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Kenneth R. Smith

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Controls on ecosystem nitrogen (N) status and carbon (C) balance in red spruce forests located in a region of historically high atmospheric N deposition

Kenneth R. Smith

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Richard B. Thomas, Ph. D., Chair
Amy Hessl, Ph. D.
James B. McGraw, Ph. D.
Brenden McNeil, Ph. D.
William T. Peterjohn, Ph. D.

Department of Biology
Morgantown, West Virginia
2016

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Abstract

Controls on ecosystem nitrogen (N) status and carbon (C) balance in red spruce forests located in a region of historically high atmospheric N deposition

Kenneth R. Smith

Atmospheric deposition of reactive nitrogen (N) can have a strong influence on patterns of carbon (C) and N cycling in forest ecosystems. High-elevation red spruce (*Picea rubens* Sarg.) forests in the central Appalachian Mountains have historically been among the most sensitive ecosystems to the negative effects of N deposition. I examined the primary environmental factors that influence 1) N status, 2) belowground C balance, and 3) red spruce aboveground growth in seven high-elevation study sites that are located in an area where N deposition has been historically moderate to high. I established the seven sites in 2011 along a transect of N deposition within the central Appalachian Mountains with the goal of determining whether historically elevated rates of N deposition were continuing to have long-term effects on ecosystem function.

With respect to N-induced changes to forest N status, I found no evidence that patterns of N availability were associated with N deposition; instead, I found that site-level differences in the abundance of broadleaf deciduous tree species were a consistently better predictor of N status at these sites. Likewise, annual rates of soil respiration and total belowground C allocation (TBCA) were positively correlated with a higher abundance of broadleaf species, but were unrelated to N deposition. These results suggest that the abundance of broadleaf tree species had a strong influence on N status and soil C balance via differences in the quality and decomposability of aboveground litterfall inputs. I found that the variation in red spruce aboveground growth along this transect was primarily related to differences in mean annual temperature between sites. I observed a weak negative trend relating red spruce growth to imbalances in foliar gas exchange (i.e. ratio of foliar dark respiration to net photosynthesis), suggesting that tree growth was reduced in locations where photosynthetic C uptake was more significantly offset by C losses via respiration. Overall, these red spruce growth patterns indicate that cooler temperatures were a stronger limiting factor to aboveground production than N availability or N deposition.

This research suggests that environmental factors apart from N deposition control C and N cycling dynamics in these forests, despite the fact that red spruce ecosystems throughout the Northeast have been negatively affected by acidic deposition in the past. However, while rates of N deposition have significantly declined since their peak in the mid 1980s, it is possible that current rates of N deposition may still have some impacts on ecosystem function, but these effects were undetectable given the overwhelming influence of tree species. Overall, these results are encouraging and could indicate that red spruce forests in this region are in a period of recovery from pollution.

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Chapter 1. General Introduction.

1.1. Research Overview.

Since the early 1900s increased industrial processes and fossil fuel emissions have led to a significant escalation in global atmospheric CO_2 concentrations compared with preindustrial times. Forest ecosystems are the primary terrestrial sink for atmospheric CO_2, sequestering about 2.4 Pg C y^{-1} or roughly 30% of fossil fuel C emissions (Pan et al. 2011; Le Quéré et al. 2013). However, while increases in atmospheric CO_2 may stimulate the productivity of forests over short timescales, there is now a growing uncertainty as to whether increased CO_2 uptake and sequestration by forests can be maintained (Hayes et al. 2011; Ma et al. 2012). Current models of climate and C cycling indicate that CO_2 uptake may decrease as plant productivity declines and ecosystem respiration increases (Friedlingstein et al. 2006). The ability of forests to sequester atmospheric C depends on numerous factors, including species composition, forest age, climate, and nutrient availability. In forests that are nitrogen (N) limited, CO_2 assimilation may be constrained by soil N availability or stimulated if N concentrations are replenished through fertilization or atmospheric deposition (Jain et al. 2009).

In addition to releasing greenhouse gases like CO_2, industrial and automotive fossil fuel burning also emits nitrogen oxides (NO_x) and sulfur oxides (SO_x) into the atmosphere. These primary pollutants undergo reactions in the atmosphere to form secondary compounds, such as nitric acid (HNO_3) and sulfuric acid (H_2SO_4). Prevailing winds transport these secondary compounds away from their emission source and deposit them on terrestrial landscapes as acidic deposition. For many forest ecosystems, high rates of acidic deposition can lead to important changes in soil chemistry and forest productivity via increases in soil acidification, leaching of essential base cations, heavy metal mobilization, and sulfur (S) and N accumulation (Driscoll et al. 2001).

