the lowest rates of soil respiration across stand age classes. These effects of stand age and indicate that the controls of soil respiration may be more complex than previously thought.

Keywords: soil respiration, fertilization, nitrogen, phosphorus, succession, stand age

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CHAPTER 1: LITERATURE REVIEW

Over the last 150 years, the global carbon cycle has been disrupted by the anthropogenic enhancement of atmospheric carbon dioxide (CO₂) concentrations (Le Quéré et al., 2018). Because of the role of carbon as the foundational element of all life on Earth, its effects upon global climate patterns, and its influence upon a number of biogeochemical processes, it is critical to understand how carbon moves through natural systems and how those systems will be affected by the disruption of the carbon cycle. The largest flux in the global carbon cycle is the movement of carbon into and out of terrestrial ecosystems (Le Quéré et al., 2018). Ecosystem respiration moves roughly 120 gigatons of carbon from terrestrial systems to the atmosphere on an annual basis, dwarfing the carbon flux attributed to human activity (Le Quéré et al., 2018). In temperate systems, about 70% of ecosystem respiration comes from soils (Goulden et al., 1996; Janssens et al., 2000; Law et al., 1999; Ryan & Law, 2005), making them the largest component of the largest source in the global carbon cycle. Simply stated, understanding soil respiration—and the mechanisms that control it—is an essential step in understanding the carbon cycle and in preparing for the implications of the abrupt disturbance to the carbon cycle that Earth has experienced in recent history.

All of the carbon released to the atmosphere by way of soil respiration is ultimately derived from the physiological processes of plants. Through photosynthesis, plants transform CO₂ into chemically stable carbohydrates, an energy source that can be transported to support vital functions such as growth, defense, reproduction, and nutrient acquisition. All of a plant's chemical assets—and these energy-rich carbohydrates in particular—are distributed such that growth-limiting resources can be acquired at higher rates (Bloom et al., 1985; Chapin et al., 1987; Rastetter & Kwiatkowski, 2020); that is, when demand for a resource is high, a plant's available assets are invested in tissues, cells, proteins, or processes specialized in the acquisition or retention of the needed resource. Should nitrogen (N) limit growth, for example, resource limitation theory (Bloom et al., 1985) would predict greater levels of investment in the processes or structures responsible for the acquisition of N, namely fine roots and the mycorrhizal communities

associated with them. These patterns of asset investment or allocation ultimately drive rates of soil respiration as the efflux of CO₂ from soils depends upon inputs from the aboveground system. And increased rates of belowground allocation allow for greater rates of many metabolic activities that together constitute soil respiration (Raich & Nadelhoffer, 1989). In this review, I will outline the ecological processes that constitute soil respiration, discussing how each may be manipulated by physiological responses to resource availability.

Components of soil respiration

Soil respiration is the total efflux of CO₂ from soils, and two categories contribute to that larger flux: autotrophic and heterotrophic respiration (Ryan & Law, 2005). In the context of belowground systems, autotrophic respiration refers specifically to respiration by roots, which release CO₂ as energy-rich compounds are catabolized to fuel growth and sustain vital functions (Hanson et al., 2000). Heterotrophic respiration in belowground systems, on the other hand, is primarily constituted by the CO₂ generated by the metabolic activity of bacteria and fungi as they decompose either detritus or root exudates. Given the symbiotic nature of roots and mycorrhizae, neatly delineating autotrophic and heterotrophic respiration can become quite challenging in experimental settings, particularly in the rhizosphere where that symbiosis occurs. I will begin this review of the components of soil respiration with a discussion of heterotrophic soil respiration. Then, I will review heterotrophic respiration in the rhizosphere specifically, followed by a discussion of autotrophic belowground respiration.

Heterotrophic soil respiration

In temperate ecosystems, between 50 and 60% of total soil CO₂ efflux can be attributed to heterotrophic respiration (Drake et al., 2011; Fahey et al., 2005). As previously discussed, that flux depends entirely upon carbon that arrived in the belowground system as a result of plant processes, primarily through foliar litter, dead roots, dead wood. Heterotrophic respiration occurs insofar as microbes are able to access and metabolize those carbon-containing compounds. Different conceptual

frameworks have evolved to categorize soil organic carbon (SOC) according to the ease with which its components are decomposed and, thus, respired as CO₂. Perhaps the most widely known model is the biochemical selectivity or chemical recalcitrance hypothesis, which categorizes SOC compounds by the ease with which their carbon can be biochemically reduced to provide energy. In this framework, “labile” compounds such as carbohydrates and amino acids are more rapidly metabolized by microbial decomposers while lignin and other “recalcitrant” macromolecules resist decomposition owing in part to the limited amount of energy they provide decomposers relative to the amount of energy they require to decompose (Burdige, 2007; Hatcher et al., 1983; Hemingway et al., 2019). Similarly, the by-products of microbial decomposition would be considered more recalcitrant than their parent material since their readily accessible energy sources have already been oxidized (Lehmann & Kleber, 2015).

More recently, however, a new framework has been developed according to protection hypotheses, which posit that resistance to decomposition is the result of interactions with minerals that physically “protect” or “occlude” organic molecular compounds from agents of decomposition such as microbes and the extracellular enzymes they exude to facilitate decomposition (Torn & Trumbore, 1997; Vogel et al., 2014). Such occlusion can cause compounds to persist in soil for much longer than would be expected under the biochemical selectivity framework because significant energy inputs are required to desorb occluded SOC compounds from mineral soils (Hemingway et al., 2019). In this model, SOC cannot be categorized into discrete pools according to its resistance to decomposition; rather, SOC compounds fall along a gradient of resistance. Ecosystem characteristics such as soil type and water availability contribute to rates of SOC occlusion (Schmidt et al., 2011), but the mechanisms of SOC occlusion are many and complex. These include—among others—the formation of large aggregates that reduce access to SOC compounds; hydrophobicity that reduces water content around SOC, limiting its potential for decomposition; and ligand exchange processes that tightly bind SOC to mineral soil (Lützow et al., 2008).

To a certain degree, the dual hypotheses of biochemical selectivity and molecular protection are thought to interact to control rates of decomposition and respiration, with biochemical selectivity

dominating early stages of decomposition and molecular protection controlling later stages (Hemingway et al., 2019). While scientific understanding of this interaction is still developing, it is clear that “younger” sources of carbon (i.e., those that have been present in the soil for no more than a year) contribute most to heterotrophic soil respiration. Indeed, only about 10% of total soil respiration—or roughly 20% of total heterotrophic respiration—is generated through microbial decomposition of older SOC compounds (Ryan & Law, 2005). The bulk of heterotrophic respiration, therefore, involves the decomposition of recently produced carbon compounds, a process that occurs primarily in the immediate surroundings of fine roots, or the rhizosphere.

Heterotrophic respiration in the rhizosphere

As fine roots exude labile carbohydrates, a community of fungi, microbes, and other soil-borne organisms colonizes the surrounding rhizosphere (Courty et al., 2010). By attracting microbial decomposers and detritivores into the rhizosphere, plants induce a “priming effect” that enhances soil fertility (Alexander, 1977; Kuzyakov et al., 2000). The release of easily decomposed exudates accelerates the decomposition of more resistant materials, thereby increasing the mineralization of nutrients into the soil matrix and making them readily available for plant uptake. As much as 21% of net primary production (NPP) has been found to be partitioned to ectomycorrhizae alone (Hobbie, 2006), and the belowground microbial community relies heavily upon root exudates for survival. In fact, a tree girdling study in Sweden detected reductions of up to 32% of the total soil microbial biomass when carbon inputs to soil from root exudates are removed (P. Högberg & Read, 2006).

Soil nutrient concentrations have been thought to control the rate at which these exudates are released, but experimental evidence in support of that hypothesis is inconsistent. Fertilization studies have shown that when the support system of soil organisms is no longer needed for N mineralization, trees invest much less of their carbohydrate assets to sustaining that belowground community. Isotopically labeled CO₂ was used to demonstrate that N additions decreased root exudation by 60% in a Swedish boreal forest (M. N. Högberg et al., 2010). Although maintaining a rhizosphere

community constitutes a steep carbon cost, that mechanism appears to be the most economical way for certain species to acquire nutrients. Verlinden et al. (2018) demonstrated, for example, that plants growing in soil inoculated with arbuscular mycorrhizae allocated less carbon belowground in mesocosms without added phosphorus (P). Despite these relationships between nutrient conditions and root exudation, still other studies found no correlation between the chemistry of soil organic matter and rates of root exudation (Brzostek et al., 2013). To explore further these relationships, a range of methods including root exclusion and isotope labeling have been used to quantify microbial contributions to soil respiration and—more specifically—the extent to which microbial respiration depends upon root exudates (Kuzyakov & Larionova, 2006). In a review of those methods, Kuzyakov and Larionova (2006) report that on average roughly 40% of soil respiration can be attributed to roots, with the remaining 60% originating from microbial decomposition or the metabolism of root exudates.

Autotrophic soil respiration

Roots respire as they expend energy to create and maintain tissue (Vose & Ryan, 2002). Photosynthates are the fuel that drive those processes, and the turnover between the production of photosynthate and its respiration belowground can be quite fast. Indeed, Högberg et al. (P. Högberg et al., 2001) found that total soil respiration fell as much as 37% just 5 days after girdling was applied to stop the supply of photosynthate to the belowground system. 1-2 months after girdling, respiration had fallen an additional 17%. When the supply of photosynthate had been cut off, roots turned to starch reserves to meet their energy-production needs, nearly depleting those reserves entirely over the course of a single growing season. In agreement with the previously cited study by Kuzyakov and Larionova (2006), Högberg et al. estimate that total root respiration is comparable to heterotrophic respiration.

Rates of respiration, however, are not consistent across all roots. Respiration per unit mass is inversely related to root diameter, with highly physiologically active fine roots respiring at much greater rates than their coarse counterparts. In one study of sugar maple (*Acer saccharum* Marsh.), for example, fine roots (<0.5 mm in diameter) respired at rates as much as 5 times greater than those of larger roots

(2.0-10.0 mm in diameter, Pregitzer et al., 1998). Despite such estimates of respiration per unit mass, it is difficult to estimate the per-unit-area contributions of coarse and fine roots to total soil respiration due to the challenge of reliably quantifying relative proportions of coarse- and fine-root biomass (Addo-Danso et al., 2016). However, studies of observational and modeled data agree that coarse root biomass is roughly 6-8 times that of fine roots in temperate forests (Fahey et al., 2005; Smyth et al., 2013). That relative imbalance in biomass allows coarse roots to contribute more to total root respiration than rates of specific root respiration may suggest. Nevertheless, Burton et al. (2012) report that while fine roots (<0.5 mm diameter) constitute only 27% of total root biomass, they contribute 69% of total root respiration.

Among foliage, stems, coarse roots, and fine roots, N concentrations are positively correlated with respiration (Reich et al., 2008; Vose & Ryan, 2002). One mechanism proposed to explain this correlation is that many processes that maintain homeostasis require N-rich proteins, and energy inputs are necessary to build and repair those proteins (de Vries, 1975). However, foliar respiration has been found to decrease by a maximum of just 37% when protein synthesis is inhibited, suggesting that that process is not the primary driver of respiration, at least in leaves (Bouma et al., 1994). In addition to protein synthesis and turnover, a long list of other physiological processes are known to require the energy inputs of respiration: the creation of new structural biomass, the movement of photosynthate, nutrient uptake, and maintaining ion gradients across cellular membranes (Amthor, 2000). All of these processes are highly relevant to fine roots, helping to explain why rates of respiration at a given tissue N concentration tend to be higher in roots than in leaves (Reich et al., 2008).

Climatic controls of soil respiration

Many models of soil respiration represent it as being tightly correlated with soil temperature because microbial and root activity increase with temperature (Kicklighter et al., 1994; Raich & Potter, 1995). It stands to reason that autotrophic respiration would, to a certain extent, increase with temperature since plant activity is greatest during the warmest part of the year. Interestingly, however, temperature-corrected respiration measurements indicate that seasonal patterns are no less important than

soil temperature. In their study of a temperate forest in North Carolina, for example, Vose and Ryan (Vose & Ryan, 2002) found that temperature-corrected fine root respiration values were greatest in late September, when the temperature at the time of measurement was 5.7 °C lower than it had been in mid-July. Fahey and Hughes (Fahey & Hughes, 1994) found that fine root respiration was greater in June than in August, proposing that root respiration was highest early in the growing season when root biomass was being actively generated by trees. These findings may suggest that the highest rates of soil respiration do not tightly follow the climactic patterns that most favor photosynthesis, despite the rapid turnaround between the production of photosynthate and its consumption in roots.

Soil moisture also affects soil respiration rates, which increase when dry soils are rewetted. This increase is driven by both biotic and abiotic factors. Rewetting events such as heavy rains fill soil macropores, preventing the movement of gases into and out of the soil system until that water is drained (Hirano et al., 2003). This not only traps CO₂ in the soil—temporarily reducing soil CO₂ efflux—it also gives soil microbes access to energy resources that had previously been inaccessible due to their location outside of water-filled micropores (Fierer & Schimel, 2003), causing newly respired CO₂ to accumulate. When the soils finally drain, that accumulated CO₂ is released in a pulse that can last 2 to 6 days (Fierer & Schimel, 2003). Finally, wind and pressure gradients also create spatial and temporal heterogeneity in rates of soil respiration as low-pressure events draw CO₂ from the soil (Ryan & Law, 2005).

Nutrient availability and soil respiration

The responses of soil respiration to different nutrient conditions are well documented for N but less so for other macronutrients. Generally, rates of belowground carbon flux are higher in low-fertility sites because greater inputs are required for resource acquisition (Bae et al., 2015; Bloom et al., 1985; Gower et al., 1994). Studies of soil respiration across natural fertility gradients, however, are much less common than those that evaluate the response of soil respiration to experimental nutrient additions. The preponderance of N fertilization studies is due in large part to concerns about the impacts of decades of

anthropogenic N additions to forest ecosystems by way of atmospheric deposition, particularly in the northeastern United States where forest productivity is thought to be N-limited (Janssens et al., 2010). These experiments have yielded varying results depending upon the duration of N addition (Bowden et al., 2004; Burton et al., 2004; Nohrstedt et al., 1989), the rate of that addition (Bowden et al., 2004; Lu et al., 1998; Mo et al., 2008), stand species composition (Lee & Jose, 2003), inherent site fertility (Arnebrant et al., 1996; Bae et al., 2015; Kang et al., 2016; Martikainen et al., 1989), and the type of fertilizer added (Thirukkumaran & Parkinson, 2000). Despite the wealth of knowledge regarding relationships between soil respiration and N, no studies have tested how N fertilization may interact with additions of other nutrients to affect soil respiration. Given recent evidence for P limitation of aboveground tree growth in northern hardwood forests, the effect of added P on microbial enzyme activity (Shan, 2019), and the detection of increased carbon mineralization under P addition (Fisk et al., 2015), there is potential for soil respiration to be affected by P availability, and further investigation into that relationship is merited.

Duration of N addition

Multiple studies have detected an increase in soil respiration in the first two years of low annual N additions ($5 \text{ g N m}^{-2} \text{ y}^{-1}$; Bowden et al., 2004; Contosta et al., 2011). In their study of fertilization effects in a northern hardwood ecosystem, Bowden et al. (2004) report increases of 26% and 14% in rates of soil respiration in the first and second years of fertilization, respectively. One mechanism that has been proposed to explain this initial increase in soil respiration include an improved capacity on the part of soil microbes to metabolize labile carbon sources that have accumulated in soil (Aber et al., 1998). Given that current models of decomposition downplay the role of biochemical selectivity, however, this explanation is not sufficient on its own. Bowden et al. (2004) suggest that increases in soil respiration may be traced back to increases in fine root biomass that were detected in the same study after four years of N addition (Magill et al., 1997). That increase in biomass would be expected to result in greater levels of root and rhizosphere respiration. With regard to heterotrophic respiration in particular, Waldrop et al. (2004)

detected an increase in microbial activity one year after low and high N additions in stands containing sugar maple but not in oak stands. This could also help to explain increased soil respiration.

As fertilization continues, soil respiration shows a negative response to N addition (Bowden et al., 2004; Burton et al., 2004; Nohrstedt et al., 1989). In the case of Bowden et al. (2004), high-rate additions of N reduced soil respiration by 41% relative to controls. This effect could be attributed to multiple potential causes: the loss of recalcitrant carbon sources following an initial pulse in their decomposition following fertilization (Neff et al., 2002), a reduction in microbial biomass in N-fertilized plots (Compton et al., 2004), or a loss of fungal diversity following N fertilization (Allison et al., 2007). Frey et al. (2004) report fungal biomass losses as great as 27-61% following chronic fertilization, and mycorrhizal taxa were found to be particularly affected by N addition, effectively failing to produce reproductive structures after just four years of low N additions (Rühling & Tyler, 1991).

Rate of N addition

Generally speaking, chronic N additions appear to exhibit some additive characteristics, with low-rate additions behaving similarly to high-rate additions over time. For example, in the first year of one long-term fertilization experiment, both low and high rates of fertilization increased the growing season net carbon flux (40% and 34%, respectively; Bowden et al., 2004). By the second year of fertilization, however, the plots receiving high rates of N addition had already begun to exhibit the suppressive effect (15%) that N additions typically have upon soil respiration over time. At the same time, soil respiration was still enhanced (14%) in plots with low rates of N addition. In the 14th year of nutrient additions, soil respiration was suppressed in both N-treated plots, but the magnitude of the effect was much greater in plots receiving high-rate additions (15% in low-rate plots versus 41% in high-rate plots).

Similarly, Lu et al. (1998) found that while specific root respiration of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings increased at low soil N concentrations (50 mg N L⁻¹), high concentrations of soil N (200 mg N L⁻¹) caused a decrease in root dry weight. If these responses are consistent for larger trees, and if chronic additions can be considered to sum over time in a manner that

would be comparable to high-rate additions, they could help to explain the patterns in soil respiration that emerge over time in long-term N addition experiments.

Inherent site fertility

Soil respiration has been found to be lowest on sites with high N mineralization and net nitrification (Bae et al., 2015). This is consistent with the economic model of asset allocation that would predict lower levels of asset investment in the belowground system in sites where resources are readily available (Bloom et al., 1985; Rastetter & Kwiatkowski, 2020). Similarly, soil respiration responses to added N depend upon the inherent fertility of the site where they are applied. Generally, sites with high pretreatment fertility tend to have a less pronounced response to nutrient addition, perhaps because fewer assets are being allocated to resource acquisition in the pretreatment conditions. Martikainen et al. (1989) report that N addition reduced soil respiration in all but the most fertile study site, where N addition actually increased respiration. Similarly, Kang et al. (2016) report correlations between pretreatment rates of N mineralization and ratios of the rate of soil respiration in N-treated plots to soil respiration values in their respective control plots ($p = 0.09$ for N plots; $p = 0.02$ for NP plots). The effect of N addition upon soil respiration was positive in sites with high pretreatment N mineralization and negative in sites with low pretreatment N mineralization.

Martikainen et al. (1989) hypothesize that the high apatite and biotite content of their high-fertility site may have provided a buffer against the acidifying effects of N addition, potentially allowing for the soil respiration enhancement observed there. Microbial communities could offer another potential mechanistic explanation, as Arnebrant et al. (1996) detected reductions in microbial biomass and microbial respiration following N addition in all sites except those with the highest site fertility. Whatever the mechanism may be, site fertility is an important consideration when analyzing the effects of N addition on soil respiration.

Stand age

In temperate ecosystems, rates of soil respiration change as stands age, but the direction of that change is inconsistent, falling with time in some ecosystems (Tedeschi et al., 2006) and rising in others (Gough et al., 2005). Where soil respiration decreased with increasing stand age, both the falling soil temperatures that accompanied crown closure and reductions in detritus were credited with causing decreased soil respiration (Gough et al., 2005). Where the opposite effect was seen, researchers attributed higher rates of soil respiration to increasing root biomass development and total root respiration over time (Gough et al., 2005).

In northern hardwood ecosystems, fine root biomass regenerates rapidly after clearcut, reaching 71% of levels in mature stands as soon as four years after clearcutting (Fahey & Hughes, 1994), so—assuming specific root respiration is constant throughout the life cycle of a tree—autotrophic contributions to soil respiration would be expected to be consistent throughout stand development. However, Kang et al. (2016) report higher rates of soil respiration in mature stands (84-130 years old) when compared to successional stands (23-43 years old). Furthermore, no effect of stand age on microbial respiration was detected in Oe-horizon soils from those stands. While the possibility of such an effect can not be eliminated, these results may suggest that the stand age effect on soil respiration could be attributed to differences in autotrophic respiration.

Conclusion

Understanding soil respiration is essential in developing a complete picture of the global carbon cycle, but given the many different and interacting communities that contribute to the generation of CO₂ belowground, it can be a challenging subject of study. However, because of that integrative nature, soil respiration measurements offer a holistic picture of the ecosystem impacts of fertilization and N deposition. Relationships between N availability and soil respiration are well studied due to the limiting role of N in North American forests, but the role of other macronutrients in the processes described above is under-studied. Exploring the extent to which other macronutrients control soil respiration will help to

refine our understanding of the terrestrial carbon cycle generally and belowground processes more specifically. This is particularly urgent given the enormous disruption to the carbon cycle that has occurred in recent history. Chapter 2 aims to address this deficit by describing soil respiration outcomes in a full-factorial NxP fertilization experiment.

CHAPTER 2: INDEPENDENT AND INTERACTIVE EFFECTS OF NITROGEN AND PHOSPHORUS ADDITION ON FOREST SOIL RESPIRATION

Introduction

The largest flux in the global carbon cycle is the movement of carbon into and out of terrestrial ecosystems (Le Quéré et al., 2018), and in temperate systems, about 70% of ecosystem respiration comes from soils (Goulden et al., 1996; Janssens et al., 2001; Law et al., 1999; Ryan & Law, 2005), making them the largest source in the global carbon cycle. The effects of nitrogen (N) addition on soil respiration are well studied in temperate forest ecosystems. Generally, rates of carbon partitioning to the belowground system—and therefore soil respiration—are higher in low-fertility sites because greater inputs are required for resource acquisition (Bae et al., 2015; Bloom et al., 1985; Gower et al., 1994; Litton et al., 2007). Furthermore, N addition has been found to reduce rates of decomposition through the down-regulation of the activity of ligninolytic enzymes (Carreiro et al., 2000; Knorr et al., 2005). Experiments testing for the effects of N addition on soil respiration yield varying results, however, depending upon the duration of fertilization (Bowden et al., 2004; Burton et al., 2004; Nohrstedt et al., 1989), and stand age has also been found to affect soil respiration (Gough et al., 2005; Kang et al., 2016; Tedeschi et al., 2006).

In a study of a northern hardwood system at Harvard Forest, Bowden et al. (2004) report increases of 40% and 14% in rates of soil respiration in the first and second years of low-rate N addition ($5 \text{ g N ha}^{-1} \text{ yr}^{-1}$), respectively. Thirteen years after fertilization began, however, soil respiration showed a negative response to N addition, with soil respiration reduced by 15% relative to controls (Bowden et al., 2004). This effect could be attributed to multiple potential causes: reduced root exudation commensurate with a decreased dependence on N resources generated by microbes by way of soil priming (Kuzyakov et al., 2000), loss of recalcitrant carbon sources following the initial pulse in decomposition after the first few years of fertilization (Neff et al., 2002), a reduction in microbial biomass in N-fertilized plots (Compton et al., 2004), or a loss of fungal diversity or biomass following N fertilization (Allison et al., 2007; Frey et al., 2004).

Rates of soil respiration change as stands progress through successional development, but the direction of that change is inconsistent, rising over time in northern hardwood ecosystems (Kang et al., 2016) and decreasing in a Mediterranean oak ecosystem (Tedeschi et al., 2006). In loblolly pine plantations, soil respiration increased with time in some locations but was not affected by stand age in others (Gough et al., 2005). Where soil respiration decreased with stand age, both the falling soil temperatures that accompanied crown closure and reductions in detritus—both woody debris on the soil surface and fine roots left behind from removed vegetation—were credited with causing decreased soil respiration (Tedeschi et al., 2006). Where the opposite effect was seen, researchers have attributed higher rates of soil respiration to increasing root development and, therefore, total root respiration (Gough et al., 2005). In northern hardwood ecosystems, however, fine root biomass regenerates rapidly after clearcutting, reaching 71% of fine root biomass in mature stands as soon as four years after clearcutting (Fahey & Hughes, 1994), suggesting that fine root biomass alone is unlikely to explain stand age effects on soil respiration.

While effects of N addition and stand age on soil respiration have each been explored individually, few studies report interactions between those factors. Furthermore, no studies have tested how N fertilization may interact with additions of other macronutrients. Given recent evidence for phosphorus (P) limitation of aboveground growth in northern hardwood forests (Goswami et al., 2018), the effect of added P on microbial enzyme activity (Shan, 2019), and the detection of increased carbon mineralization under P addition (Fisk et al., 2015), there is potential for soil respiration to be affected by P availability. Furthermore, with the potential for productivity to be co-limited by multiple elements (Rastetter & Shaver, 1992), there is a paucity of literature reporting how P controls soil respiration.

In this study, we tested for interactive effects of N and phosphorus (P) on soil respiration across stands of three age classes in the longest-running N by P manipulation experiment in a temperate forest. Our dataset spans 10 years (2011-2020) and includes three seasons of soil respiration measurements. We hypothesized (1) that soil respiration would be reduced by N addition and increased by P addition and (2) that soil respiration would be consistent across stand age classes.

Methods

Site description

This study was conducted in 13 stands in the White Mountain National Forest of New Hampshire, USA (Table 1). Stands were of three successional stages: two early successional stands (19-21 years old

Table 1. Stand characteristics

Site	Stand	Age class	Year cut	Elevation (m)	Aspect	Slope (%)	Basal area (m ² ha ⁻¹)	Dominant species based on basal area
BEF	C1	Early successional	1990	570	Flat to SE	5-20	9.5	<i>Betula papyrifera</i> , <i>Prunus pensylvanica</i> , <i>Fagus grandifolia</i>
	C2	Early successional	1988	340	NE	15-30	10.8	<i>Acer rubrum</i> , <i>F. grandifolia</i> , <i>B. papyrifera</i>
	C3	Mid-successional	1985	590	NNE	8-20	20.9	<i>F. grandifolia</i> , <i>A. rubrum</i> , <i>P. pensylvanica</i>
	C4	Mid-successional	1978	410	NE	20-25	26.3	<i>B. papyrifera</i> , <i>Populus grandidentata</i> , <i>A. rubrum</i>
	C5	Mid-successional	1976	550	NW	20-30	19.7	<i>B. papyrifera</i> , <i>F. grandifolia</i> , <i>A. rubrum</i>
	C6	Mid-successional	1975	460	NNW	13-20	29.6	<i>A. rubrum</i> , <i>B. papyrifera</i> , <i>B. alleghaniensis</i>
	C7	Mature	~1890	440	ENE	5-10	32.8	<i>F. grandifolia</i> , <i>A. saccharum</i> , <i>Tsuga canadensis</i>
	C8	Mature	1883	330	NE	5-35	40.5	<i>F. grandifolia</i> , <i>A. saccharum</i> , <i>B. alleghaniensis</i>
	C9	Mature	1890	440	NE	10-35	31.7	<i>A. saccharum</i> , <i>F. grandifolia</i> , <i>B. alleghaniensis</i>
HBEF	HBM	Mid-successional	1970	500	S	10-25	27.6	<i>B. alleghaniensis</i> , <i>B. papyrifera</i> , <i>F. grandifolia</i>
	HBO	Mature	1911-1913	500	S	25-35	27.1	<i>B. alleghaniensis</i> , <i>F. grandifolia</i> , <i>A. saccharum</i>
JB	JBM	Mid-successional	~1974	730	WNW	25-35	24.0	<i>B. alleghaniensis</i> , <i>A. saccharum</i> , <i>B. papyrifera</i>
	JBO	Mature	1915-1929	730	WNW	30-40	35.6	<i>A. saccharum</i> , <i>B. papyrifera</i> , <i>F. grandifolia</i>

Note: Basal area calculations are based upon inventory data collected in 2015. Dominant species are listed in descending order of stand basal area per species.

at the beginning of the study in 2009), six mid-successional stands (24-39 years old in 2009), and five mature stands (80-126 years old in 2009). Stands were located in three sites: nine at Bartlett

Experimental Forest (BEF; 44°2–4` N, 71°9–19` W; elevation 250–500 m), two at Hubbard Brook

Experimental Forest (HBEF; 43°56` N, 71°44` W; elevation 500 m), and two at Jeffers Brook (JB; 44°2`

N, 71°53` W; elevation 730 m). Tree species composition varied across stands, but mature stands were

representative of the typical northern hardwood forest: sugar maple (*Acer saccharum* Marsh.), yellow

successional stands consisted of red maple (*A. rubrum* L.), striped maple (*A. pensylvanicum* L.), pin cherry (*Prunus pensylvanica* L.f.), paper birch (*Betula papyrifera* Marsh.), yellow birch, and American beech. Soils in all stands were formed in glacial drift and are predominantly Spodosols with a range of drainage characteristics (Bailey, 2020; Vadeboncoeur et al., 2012). Precipitation is evenly distributed throughout the year and amounts to about 127 cm annually. Annual temperatures in this humid, continental climate range from an average low of -5 °C to an average high of 32 °C (Adams et al., 2008), but differences in elevation and slope aspect across the stands result in considerable temperature differences.

The study stands are part of the Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) study. Within each of the 13 stands are four treatment plots, each of which has received one of four nutrient treatments annually beginning in 2011: N addition (3 g N m⁻² yr⁻¹ in the form of granular NH₄NO₃), P addition (1 g P m⁻² yr⁻¹ in the form of granular NaH₂PO₄), N plus P (at the same rates), or neither (control). In 10 stands, plots measure 50 m x 50 m including a 10 m buffer on each side to ensure that trees with roots that extend beyond the 30x30 measurement area receive the full fertilization. In two of the mid-successional stands (HBM and JBM), each plot measures 30 m x 30 m including a 5 m buffer. In one early successional stand (C2), each plot measures 45 m x 45 m including a 7.5 m buffer.

Soil respiration

Soil CO₂ efflux (soil respiration) was measured in all 13 stands from 2009 to 2020 (i.e., including two pretreatment years) using the LiCOR 8100 F_{CO2} system (Licor Biosciences, Lincoln, NE). Measurements were made between 9 AM and 4 PM with most falling between the hours of 10 AM and 2 PM. The number of measurements recorded and the stands in which they were made varied across years. In 2009, five collars were haphazardly arranged in each plot. Locations near boles, boulders, large roots, and areas that would collect water were avoided. In 2009 and each subsequent year, soil respiration was measured at each collar with a single 90-second reading. In 2010, those collars were removed and systematically arranged in their final locations. In 2014, two additional collars were added to each plot in

systematic locations. Soil temperature was measured simultaneously with soil respiration using a handheld resistance thermometer at a depth of 20 cm adjacent to each collar.

Data analysis

We tested for effects of nutrient addition and stand age on soil respiration with a repeated-measures, mixed effect analysis of variance (ANOVA) using the lme4 package in R (Bates et al. 2020). The dataset was broken into five distinct phases to capture transient effects (Bowden et al., 2004): 2011 (the first year of nutrient additions), 2012-2014, 2015-2017, and 2018-2020. Each phase was then analyzed by season: spring (March 15 - May 31), summer (June 1 - August 30), and fall (August 31 - November 26). Each season within a three-year phase of the study is referred to as an “analysis period” throughout this study.

Median plot soil respiration values were used as the response variable to control for undue influence by individual collars that had unusually high rates of soil respiration on a given visit to a plot. Those median values were log-transformed to improve normality of residuals. Treatments (N addition, P addition, and stand age) were replicated across stands. Fixed effects included a covariate to capture pretreatment differences across plots, median soil temperature within the plot at the time of measurement, and a four-way interaction term including N addition, P addition, stand age, and year. The pretreatment covariate was calculated by averaging the median soil respiration values for each plot in 2009 and 2010. The pretreatment covariate was correlated with post-treatment soil respiration only in summer 2011 ($p = 0.08$), so it was removed from the model in every other analysis period. Stand age was a categorical factor, with stands C1 and C2 classified as early successional; C3, C4, C5, C6, HBM, and JBM classified as mid-successional; and C7, C8, C9, HBO, and JBO classified as mature (Table 1). Year was coded as a categorical factor. Each of the four factors included in the interaction term was also tested for its main effect.

Random effects included plot nested within stand nested within site. Assumptions of normality of residuals and homoscedasticity were evaluated visually using residual plots. When significant effects

interacted with year, seasons within years were analyzed individually to offer a more complete picture of how treatment effects evolved over the course of the study.

Results

Because many complex interactions were detected in the analysis of this data, results are presented focusing on one factor or interaction at a time. We begin by analyzing how the N effect developed over the course of the study. Next, we present effects of P, followed by the interaction of N and P, and concluding with stand age and interactions with that factor. Throughout this study, “analysis period” is used to identify each season within a three-year window of data collection.

Nitrogen

Nitrogen affected soil respiration at $\alpha=0.05$ in five of 10 analysis periods, but in three of those five, N affected soil respiration in interaction with some other factor (Table 2). In 2 of the 5 analysis periods in which the main effect of N was not significant, N was involved in a significant interaction with at least one other factor. Two of the three remaining analysis periods in which N was not significant either as a main effect or in interaction were in 2011, the first year of fertilization.

Spring 2012-2014 was the first analysis period in which an N effect was detected (N*year, $p = 0.01$). When the spring of 2012 was analyzed individually, N addition enhanced soil respiration 32%, making spring 2012 the only time frame in the course of the study during which an enhancement of soil respiration by N addition was detected ($p = 0.02$). By the spring of 2013, plots receiving added N had lower soil respiration than those that did not, but that reduction was not statistically significant ($p = 0.64$). After that initial pulse, soil respiration was consistently reduced by N addition, with the average magnitude of the reduction in summertime measurements increasing over time from 8% in 2012-2014, to 13% in 2015-2017, to 14% in 2018-2020 (Table 3a).

Table 2. Summary of data availability, main effects, and interaction effects on soil respiration by analysis period

A) Data extent by analysis period												
	2011			2012-2014			2015-2017			2018-2020		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Plot-level observations	36	172	65	76	488	236	0	345	45	108	357	212
Stands represented	9	13	9	13	13	13	0	13	11	13	13	13
Rounds	1	4	2	2	10	5	0	9	1	3	8	4
Years represented	1	1	1	2	3	3	0	3	1	1	3	1

B) P-values of significant main effects on soil respiration													
	2011			2012-2014			2015-2017			2018-2020			
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	
Pretreatment covariate		0.07					No data						
Median soil temperature		0.01	0.01		0.01	0.01		0.01		0.01	0.01	0.01	
N					0.01	0.01		0.01				0.01	0.02
P													
Stand age					0.05	0.08		0.01		0.03			0.06
Year					0.01	0.01		0.01				0.01	

C) P-values of significant two-way interaction effects on soil respiration												
	2011			2012-2014			2015-2017			2018-2020		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
N*P					0.02	0.02	No data					
N*year				0.01								
P*year								0.07				
N*Stand age												0.01
Stand age*Year				0.03		0.03						0.01

D) P-values of significant three-way interaction effects on soil respiration												
	2011			2012-2014			2015-2017			2018-2020		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
N*age*year							No data	0.07			0.08	
P*age*year							data	0.07				

Note: Values in effects charts are p-values. Empty cells indicate that the factor was not statistically significant in that analysis period. Bold values are less than 0.05, and script that is not bold indicates a p-value greater than 0.05 and less than 0.10. Cells in gray indicate that the factor was not tested for that analysis period.

Table 3. Summary of main effects and interaction effects of nitrogen addition on soil respiration

A) Main effect of nitrogen All analysis periods												
	2011			2012-2014			2015-2017			2018-2020		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Magnitude of N effect					-8%	-11%	No data	-13%			-14%	-13%

B) Interaction: N * Year Spring 2012-2014			
	Spring 2012	Spring 2013	Spring 2014
Magnitude of N effect	+32%	-5%	No data

C) Interaction: N * Year * Stand age Summer 2015-2017, Summer 2018-2020						
	Summer 2015	Summer 2016	Summer 2017	Summer 2018	Summer 2019	Summer 2020
N effect in early successional stands	-4%	-16%	-10%	-61%	-23%	-19%
N effect in mid-successional stands	-31%	-7%	0%	0%	-10%	-9%
N effect in mature stands	No data	-19%	-19%	+6%	-8%	-12%

Note: Empty cells indicate that the factor was not statistically significant in that analysis period.

In summer measurements from 2015 to 2020, the effect of N addition on soil respiration depended on stand age and year (2015-2017: $p = 0.07$, 2018-2020: $p = 0.08$). Between 2015 and 2017, there was no single stand age that was most strongly affected by N addition (Table 3c). However, beginning in 2018, early successional stands were most negatively affected by N addition, with soil respiration reductions of 61%, 23%, and 19% in 2018, 2019, and 2020, respectively.

Phosphorus

P addition did not affect soil respiration as a main effect in any of the analysis periods, and the more complex, interactive P effects were mostly marginally significant. For example, in the summer 2015-2017 analysis period, P effects depended on year ($p = 0.07$). We found that P addition enhanced soil respiration 18% in 2015 and that this effect was significant when summer 2015 was analyzed individually ($p = 0.01$). P appeared to suppress soil respiration in 2016 and 2017, but those effects were

not significant when those years were analyzed individually ($p = 0.16$ in 2016; $p = 0.30$ in 2017). Those average annual effects can be misleading, however, as the effect of P addition within each year depended on stand age ($p = 0.07$, Table 4b).

Table 4. Summary of interaction effects of phosphorus addition on soil respiration

A) Interaction: P * Year Summer 2015-2017			
	Summer 2015	Summer 2016	Summer 2017
Magnitude of P effect	+18%	-1%	-7%

B) Interaction: P * Year * Stand age Summer 2015-2017			
	Summer 2015	Summer 2016	Summer 2017
P effect in early successional stands	+20%	+30%	-2%
P effect in mid-successional stands	+15%	-11%	-7%
P effect in mature stands	No data	-1%	-7%

P addition enhanced soil respiration in both early successional and mid-successional stands in summer 2015, and the magnitude of the P effect did not vary by stand age when summer 2015 was analyzed individually ($p = 0.70$). In summer 2016, P addition enhanced soil respiration 30% in early successional stands but had a suppressive effect in mid-successional stands (11%) and mature stands (1%). Unlike summer 2015, these differences were significant when summer 2016 was analyzed individually ($p = 0.02$). Finally, in summer 2017, P addition reduced soil respiration across all stand ages, and the magnitude of that reduction was greater in mid-successional and mature stands (7%) than in early successional stands (2%). However, the P*stand age interaction was not significant when summer 2017 was analyzed individually ($p = 0.93$).

*N*P interactions*

Interactions between N and P were detected in two analysis periods (summer 2012-2014 and fall 2012-2014). In both cases, the magnitude of the suppressive N effect was reduced by P addition. In

summer 2012-2014, soil respiration was 8% lower in plots receiving N than in their respective controls ($p < 0.01$). However, while N reduced soil respiration 14% in the absence of P, it only reduced soil respiration 1% when P was present ($p = 0.02$, Table 5). When the summer data of each year is analyzed individually, the N*P interaction is significant in 2012 ($p = 0.03$) and 2014 ($p = 0.01$).

Table 5. Summary of interaction effects of N and P addition

Interaction: N * P								
Summer 2012-2014, Fall 2012-2014								
	Summer 2012-2014			Fall 2012-2014				
	Average soil respiration [95% CI] ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Main effect of N addition	Simple effects of N addition	Average soil respiration [95% CI] ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Main effect of N addition	Simple effects of N addition		
Control	4.61 [4.30, 4.92]	-8%	-14%	3.23 [2.92, 3.55]	-11%	-18%		
Nitrogen	3.95 [3.71, 4.20]			2.64 [2.42, 2.87]				
Phosphorus	4.22 [3.96, 4.48]			-1%			2.70 [2.47, 2.92]	-3%
Nitrogen + Phosphorus	4.20 [3.93, 4.47]						2.61 [2.40, 2.83]	

In fall 2012-2014, soil respiration was 11% lower in N-treated plots than in their respective controls ($p < 0.01$). When no P was added, N addition reduced soil respiration 18%, but in the presence of added P, the suppressive effect of N addition was reduced to just 3% ($p = 0.02$, Table 5). When spring data from each year in the 2012-2014 period is analyzed individually, the interaction between N and P is detected only in 2014 ($p = 0.01$).

Stand age

In seven of the 12 analysis periods for which soil respiration was measured, stand age affected soil respiration either independently or in interaction with another treatment factor. In four of 12 analysis periods, that effect was significant at $\alpha = 0.05$, and in three it was significant at $\alpha = 0.10$. Generally, soil respiration was greatest in early successional stands and lowest in mid-successional stands, but those trends were only sporadically detected (Figure 2). In mid-successional stands, the 95% CIs of soil respiration rates were lower than and exclusive of those of early successional and mature age classes in

effect varied by year within three analysis periods (spring 2012-2014, $p = 0.03$; fall 2012-2014, $p = 0.03$; and summer 2018-2020, $p = 0.01$; Table 6).

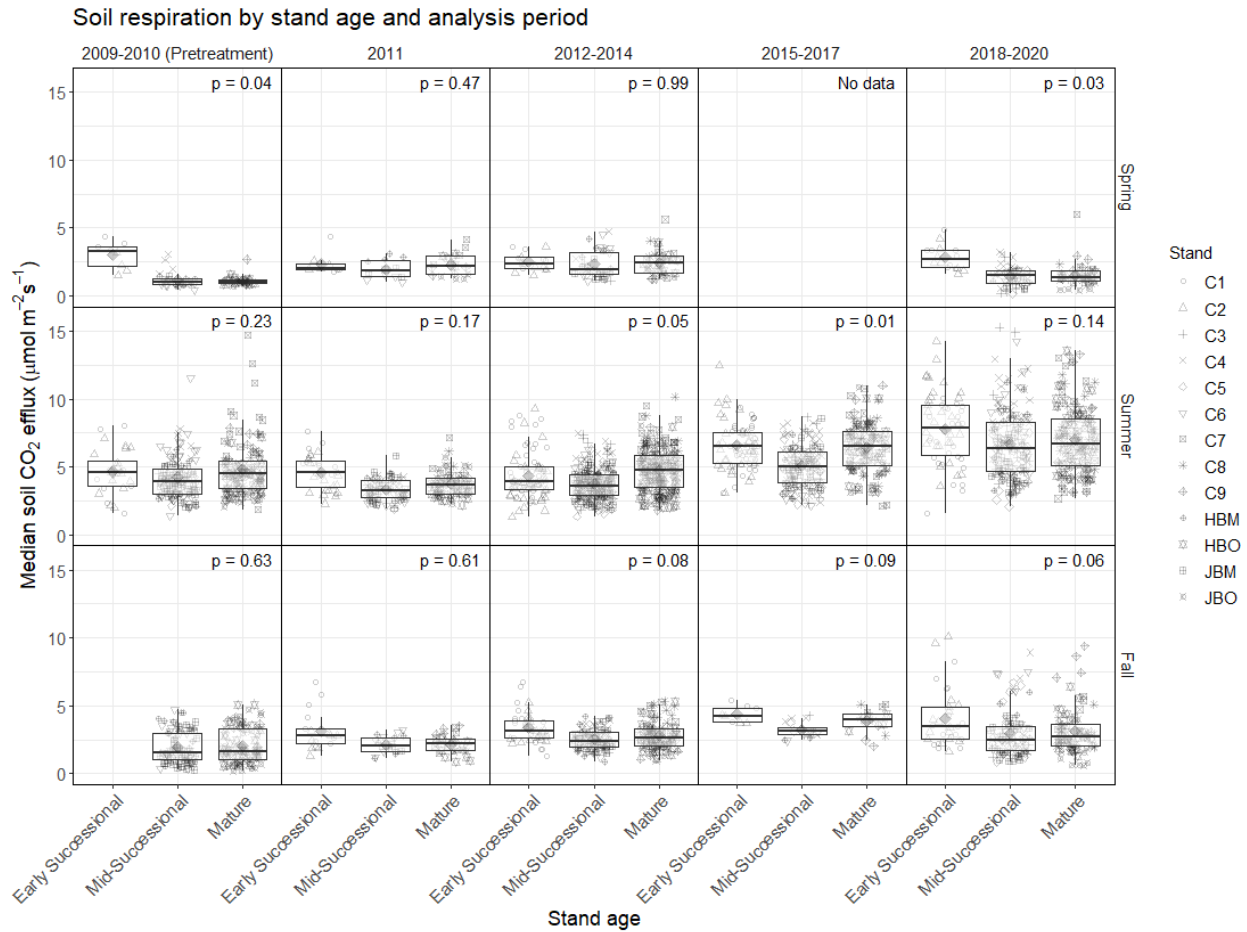


Figure 2. Boxplots of soil respiration by stand age and analysis period. Gray diamonds indicate the average for a given stand age class. Two exceptionally high points [(1) treatment = C, median soil CO₂ efflux = 28.60 µmol m⁻² s⁻¹; (2) treatment = P, median soil CO₂ efflux = 32.00 µmol m⁻² s⁻¹] were removed from the summer 2018-2020 plot to improve the scale.

Table 6. Summary of main effects of stand age on soil respiration

Main effect: Stand age All analysis periods				
		Average soil respiration [95% CI] ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
		Early successional	Mid-successional	Mature
2011	Spring			
	Summer			
	Fall			
2012-2014	Spring	Interaction: Stand age*Year		
	Summer	4.36 [3.98, 4.75]	3.74 [3.58, 3.90]	4.79 [4.55, 5.02]
	Fall	3.38 [3.02, 3.74]	2.53 [2.38, 2.68]	2.82 [2.62, 3.03]
2015-2017	Spring	No data		
	Summer	6.58 [6.18, 6.98]	5.06 [4.82, 5.30]	6.59 [6.21, 6.97]
	Fall	4.38 [3.94, 4.81]	3.21 [2.97, 3.45]	3.94 [3.49, 4.39]
2018-2020	Spring	2.82 [2.38, 3.27]	1.45 [1.24, 1.65]	1.53 [1.27, 1.79]
	Summer	Interactions: Stand age*N, Stand age*Year		
	Fall	4.08 [3.31, 4.84]	2.97 [2.62, 3.33]	3.18 [2.80, 3.57]

Note: Empty cells indicate that the main effect of stand age was not significant for that analysis period. 95% CIs were seldom mutually exclusive across age classes, and tests of least square means indicated that no stand age classes were statistically different from one another.

Table 7. Summary of interaction effects of stand age and year on soil respiration

Interaction: Stand age * Year Spring 2012-2014, Fall 2012-2014, Summer 2018-2020										
		Average soil respiration [95% CI] ($\mu\text{mol m}^{-2} \text{s}^{-1}$)								
		Spring 2012-2014			Fall 2012-2014			Summer 2018-2020		
		2012	2013	2014	2012	2013	2014	2018	2019	2020
Early successional		2.75 [2.20, 3.30]	2.22 [1.96, 2.48]	No data	3.42 [2.77, 4.08]	3.72 [3.23, 4.21]	2.60 [2.12, 3.09]	11.21 [5.09, 17.33]	9.03 [8.07, 9.99]	7.32 [6.35, 8.28]
Mid-successional		3.05 [2.51, 3.59]	1.97 [1.63, 2.31]		1.98 [1.78, 2.19]	3.03 [2.85, 3.20]	2.09 [1.89, 2.82]	5.30 [4.12, 6.48]	7.26 [6.78, 7.74]	6.70 [6.15, 7.24]
Mature		2.67 [2.17, 3.17]	2.24 [1.86, 2.62]		2.43 [2.07, 2.78]	3.36 [3.08, 3.63]	2.57 [2.30, 2.84]	7.02 [5.79, 8.25]	7.28 [6.50, 8.05]	7.21 [6.64, 7.79]

Note: 95% CIs were seldom mutually exclusive across age classes, and tests of least square means indicated that no stand age classes were statistically different from one another.

Discussion

Nitrogen effects

The interactive effects of N and P on soil respiration present an interesting wrinkle in the long history of research into the effects of N addition on soil respiration, and these effects are explored in further detail in the next section of this discussion. Those effects are best understood, however, with a foundational understanding of how N additions affected soil respiration. Overall, the results of this experiment are consistent with those of other studies with regard to the effects of low-rate N addition on soil respiration. No significant effect of N addition ($3 \text{ g N m}^{-2} \text{ yr}^{-1}$) was detected in the first year of nutrient additions, but soil respiration was enhanced 32% by N addition the following spring. Similarly, Bowden et al. (2004) report increases in soil respiration of 40% and 14% in the first two years of low-rate N addition ($5 \text{ g N m}^{-2} \text{ yr}^{-1}$) in a long-term fertilization experiment at Harvard Forest. The relatively rapid response seen in that study could be attributed to the fact that they used an aqueous fertilizer that was more readily taken up by soil biota than the granular fertilizer used in our study. Following the pulse in the spring of 2012, soil respiration was consistently reduced by N addition, reaching a maximum reduction of 14% in summer 2018-2020 (Figure 3). Soil respiration was reduced 15% in response to 13 years of low-rate N additions at Harvard Forest (Bowden et al., 2004), suggesting that a 15% reduction may be an upper limit for the effect of long-term N additions of soil respiration.

Generally, there was no interaction between season and N addition; instead, the effects seen in shoulder seasons typically reflected those detected during the summer for each analysis period. There were, however, a few exceptions to this trend. First, we did not detect N effects in fall measurements for the 2015-2017 period. This is most likely due to a sample size ($N = 45$) that was too small to detect the differences that were consistently recorded for the other analysis periods that had much larger sample sizes ($N = 236$ for 2012-2014 and $N = 203$ for 2018-2020). Second, no N effect was detected in spring measurements during the 2018-2020 period. This is less likely due to sample size alone as N effects were

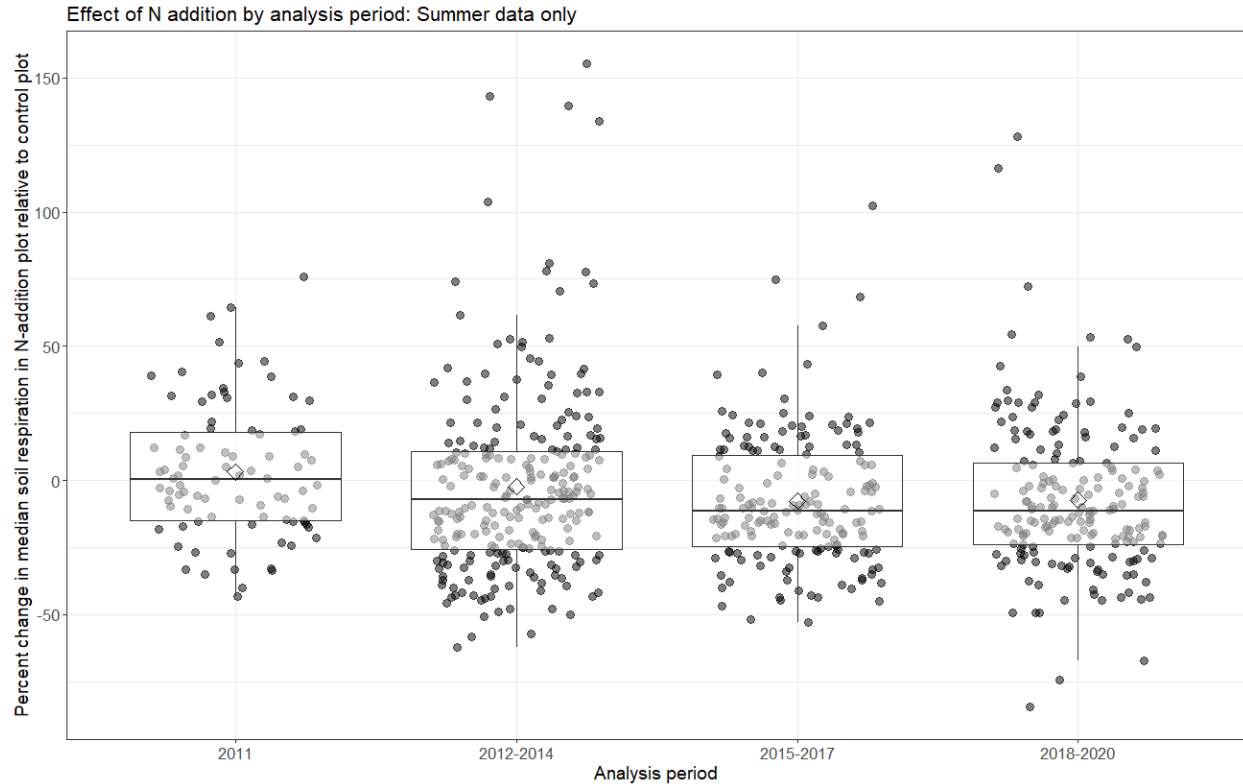


Figure 3. Boxplots of percent change in summertime soil respiration values in response to nitrogen addition. White diamonds indicate the average percent change for a given analysis period.

detected in the spring 2012-2014 analysis period with fewer observations ($N = 76$ for 2012-2014 versus $N = 108$ for 2018 -2020).

Interactions with P

An interaction of N and P in summer and fall 2012-2014 indicated that soil respiration responses to N addition were tempered by P availability. In summertime measurements from 2012-2014, soil respiration was 16% lower in plots receiving just N than in untreated controls, but in the presence of added P, the N addition had no effect (Figure 1). Similarly, in fall measurements from 2012-2014, N reduced soil respiration 18% relative to untreated controls, but the N suppression was limited to just 3% in the presence of added P (Figure 1). This interaction was not detected in any subsequent analysis period, suggesting that the effect of added P was transient.

A variety of mechanisms have been considered to explain reductions in soil respiration in response to N addition. The first is reductions in root exudation and the subsequent respiration of that carbon by soil microbes (Kuzyakov et al., 2000). Nitrogen fertilization, it is thought, reduces the extent to which plants depend upon microbial communities to mineralize organic N pools, so plants respond by partitioning less carbon to sustaining those communities. Perhaps when N and P are added together, however, plants respond to greater P availability with a greater demand for N, causing resource allocation for N acquisition to be suppressed less than it would be if N were added alone. Such an interaction would be consistent with the multiple-element limitation hypothesis, which posits that vegetation responds to changes in nutrient concentrations in such a way that all nutrients limit productivity (Rastetter & Shaver, 1992).

An alternative explanation for the N effect suggests that N fertilization reduces pools of soil organic carbon, ultimately resulting in lower rates of heterotrophic soil respiration (Neff et al., 2002). This hypothesis suggests that N addition initially accelerates decomposition by lowering ratios of carbon to N in the soil system. This allows for otherwise recalcitrant substrates to be more easily mineralized, causing an initial pulse in respiration followed by a reduction. If this were to explain the interaction of N and P detected in this study, P addition would have to inhibit the depletion of soil carbon pools. Our results do not support that explanation P had no independent effects prior to the interaction of N and P detected in 2012-2014. Furthermore, an analysis of carbon mineralization in incubated soils from these sites found that microbial respiration increased in response to added P and added leaf litter and decreased in response to added N (Fisk et al., 2015). This suggests that if P addition were to affect recalcitrant carbon pools in any way, it would be to cause them to be reduced and, therefore, reduce long-term soil respiration.

Yet another mechanism for the N effect could be reductions in rates of decomposition: as decomposition is reduced, soil respiration would be expected to decrease as well. Nitrogen fertilization has been found to stimulate, reduce, and have no effect on decomposition, with the nature of the effect depending upon litter quality (Knorr et al., 2005). Where decomposition is reduced, N addition has been

found to down-regulate the activity of the microbial enzyme polyphenol oxidase, which facilitates the acquisition of carbon from lignin-rich detritus (Carreiro et al., 2000). In an analysis of enzyme activity at the sites used in this study, N addition had no effect on the activity of polyphenol oxidase; instead, its activity was limited under P addition (Shan, 2019). Based upon these outcomes alone, one would expect soil respiration to be reduced by P addition and unaffected by N addition. However, enzyme activity in that study also suggests that N fertilization promotes microbial acquisition of carbon, carbon that would ultimately be respired or immobilized in microbial necromass (Kallenbach et al., 2016). Our observed reductions of soil respiration in N-treated plots indicate that the latter is the most likely outcome. If P additions in the presence of added N facilitated the decomposition of that otherwise immobilized microbial carbon pool, the interactive effect of N and P observed in this study could be more easily understood.

Reductions in microbial biomass and diversity in N-fertilized plots are also a potential explanation for the effects of N addition on soil respiration (Allison et al., 2007; Compton et al., 2004; Frey et al., 2004), but there is little evidence to suggest that shifts in fungal biomass and diversity alone could explain the N*P interaction on soil respiration. In a study of the abundance, biomass, and species richness of ectomycorrhizal fungal fruiting bodies in these study plots, fungal community composition was altered by both N and P, but the biomass of fruiting bodies was not affected by the addition of either nutrient (Victoroff, 2020). Only N addition was found to reduce sporocarp abundance, and N x P interactions were not detected for any response variable (Victoroff, 2020). Admittedly, fungal fruiting bodies offer only a very limited picture of the belowground community. However, if they can be considered a proxy for microbial biomass and diversity, these results would indicate that any role in the N*P interaction described previously would be the result of shifts in community composition.

Given the evidence for interactive effects of N and P on soil respiration, further research will be valuable for identifying the mechanisms causing that interaction. Two studies conducted in the MELNHE experiment may help to inform that research. First, P additions increased tree diameter growth from 2011-2015 (Goswami et al., 2018), a window that intersects with the N*P interaction observed

here. Fine-root growth, however, increased in N-treated plots rather than P-treated plots from 2013-2015 (Shan, 2019). The contrasting nutrient limitations of above- and belowground processes may be related to the N*P interaction observed in soil respiration: if P addition affects aboveground activity—and its implications for belowground allocation—in one manner while N addition affects belowground activity in another, potential interaction effects seem likely. Future studies to further elucidate these interactions could include testing for P effects on root exudation and allocation to mycorrhizae, testing for increased litter production in P-treated plots, testing for the effects of N and P on the decomposition of microbial biomass, and testing for effects of nutrient addition on specific root respiration.

Stand age

Previous studies of the relationship between stand age have found conflicting results, with some reporting positive relationships between stand age and soil respiration (Gough et al., 2005), some reporting the opposite (Ewel et al., 2011; Tedeschi et al., 2006), and still others reporting interannual differences in the effect of stand age on soil respiration (Irvine & Law, 2002). Negative relationships between stand age and soil respiration are often attributed to high soil temperatures in aggrading stands and the high quantities of detritus in post-harvest slash (Ewel et al., 2011). Positive relationships, on the other hand, are typically explained by increases in fine-root biomass (Gough et al., 2005). As mentioned previously, fine root biomass recovers quickly after clearcutting in northern hardwood ecosystems, achieving as much as 71% of the fine root biomass in mature forest after just four years (Fahey & Hughes, 1994).

Assuming that soil respiration is greatest in early successional stands and lowest in mid successional stands, it seems—based on known trends of fine-root biomass production—difficult to attribute the stand age effect to differences in biomass alone. Otherwise, the stand age effect could be related to changes in specific fine-root respiration, which has been found to increase over time following fire in a boreal forest (Makita et al., 2016) and to be positively correlated with specific root length (Makita et al., 2012). However, fire is likely to introduce factors that would not explain the stand age

effect in this study, and specific root length was not found to be affected by stand age in a study conducted in these stands (Naples & Fisk, 2010), meaning those explanations can likely be ruled out as well. Perhaps high rates of soil respiration in young stands can be attributed to the high degree of competition for resources. It stands to reason that this may require elevated allocation of resources—i.e., greater root exudation—to the belowground system and thus higher levels of soil respiration. Why that effort would increase again after the mid-successional stage is unclear but could, perhaps, be related to the high degree of resources required to sustain large trees. Clearly, as for the N*P interaction before, further investigation into stand age effects on belowground allocation, root exudation, and specific root respiration may be necessary to better understand the effect of stand age on soil respiration.

Finally, in analysis of summer data from 2018-2020, N effects varied by stand age ($p = 0.01$), with the suppression of soil respiration by added N greatest in early successional stands (32% in early successional stands versus 8% in mid-successional stands and 9% in mature stands). Again, perhaps this effect is related to the intense competition for nutrients by the many young trees that occupy young stands. If that competition is relieved by the addition of excess N, then belowground allocation by those trees could be expected to decrease.

Conclusion

We found that N effects on soil respiration depend upon P availability in the early phases of nutrient manipulation experiments. Traditional explanations for the suppressive effect of N on soil respiration hypothesize that greater resource availability allows for reduced belowground allocation on the part of plants. The novel finding presented here suggests that those explanations may be improved by consideration of the role of other macronutrients. Furthermore, modeled predictions of soil respiration that rely upon nutrient concentrations will be most accurate if they account for concentrations of both N and P. Our findings may also support the multiple-element limitation hypothesis by providing evidence to suggest that the responses of northern hardwood ecosystems to excess N and P are not

additive. Further research—particularly into areas of P effects on root exudation, litterfall, and specific root respiration—will be necessary to identify the mechanisms responsible for these effects.

CHAPTER 3: CONCLUSIONS AND REFLECTION

The first chapter of this thesis offers a broad overview of the scientific literature pertaining to soil respiration, including descriptions of the autotrophic and heterotrophic communities that contribute to the efflux of CO₂ from soils and the factors that control rates of that efflux from each of those sources. Particular attention was given to the extent to which nutrient availability, nutrient addition, and stand age affect soil respiration. Generally speaking, high N availability—whether due to inherent site conditions or due to artificial fertilization—is correlated with lower rates of soil respiration (Bae et al., 2015; Bowden et al., 2004). The general understanding within the scientific community is that reductions in the efflux of carbon from soil can be attributed to reductions in the belowground flux of carbon from plant communities (Haynes & Gower, 1995). In short, less carbon is released from soil in nutrient-rich conditions because less carbon is allocated belowground. This is the case—it is thought—because less “effort” is necessary on the part of plant communities to acquire limiting nutrients.

Naturally, this hypothesis is being constantly refined through further investigation into controls of decomposition and respiration among belowground communities (Burdige, 2007; Hatcher et al., 1983; Hemingway et al., 2019; Lehmann & Kleber, 2015; Torn & Trumbore, 1997; Vogel et al., 2014). However, very little evidence exists to explain how P availability may control rates of soil respiration in temperate forests. The goal of the study outlined in Chapter 2 was to address that knowledge gap. Does P availability affect soil respiration outright? Does the well studied N effect depend upon P availability? If so, do those dynamics change throughout the life cycle of a forest stand? And finally, how does the nature of these relationships evolve over the course of repeated annual nutrient additions?

Chapter 2 reports the findings of a long-term full-factorial NxP fertilization experiment in the White Mountains of New Hampshire. Hypotheses were (1) that soil respiration would be reduced by N addition and increased by P addition and (2) that soil respiration would be consistent across stand age classes. The first hypothesis was partially confirmed as soil respiration was consistently reduced by N addition but generally unaffected by P. We did discover, however, that in the summer and fall of 2012-

2014, the second, third, and fourth years of fertilization, the magnitude of the N effect depended upon P availability ($p = 0.02$ for both summer and fall). Additionally, in the summer of 2018-2020, the magnitude of the N effect depended upon stand age class ($p = 0.01$), with the strongest reductions in soil respiration (32%) measured in early successional stands and more modest reductions measured in mid-successional (8%) and mature stands (9%). The second hypothesis was rejected, as soil respiration frequently differed across stand age classes. Mid-successional stands consistently had the lowest rates of soil respiration, and whether early successional or mature stands had the highest rates of soil respiration depended upon the analysis period.

Limitations of my study

I was very fortunate to have such a thorough, long-term dataset to analyze the effects of these factors on soil respiration. I am sure that it is quite uncommon for a master's thesis to involve ten years of post-treatment data, three years of pre-treatment data, and over 16,000 observations. Analysis of a massive long-term dataset like this, however, does have its challenges. As mentioned previously, one goal of this study was to describe how the effects of nutrient addition on soil respiration may evolve over the course of repeated annual fertilization. Indeed, those dynamics did change over the course of our study, and finding a sensible and scientifically defensible way to describe those changes was challenging.

Ultimately, we chose to define analysis periods by season within three-year windows. In a sense, this decision was supported by the fact that effects on soil respiration did vary by season and across the three-year windows. However, how those analysis periods were defined was fairly arbitrary. This is particularly true for seasons, with spring defined as including all measurements prior to June 1, summer as including all measurements prior to August 31, and fall including all measurements September 1 and beyond. No measurements were taken earlier than April 18 or later than November 25. Clearly, these seasonal boundaries are not based upon breakpoints in the data or even phenological events, and future development of this manuscript will require improving how seasons are defined. This may be done either

by using statistical breaks in the data or by taking advantage of the excellent phenological record at Hubbard Brook to define seasons by natural events such as budbreak, leaf color change, or abscission.

Novel contributions of my study

As mentioned previously, the results presented in Chapter 2 make a unique contribution to the scientific understanding of soil respiration by demonstrating that N effects in temperate ecosystems are mediated by other factors. The availability of P, for example, affects the magnitude of N-induced reductions in soil respiration, particularly in the early stages of nutrient addition experiments. Stand age also affects the magnitude of N effects, which are strongest in young stands. The stand age effect on soil respiration—in which soil respiration is greatest at early and later stages of stand development—is also unique within the scientific literature but warrants further research as it was detected only sporadically across our analysis periods.

Opportunities for future study

The novel results of this study justify further investigation into potential mechanistic explanations for nutrient controls on soil respiration. I briefly propose some potential directions in Chapter 2, but, generally speaking, it will be critical to explore how exactly the effect of N addition depends upon P availability. The first step in that process would involve studying how N and P interact to affect component elements of belowground carbon cycling. Testing for N and P interactions on rhizosphere respiration could help to explain whether increased P availability raises the demand for N to the extent that root exudation for soil priming is sustained at relatively higher rates. The interaction effect detected in this study could also be attributed to higher levels of specific root respiration in P-treated plots. If P addition raises specific root respiration, that increase could help to offset N-induced reductions in root exudation for purposes of soil priming. Finally, interactive effects of N and P could be attributed to effects of fertilization on carbon fluxes into the soil system by way of litterfall. If P addition increased

litterfall, rates of decomposition may increase as well, potentially offsetting N-induced reductions in soil respiration.

Another opportunity for future study that was not explored in Chapter 2 is explaining the gradual but consistent increase in our soil respiration measurements over time (Figure 3). Given that soil

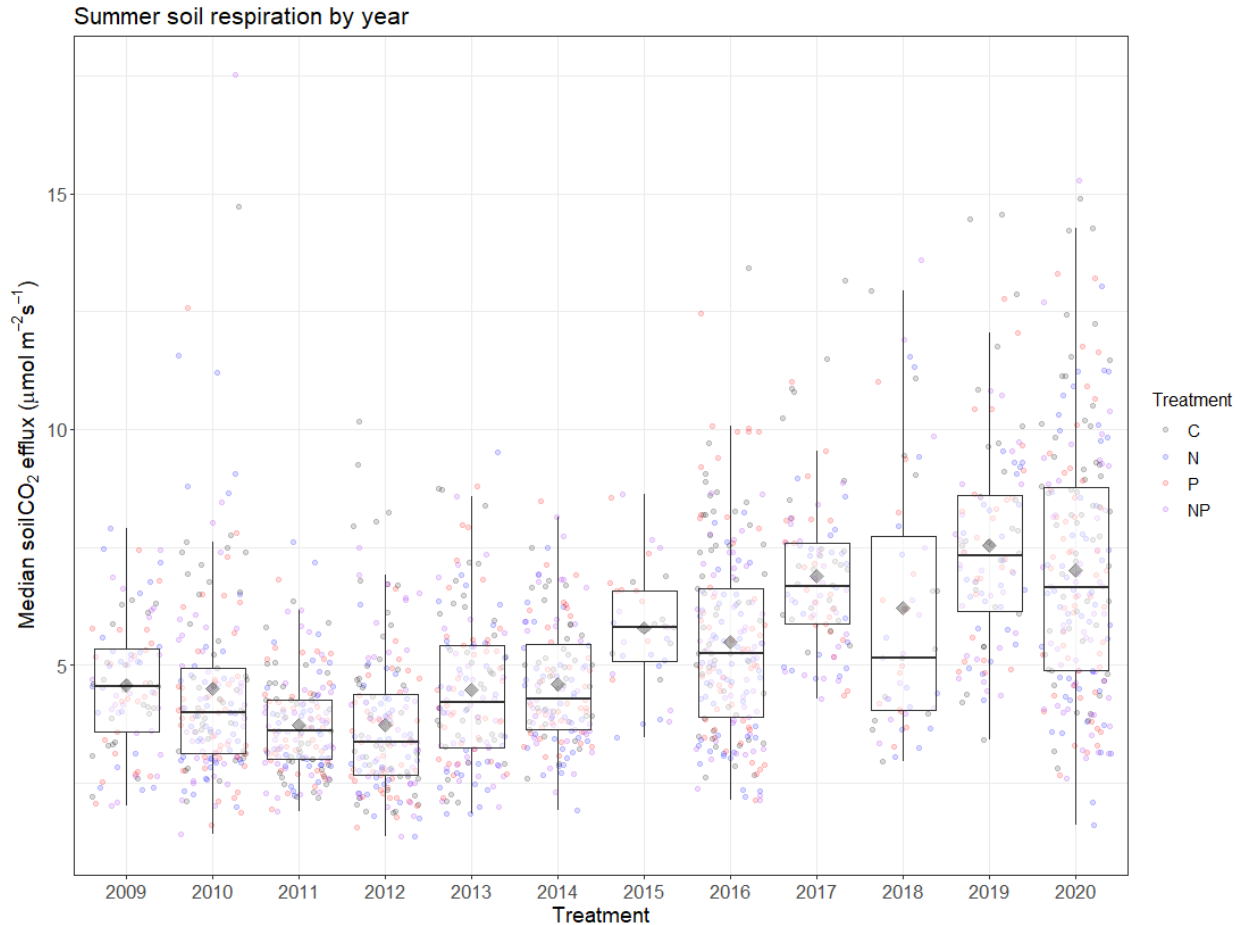


Figure 4. Boxplots of median soil respiration values from summer observations. Gray diamonds represent annual means. Two exceptionally high points [(1) year = 2018, median soil CO₂ efflux = 28.60 μmol m⁻² s⁻¹; (2) year = 2018, median soil CO₂ efflux = 32.00 μmol m⁻² s⁻¹] were removed from the plot to improve the scale.

temperatures have not increased in a manner commensurate to the increase in soil respiration, it is unlikely that a warming climate can explain this phenomenon. Tim Fahey, who will be a co-author on the manuscript of Chapter 2, suggested that the increase could be an artifact of a “collar effect.” In other words, physiological or even physical processes taking place around respiration collars are causing

increases in soil respiration in a manner that would not be reflected in undisturbed soil. Thus, another opportunity for future study could involve installing new collars beside those that have been measured repeatedly for years. If rates of soil respiration in newly installed collars are statistically lower than those in old collars, then we can be confident that the increase over time can be attributed to a collar effect. This experiment is scheduled to be conducted by an undergraduate intern in the summer of 2021.

Conclusion

I am grateful to have had the opportunity through this project to make what I hope will be at least a small contribution to our understanding of the carbon cycle. I know that there are exciting next steps relevant to the findings reported here that the MELNHE project is well equipped to take, and I look forward to seeing how those next steps will build upon this work. I am grateful, too, for having had the opportunity to develop as a scientist in the course of this project. The hard skills of data collection, management, and analysis will certainly be valuable in whatever work follows my time at ESF, and I know that I have grown in my ability to think rigorously and scientifically about ecological processes and their analysis. I am hopeful that with this experience, I will be able to continue to work toward protecting and understanding the natural world in whatever comes next.

LITERATURE CITED

- Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Bernston, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., & Fernandez, I. (1998). Nitrogen Saturation in Temperate Forest Ecosystems. *BioScience*, 48(11), 921. <https://doi.org/10.2307/1313296>
- Adams, M. B., Loughry, L., & Linda, comps P. (2008). Experimental Forests and Ranges of the USDA Forest Service. *Gen. Tech. Rep. NE-321 Revised. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 178 p. [CD ROM], 321.*
<https://doi.org/10.2737/NE-GTR-321>
- Addo-Danso, S. D., Prescott, C. E., & Smith, A. R. (2016). Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: A review. *Forest Ecology and Management*, 359, 332–351. <https://doi.org/10.1016/j.foreco.2015.08.015>
- Alexander, M. (1977). *Introduction to Soil Microbiology*. John Wiley & Sons.
- Allison, S. D., Hanson, C. A., & Treseder, K. K. (2007). Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology and Biochemistry*, 39(8), 1878–1887. <https://doi.org/10.1016/j.soilbio.2007.02.001>
- Amthor, J. (2000). The McCree–de Wit–Penning de Vries–Thornley Respiration Paradigms: 30 Years Later. *Annals of Botany*, 86(1), 1–20. <https://doi.org/10.1006/anbo.2000.1175>
- Arnebrant, K., Bååth, E., Söderström, B., & Nohrstedt, H.-Ö. (1996). Soil microbial activity in eleven Swedish coniferous forests in relation to site fertility and nitrogen fertilization. *Scandinavian Journal of Forest Research*, 11(1–4), 1–6. <https://doi.org/10.1080/02827589609382906>
- Bae, K., Fahey, T., Yanai, R., & Fisk, M. (2015). Soil Nitrogen Availability Affects Belowground Carbon Allocation and Soil Respiration in Northern Hardwood Forests of New Hampshire. *Ecosystems*, 18(7), 1179–1191. <https://doi.org/10.1007/s10021-015-9892-7>
- Bailey, S. W. (2020, July 8). *Building Blocks of Watersheds/Ecosystems*. Hubbard Brook Ecosystem Study 57th Annual Cooperators' Meeting.

- Bloom, A. J., Chapin, F. S., & Mooney, H. A. (1985). Resource Limitation in Plants—An Economic Analogy. *Annual Review of Ecology and Systematics*, *16*, 363–392.
- Bouma, T. J., Visser, R. D., Janssen, J. H. J. A., Kock, M. J. D., Leeuwen, P. H. V., & Lambers, H. (1994). Respiratory energy requirements and rate of protein turnover in vivo determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiologia Plantarum*, *92*(4), 585–594. <https://doi.org/10.1111/j.1399-3054.1994.tb03027.x>
- Bowden, R. D., Davidson, E., Savage, K., Arabia, C., & Steudler, P. (2004). Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *FEMS Microbiology Letters*. [https://doi.org/10.1016/S0378-1127\(04\)00191-4](https://doi.org/10.1016/S0378-1127(04)00191-4)
- Brzostek, E., Greco, A., Drake, J., & Finzi, A. (2013). Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. *Biogeochemistry*, *115*(1–3), 65–76. <https://doi.org/10.1007/s10533-012-9818-9>
- Burdige, D. J. (2007). Preservation of Organic Matter in Marine Sediments: Controls, Mechanisms, and an Imbalance in Sediment Organic Carbon Budgets? *Chemical Reviews*, *107*(2), 467–485. <https://doi.org/10.1021/cr050347q>
- Burton, A. J., Jarvey, J. C., Jarvi, M. P., Zak, D. R., & Pregitzer, K. S. (2012). Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biology*, *18*(1), 258–266. <https://doi.org/10.1111/j.1365-2486.2011.02527.x>
- Burton, A. J., Pregitzer, K. S., Crawford, J. N., Zogg, G. P., & Zak, D. R. (2004). Simulated chronic NO₃– deposition reduces soil respiration in northern hardwood forests. *Global Change Biology*, *10*(7), 1080–1091. <https://doi.org/10.1111/j.1365-2486.2004.00737.x>
- Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., & Parkhurst, D. F. (2000). Microbial Enzyme Shifts Explain Litter Decay Responses to Simulated Nitrogen Deposition. *Ecology*, *81*(9), 2359–2365. [https://doi.org/10.1890/0012-9658\(2000\)081\[2359:MESELD\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[2359:MESELD]2.0.CO;2)
- Chapin, F. S., Bloom, A. J., Field, C. B., & Waring, R. H. (1987). Plant Responses to Multiple Environmental Factors. *BioScience*, *37*(1), 49–57. <https://doi.org/10.2307/1310177>

- Compton, J. E., Watrud, L. S., Arlene Porteous, L., & DeGroot, S. (2004). Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *Forest Ecology and Management*, *196*(1), 143–158. <https://doi.org/10.1016/j.foreco.2004.03.017>
- Contosta, A. R., Frey, S. D., & Cooper, A. B. (2011). Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils. *Ecosphere*, *2*(3), art36. <https://doi.org/10.1890/ES10-00133.1>
- Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., & Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*, *42*(5), 679–698. <https://doi.org/10.1016/j.soilbio.2009.12.006>
- de Vries, F. W. T. P. (1975). The Cost of Maintenance Processes in Plant Cells. *Annals of Botany*, *39*(159), 77–92.
- Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., Johnsen, K. S., Lichter, J., McCarthy, H. R., McCormack, M. L., Moore, D. J. P., Oren, R., Palmroth, S., Phillips, R. P., Phippen, J. S., Pritchard, S. G., Treseder, K. K., Schlesinger, W. H., DeLucia, E. H., & Finzi, A. C. (2011). Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecology Letters*, *14*(4), 349–357. <https://doi.org/10.1111/j.1461-0248.2011.01593.x>
- Ewel, K. C., Jr, W. P. C., & Gholz, H. L. (2011). Soil CO₂ evolution in Florida slash pine plantations. I. Changes through time. *Canadian Journal of Forest Research*. <https://doi.org/10.1139/x87-054>
- Fahey, T. J., & Hughes, J. W. (1994). Fine Root Dynamics in a Northern Hardwood Forest Ecosystem, Hubbard Brook Experimental Forest, NH. *Journal of Ecology*, *82*(3), 533–548. <https://doi.org/10.2307/2261262>
- Fahey, T., Siccama, T., Driscoll, C., Likens, G., Campbell, J., Johnson, C., Battles, J., Aber, J., Cole, J., Fisk, M., Groffman, P., Hamburg, S., Holmes, R., Schwarz, P., & Yanai, R. (2005). The

- Biogeochemistry of Carbon at Hubbard Brook. *Biogeochemistry*, 75(1), 109–176.
<https://doi.org/10.1007/s10533-004-6321-y>
- Fierer, N., & Schimel, J. P. (2003). A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil. *Soil Science Society of America Journal*, 67(3), 798–805. <https://doi.org/10.2136/sssaj2003.7980>
- Fisk, M., Santangelo, S., & Minick, K. (2015). Carbon mineralization is promoted by phosphorus and reduced by nitrogen addition in the organic horizon of northern hardwood forests. *Soil Biology and Biochemistry*, 81, 212–218. <https://doi.org/10.1016/j.soilbio.2014.11.022>
- Frey, S. D., Knorr, M., Parrent, J. L., & Simpson, R. T. (2004). Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management*, 196(1), 159–171. <https://doi.org/10.1016/j.foreco.2004.03.018>
- Goswami, S., Fisk, M. C., Vadeboncoeur, M. A., Garrison-Johnston, M., Yanai, R. D., & Fahey, T. J. (2018). Phosphorus limitation of aboveground production in northern hardwood forests. *Ecology*, 99(2), 438–449. <https://doi.org/10.1002/ecy.2100>
- Gough, C. M., Seiler, J. R., Wiseman, P. E., & Maier, C. A. (2005). Soil CO₂ efflux in loblolly pine (*Pinus taeda* L.) plantations on the Virginia Piedmont and South Carolina Coastal Plain over a rotation-length chronosequence. *Biogeochemistry*, 73(1), 127–147.
<https://doi.org/10.1007/s10533-004-0566-3>
- Goulden, M. L., Munger, J. W., Fan, S.-M., Daube, B. C., & Wofsy, S. C. (1996). Measurements of carbon sequestration by long-term eddy covariance: Methods and a critical evaluation of accuracy. *Global Change Biology*, 2(3), 169–182. <https://doi.org/10.1111/j.1365-2486.1996.tb00070.x>
- Gower, S. T., Gholz, H. L., Nakane, K., & Baldwin, V. C. (1994). Production and Carbon Allocation Patterns of Pine Forests. *Ecological Bulletins*, 43, 115–135.

- Hanson, P. J., Edwards, N. T., Garten, C. T., & Andrews, J. A. (2000). Separating Root and Soil Microbial Contributions to Soil Respiration: A Review of Methods and Observations. *Biogeochemistry*, 48(1), 115–146.
- Hatcher, P. G., Spiker, E. C., Szeverenyi, N. M., & Maciel, G. E. (1983). Selective preservation and origin of petroleum-forming aquatic kerogen. *Nature*, 305, 498–501.
- Haynes, B. E., & Gower, S. T. (1995). Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiology*, 15(5), 317–325.
<https://doi.org/10.1093/treephys/15.5.317>
- Hemingway, J. D., Rothman, D. H., Grant, K. E., Rosengard, S. Z., Eglinton, T. I., Derry, L. A., & Galy, V. V. (2019). Mineral protection regulates long-term global preservation of natural organic carbon. *Nature*, 570(7760), 228–231. <https://doi.org/10.1038/s41586-019-1280-6>
- Hirano, T., Kim, H., & Tanaka, Y. (2003). Long-term half-hourly measurement of soil CO₂ concentration and soil respiration in a temperate deciduous forest. *Journal of Geophysical Research: Atmospheres*, 108(D20). <https://doi.org/10.1029/2003JD003766>
- Hobbie, E. A. (2006). Carbon Allocation to Ectomycorrhizal Fungi Correlates with Belowground Allocation in Culture Studies. *Ecology*, 87(3), 563–569. <https://doi.org/10.1890/05-0755>
- Högberg, M. N., Briones, M. J. I., Keel, S. G., Metcalfe, D. B., Campbell, C., Midwood, A. J., Thornton, B., Hurry, V., Linder, S., Näsholm, T., & Högberg, P. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, 187(2), 485–493.
<https://doi.org/10.1111/j.1469-8137.2010.03274.x>
- Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F., Ekblad, A., Högberg, M. N., Nyberg, G., Ottosson-Löfvenius, M., & Read, D. J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, 411(6839), 789–792.
<https://doi.org/10.1038/35081058>

- Högberg, P., & Read, D. J. (2006). Towards a more plant physiological perspective on soil ecology. *Trends in Ecology & Evolution*, 21(10), 548–554. <https://doi.org/10.1016/j.tree.2006.06.004>
- Irvine, J., & Law, B. E. (2002). Contrasting soil respiration in young and old-growth ponderosa pine forests. *Global Change Biology*, 8(12), 1183–1194. <https://doi.org/10.1046/j.1365-2486.2002.00544.x>
- Janssens, I. A., Dieleman, W., Luysaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E.-D., Tang, J., & Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, 3(5), 315–322. <https://doi.org/10.1038/ngeo844>
- Janssens, I. A., Kowalski, A. S., Longdoz, B., & Ceulemans, R. (2000). Assessing forest soil CO₂ efflux: An in situ comparison of four techniques. *Tree Physiology*, 20(1), 23–32. <https://doi.org/10.1093/treephys/20.1.23>
- Janssens, I. A., Lankreijer, H., Matteucci, G., Kowalski, A. S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E. J., Grelle, A., Rannik, Ü., Morgenstern, K., Oltchev, S., Clement, R., ... Valentini, R. (2001). Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, 7(3), 269–278. <https://doi.org/10.1046/j.1365-2486.2001.00412.x>
- Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, 7(1), 13630. <https://doi.org/10.1038/ncomms13630>
- Kang, H., Fahey, T. J., Bae, K., Fisk, M., Sherman, R. E., Yanai, R. D., & See, C. R. (2016). Response of forest soil respiration to nutrient addition depends on site fertility. *Biogeochemistry*, 127(1), 113–124. <https://doi.org/10.1007/s10533-015-0172-6>
- Kicklighter, D. W., Melillo, J. M., Peterjohn, W. T., Rastetter, E. B., McGuire, A. D., Stuedler, P. A., & Aber, J. D. (1994). Aspects of spatial and temporal aggregation in estimating regional carbon

- dioxide fluxes from temperate forest soils. *Journal of Geophysical Research: Atmospheres*, 99(D1), 1303–1315. <https://doi.org/10.1029/93JD02964>
- Knorr, M., Frey, S. D., & Curtis, P. S. (2005). Nitrogen Additions and Litter Decomposition: A Meta-Analysis. *Ecology*, 86(12), 3252–3257. <https://doi.org/10.1890/05-0150>
- Kuzyakov, Y. V., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, 32(11), 1485–1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)
- Kuzyakov, Y. V., & Larionova, A. A. (2006). Contribution of rhizomicrobial and root respiration to the CO₂ emission from soil (A review). *Eurasian Soil Science*, 39(7), 753–764. <https://doi.org/10.1134/S106422930607009X>
- Law, B. E., Ryan, M. G., & Anthoni, P. M. (1999). Seasonal and annual respiration of a ponderosa pine ecosystem. *Global Change Biology*, 5(2), 169–182. <https://doi.org/10.1046/j.1365-2486.1999.00214.x>
- Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., Pickers, P. A., Korsbakken, J. I., Peters, G. P., Canadell, J. G., Arneeth, A., Arora, V. K., Barbero, L., Bastos, A., Bopp, L., Chevallier, F., Chini, L. P., Ciais, P., Doney, S. C., ... Zheng, B. (2018). Global Carbon Budget 2018. *Earth System Science Data*, 10(4), 2141–2194. <https://doi.org/10.5194/essd-10-2141-2018>
- Lee, K.-H., & Jose, S. (2003). Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. *Forest Ecology and Management*, 185(3), 263–273. [https://doi.org/10.1016/S0378-1127\(03\)00164-6](https://doi.org/10.1016/S0378-1127(03)00164-6)
- Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, 528(7580), 60–68. <https://doi.org/10.1038/nature16069>
- Litton, C. M., Raich, J. W., & Ryan, M. G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology*, 13(10), 2089–2109. <https://doi.org/10.1111/j.1365-2486.2007.01420.x>

- Lu, S., Mattson, K. G., Zaerr, J. B., & Marshall, J. D. (1998). Root respiration of Douglas-fir seedlings: Effects of N concentration. *Soil Biology and Biochemistry*, 30(3), 331–336.
[https://doi.org/10.1016/S0038-0717\(97\)00134-X](https://doi.org/10.1016/S0038-0717(97)00134-X)
- Lützw, M. von, Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., & Kalbitz, K. (2008). Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Journal of Plant Nutrition and Soil Science*, 171(1), 111–124. <https://doi.org/10.1002/jpln.200700047>
- Magill, A. H., Aber, J. D., Hendricks, J. J., Bowden, R. D., Melillo, J. M., & Steudler, P. A. (1997). Biogeochemical Response of Forest Ecosystems to Simulated Chronic Nitrogen Deposition. *Ecological Applications*, 7(2), 402–415. [https://doi.org/10.1890/1051-0761\(1997\)007\[0402:BROFET\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1997)007[0402:BROFET]2.0.CO;2)
- Makita, N., Kosugi, Y., Dannoura, M., Takanashi, S., Niiyama, K., Kassim, A. R., & Nik, A. R. (2012). Patterns of root respiration rates and morphological traits in 13 tree species in a tropical forest. *Tree Physiology*, 32(3), 303–312. <https://doi.org/10.1093/treephys/tps008>
- Makita, N., Pumpanen, J., Köster, K., & Berninger, F. (2016). Changes in very fine root respiration and morphology with time since last fire in a boreal forest. *Plant & Soil*, 402(1/2), 303–316.
<https://doi.org/10.1007/s11104-016-2801-9>
- Martikainen, P. J., AARNIO, T., TAAVITSAINEN, V.-M., PÄIVINEN, L., & SALONEN, K. (1989). Mineralization of carbon and nitrogen in soil samples taken from three fertilized pine stands: Long-term effects. *Plant and Soil*, 114(1), 99–106.
- Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., & Wang, H. (2008). Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. *Global Change Biology*, 14(2), 403–412. <https://doi.org/10.1111/j.1365-2486.2007.01503.x>
- Naples, B., & Fisk, M. (2010). Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry*, 97, 109–121. <https://doi.org/10.1007/s10533-009-9354-4>

- Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., & Bowman, W. D. (2002). Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature*, *419*(6910), 915–917. <https://doi.org/10.1038/nature01136>
- Nohrstedt, H.-Ö., Arnebrant, K., Bååth, E., & Söderström, B. (1989). Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. *Canadian Journal of Forest Research*, *9*, 323–328. <https://doi.org/10.1139/x89-048>
- Pregitzer, K. S., Laskowski, M. J., Burton, A. J., Lessard, V. C., & Zak, D. R. (1998). Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology*, *18*(10), 665–670. <https://doi.org/10.1093/treephys/18.10.665>
- Raich, J. W., & Nadelhoffer, K. J. (1989). Belowground Carbon Allocation in Forest Ecosystems: Global Trends. *Ecology*, *70*(5), 1346–1354. <https://doi.org/10.2307/1938194>
- Raich, J. W., & Potter, C. S. (1995). Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles*, *9*(1), 23–36. <https://doi.org/10.1029/94GB02723>
- Rastetter, E. B., & Kwiatkowski, B. L. (2020). An approach to modeling resource optimization for substitutable and interdependent resources. *Ecological Modelling*, *425*, 109033. <https://doi.org/10.1016/j.ecolmodel.2020.109033>
- Rastetter, E. B., & Shaver, G. R. (1992). A Model of Multiple-Element Limitation for Acclimating Vegetation. *Ecology*, *73*(4), 1157–1174. <https://doi.org/10.2307/1940666>
- Reich, P. B., Tjoelker, M. G., Pregitzer, K. S., Wright, I. J., Oleksyn, J., & Machado, J.-L. (2008). Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecology Letters*, *11*(8), 793–801. <https://doi.org/10.1111/j.1461-0248.2008.01185.x>
- Rühling, Å., & Tyler, G. (1991). Effects of Simulated Nitrogen Deposition to the Forest Floor on the Macrofungal Flora of a Beech Forest. *Ambio*, *20*(6), 261–263.
- Ryan, M. G., & Law, B. E. (2005). Interpreting, Measuring, and Modeling Soil Respiration. *Biogeochemistry*, *73*(1), 3–27.

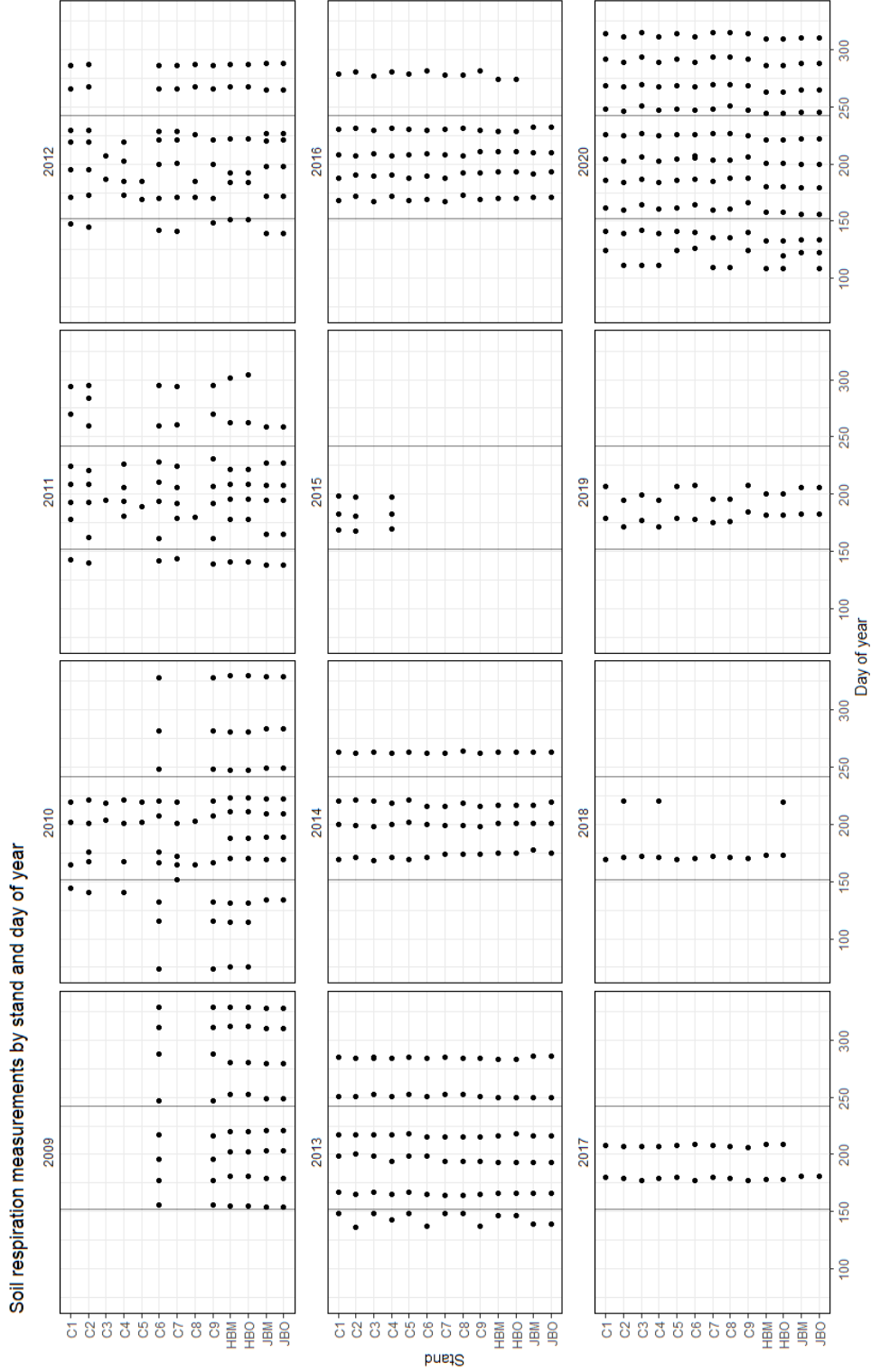
- Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49–56. <https://doi.org/10.1038/nature10386>
- Shan, S. (2019). *The controls of nutrient limitation on resource allocation belowground* [Unpublished doctoral dissertation]. Miami University.
- Smyth, C. E., Kurz, W. A., Neilson, E. T., & Stinson, G. (2013). National-scale estimates of forest root biomass carbon stocks and associated carbon fluxes in Canada. *Global Biogeochemical Cycles*, *27*(4), 1262–1273. <https://doi.org/10.1002/2012GB004536>
- Tedeschi, V., Rey, A., Manca, G., Valentini, R., Jarvis, P. G., & Borghetti, M. (2006). Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. *Global Change Biology*, *12*(1), 110–121. <https://doi.org/10.1111/j.1365-2486.2005.01081.x>
- Thirukkumaran, C. M., & Parkinson, D. (2000). Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. *Soil Biology and Biochemistry*, *32*(1), 59–66. [https://doi.org/10.1016/S0038-0717\(99\)00129-7](https://doi.org/10.1016/S0038-0717(99)00129-7)
- Torn, M. S., & Trumbore, S. E. (1997). Mineral control of soil organic carbon storage and turnover. *Nature*, *389*(6647), 170. <https://doi.org/10.1038/38260>
- Vadeboncoeur, M. A., Hamburg, S. P., Blum, J. D., Pennino, M. J., Yanai, R. D., & Johnson, C. E. (2012). The Quantitative Soil Pit Method for Measuring Belowground Carbon and Nitrogen Stocks. *Soil Science Society of America Journal*, *76*(6), 2241–2255. <https://doi.org/10.2136/sssaj2012.0111>
- Verlinden, M. S., Ven, A., Verbruggen, E., Janssens, I. A., Wallander, H., & Vicca, S. (2018). Favorable effect of mycorrhizae on biomass production efficiency exceeds their carbon cost in a fertilization experiment. *Ecology*, *99*(11), 2525–2534. <https://doi.org/10.1002/ecy.2502>

- Vogel, C., Mueller, C. W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., & Kögel-Knabner, I. (2014). Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications*, *5*(1), 2947. <https://doi.org/10.1038/ncomms3947>
- Vose, J. M., & Ryan, M. G. (2002). Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis. *Global Change Biology*, *8*(2), 182–193. <https://doi.org/10.1046/j.1365-2486.2002.00464.x>
- Waldrop, M. P., Zak, D. R., & Sinsabaugh, R. L. (2004). Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biology and Biochemistry*, *36*(9), 1443–1451. <https://doi.org/10.1016/j.soilbio.2004.04.023>

APPENDICES

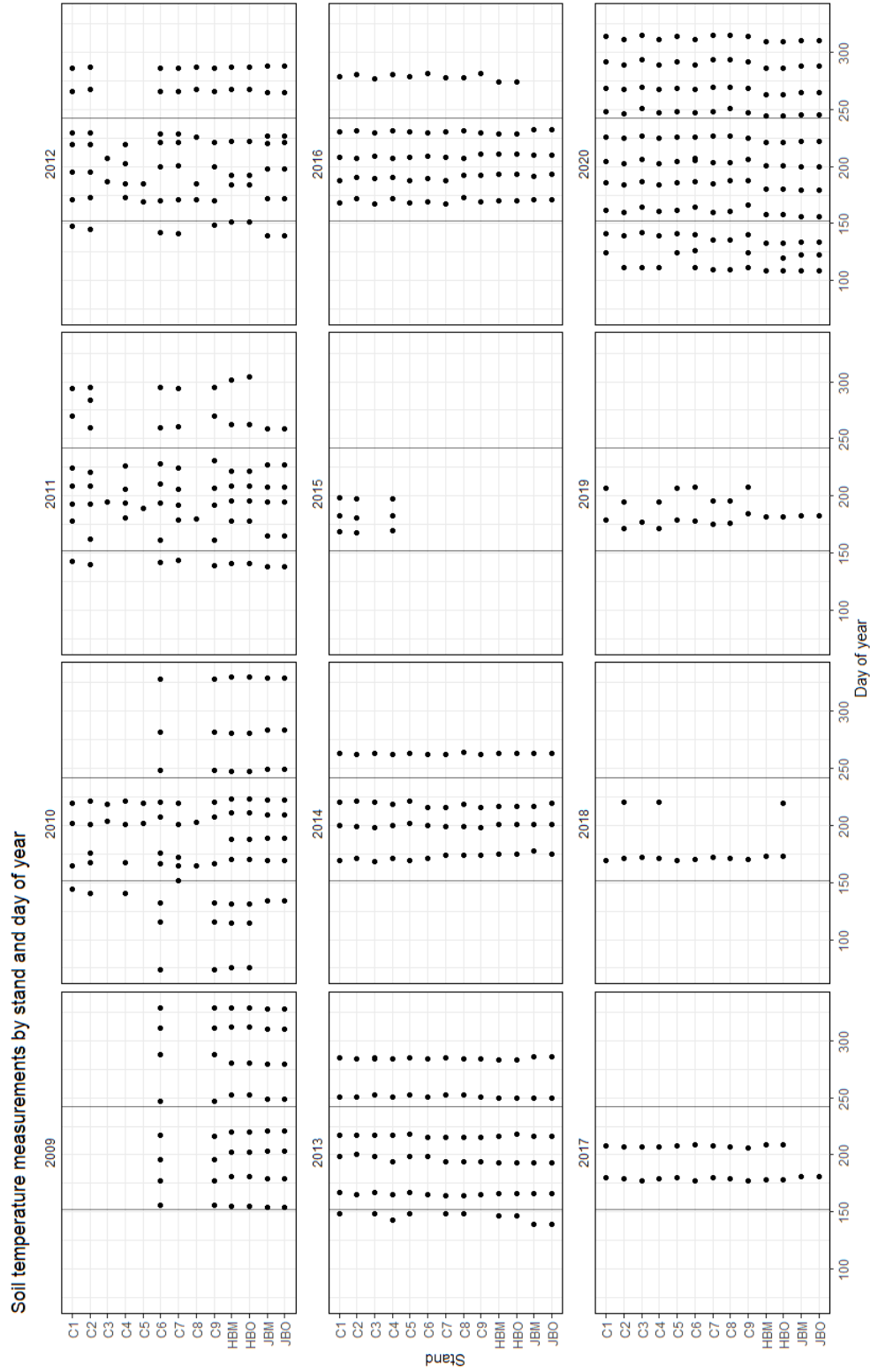
Appendix A

Availability of soil respiration measurements by stand and day of year



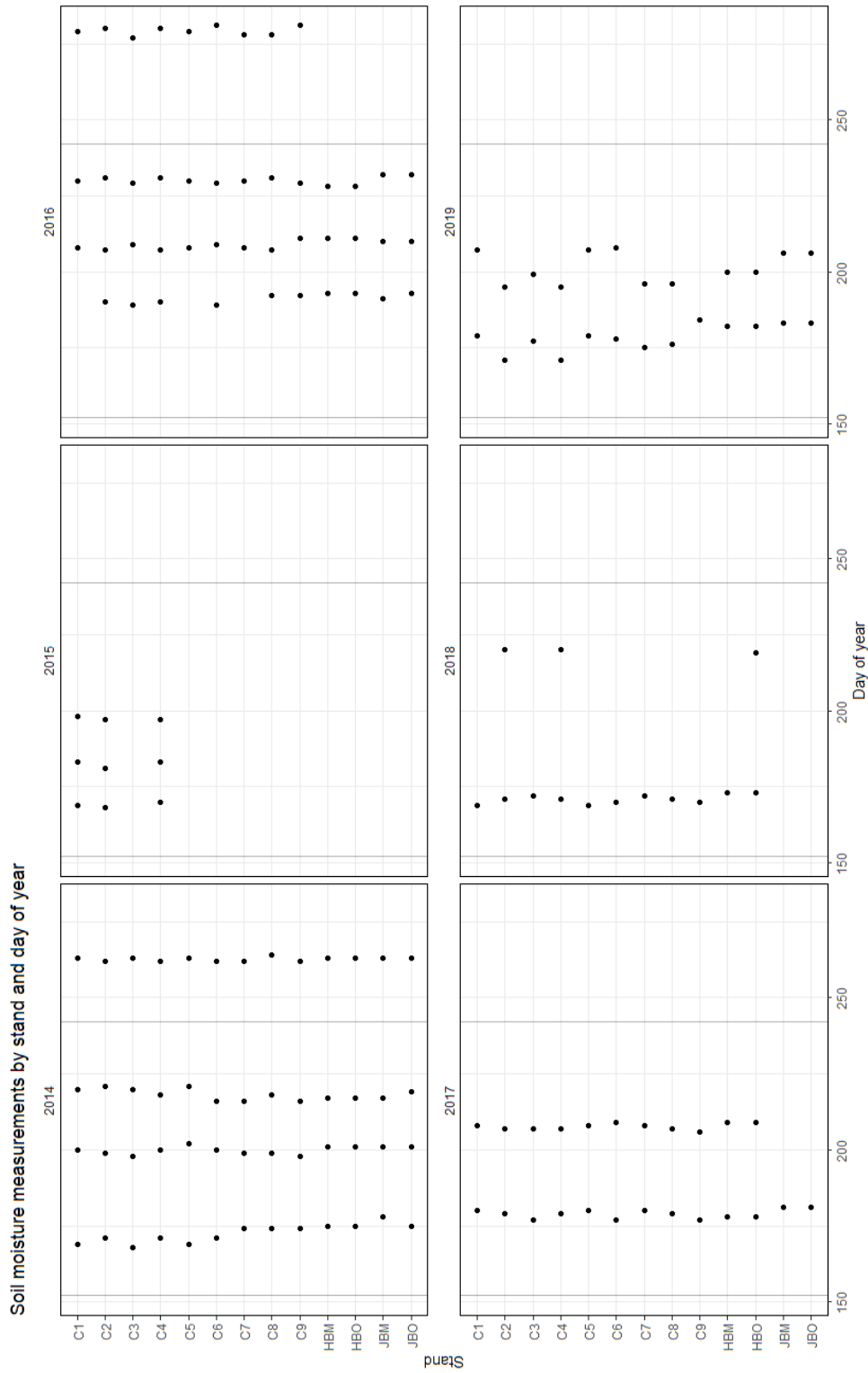
Appendix B

Availability of soil temperature measurements by stand and day of year



Appendix C

Availability of soil moisture measurements by stand and day of year



RESUME

Thomas Mann

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· 502-410-9245

· ttomjr2@gmail.com

RELEVANT SKILLS

- Managing, analyzing, and visualizing large longitudinal datasets in R using base R and the dplyr, lmer, and ggplot2 packages
- Using GitHub and markdown for website development, project management, version control
- Collecting and managing ecological data from soil sampling, tree inventory, litterfall production, and soil respiration
- Using ArcMap and ArcGIS Pro for the creation, management, editing, and analysis of spatial data
- Using the USFS Forest Vegetation Simulator to model stand growth under a variety of silvicultural treatments
- Fluency in spoken and written Spanish

RESEARCH AND WORK EXPERIENCE

State University of New York College of Environmental Science and Forestry, Graduate Student

Soil respiration and litterfall production in the context of a long-term NxP fertilization experiment

- Analyzed the effect of nitrogen and phosphorus additions on soil respiration over 13 years and across 15 forest stands
- Used subsampling design for data collection using ground-based litter baskets and the LI-COR 8100 device
- Used visual analysis to detect errors in historical data
- Mentored and managed undergraduate interns assisting with data collection
- Reorganized and transformed data for analysis in a mixed-effect model using the nlme and lme4 packages in R
- Developed project in collaboration with a committee of advisors

Nitrogen and phosphorus additions affect the population densities of sugar maple and American beech germinants

- Analyzed the effect of nitrogen and phosphorus additions on germinant counts of sugar maple and American beech
- Mapped sampling plots and tree location data for a distance-weighted analysis of fertilization effects on regeneration
- Used Focal Statistics tool in ArcMap to generate a “germinant prediction index” raster that quantified the likelihood of germination in a given location based upon proximity to trees of interest and the size of those trees
- Used Geostatistical Wizard tool to generate a raster interpolating count data among sampling points
- Mentored and managed undergraduate interns assisting with data collection
- Analyzed non-normal count data with a mixed-effect model using the glmmTMB package in R

The current state of uncertainty reporting in ecosystem studies: A systematic evaluation of peer-reviewed literature

- Second author on accepted manuscript reviewing rates at which measures of uncertainty are reported in scientific literature
- Randomly sampled 132 articles from 100 of the top journals in ecosystem sciences to evaluate whether they reported measures of uncertainty relevant to studies of soils, surface water, precipitation, and vegetation
- Managed dataset in R and google sheets for collaboration among authors
- Compared rates of uncertainty reporting across topic areas using ANOVA in R

Quantifying Uncertainty Estimates and Risk for Carbon Accounting (QUERCA)

- Assisted in research to improve measures of uncertainty in carbon accounting practices for nations participating in the REDD+ program
- Reviewed reports of countries participating in REDD+ program to identify issues in error propagation in the calculation of carbon budgets
- Began to develop recommendations to help participating countries appropriately apply error propagation techniques

EDUCATION

State University of New York College of Environmental Science and Forestry, Syracuse, NY

- *Master of Science*

Coursework: Advanced GIS, advanced silviculture, REDD+ carbon accounting, biogeochemistry,
plant physiology, sampling methods, ANOVA, regression analysis

Expected Spring 2021

GPA: 4.00/4.00

University of Kentucky, Lexington, KY

- *Post-baccalaureate studies*

Coursework: Forest ecology, fundamentals of soil science, plant pathology, horticultural entomology

Fall 2018

GPA: 4.00/4.00

University of Notre Dame, Notre Dame, IN

- *Master of Education*

July 2017

- *Master of Arts in Iberian and Latin American Studies*

May 2015

- *Bachelor of Arts in Spanish, sociology, and pre-health*

May 2014