Atmospheric N deposition influences ecosystem function primarily through its effects on N availability and C balance. In many temperate forests, the availability of N places a significant nutrient constraint on net primary productivity (NPP) (Vitousek and Howarth 1991; LeBauer and Treseder 2008); therefore, increased inputs of NO_3^- and NH_4^+ through atmospheric deposition have the potential to stimulate C assimilation and tree growth (McNulty and Aber 1993; Pregitzer et al. 2008). However, chronic N deposition has also been shown to have negative effects on forest productivity, including decreased frost hardiness in coniferous stands (Friedland et al. 1984; McNulty et al. 1996) and low root and mycorrhizal biomass (Dighton and Jansen 1991; Brandrud 1995). Whether forests exhibit positive or negative responses to atmospheric N deposition largely depends on the factors that determine the strength of ecosystem N sinks. For example, the most pronounced negative effects of deposition occur when N additions far exceed the demands of a N-limited ecosystem, resulting in a phenomenon known as N saturation (Aber et al. 1989, 1998).

The classic conceptual model of N saturation proceeds across four general stages (Aber et al. 1989, 1998). Under normal conditions most temperate forests are characterized as being N-limited (stage 0), with relatively low foliar N concentrations and low NPP. At this stage, soil NH_4^+ is the primary source of inorganic N that is available for plant and mycorrhizal uptake, while rates of nitrification and NO_3^- leaching are low. As N additions increase through atmospheric deposition (stage 1), soil N is in greater supply and invariably stimulates plant growth. During this stage, soil N mineralization increases along with increases in foliar N content, biomass, and ultimately net primary productivity. With chronic addition of N, net nitrification becomes induced (stage 2), thus further contributing to high soil NO_3^- content. Once N inputs exceed plant demand (stage 3), critical changes in soil nutrient cycling begin to occur. At this point, rates of nitrification typically increase above ambient levels resulting in detectable NO_3^- losses through leaching, which can be accompanied by the loss of base cations (Aber et al. 1989; Fenn et al. 1998). Additionally, increased rates of denitrification may occur wherein N_2O and N_2 are released back into the atmosphere (Davidson et al. 2000).

Recent empirical evidence has challenged the model of progressive N saturation proposed by Aber et al. (1989, 1998). For example, elevated rates of N deposition may stimulate net N mineralization, nitrification, and nitrate leaching for some sites but not others (Lovett and Rueth 1999; Nave et al. 2009). Moreover, tree growth responses to atmospheric N deposition appear to be species-specific (Thomas et al. 2010), and while foliar N content tends to be higher for sites that receive greater N inputs (Pardo et al. 2006; McNeil et al. 2007), this is not always the case (Aber et al. 2003). Much of this uncertainty likely reflects differences in N retention due to variability in climate, stand age, and species composition (Aber et al. 2003; Phillips et al. 2013).

To resolve some of these uncertainties, Lovett and Goodale (2011) developed a new conceptual model of N saturation, suggesting that the degree to which an ecosystem exhibits symptoms of N saturation depends on the strength of plant and soil N sinks, as well as the factors that influence how N is transferred between sinks. A key distinction between this model and the model proposed by Aber et al. (1989, 1998) is that N saturation can proceed through numerous pathways simultaneously, rather than through a stepwise series of events. While this model may explain why some ecosystems are more resilient to the negative impacts of N deposition, it does not provide an explicit framework to predict ecosystem responses to deposition due to our limited understanding of how state factors (e.g. climate, topography, geology, biota, and time) (Jenny 1994) interactively control the transfer of N to various sinks.

High-elevation red spruce (*Picea rubens* Sarg.) forests in the eastern United States are among the more sensitive ecosystems to environmental stressors like atmospheric N deposition; thus, they are an ideal model system to compare the strength of various state factor controls on forest C and N cycling dynamics. In the Central Appalachian Mountains of West Virginia and Virginia, red spruce communities once spanned an estimated 600,000 ha prior to European settlement (Hopkins 1899). These forests inhabited only the highest peaks and ridgelines in the region, exhibiting a strong preference for low temperature, subalpine conditions (Thomas-Van Gundy et al. 2012). During this pre-settlement time, natural disturbances like periodic extreme wind and snow storms were the primary agents responsible for canopy gap-phase dynamics (White et al. 1993; Rentch et al. 2010), while naturally-occurring wildfires were rare due to the cool, humid microclimates that red spruce forests occupy (White and Cogbill 1992). However, at the onset of the Industrial Revolution, the expansion of improved railroad technologies into these high-elevation areas provided logging access to this high value timber species; as a result, many of these ecosystems were decimated by extensive clear-cutting practices and uncontrolled slash fires (Lewis 1998; Nowacki and Wendt 2010). Because of these disturbances by human activities, red spruce forests in this region currently occupy a much more limited range of about 10,000 ha (Lewis 1998).

High rates of acidic deposition have also had a severe, negative effect on these high-elevation red spruce forests. Between the mid 1960s and mid 1980s, native red spruce stands in the eastern

United States experienced periodic and unexplained growth declines throughout the species' range (Siccama et al. 1982; Johnson and Siccama 1983; Foster and Reiners 1983; Scott et al. 1984; Johnson and McLaughlin 1986). Initially, researchers were uncertain of the underlying causes of this decline and speculated that multiple factors could be responsible, including: age-related senescence, climate change, biotic pathogens, and acid deposition (Siccama et al. 1982; Johnson and McLaughlin 1986; Cook and Johnson 1989). However, a number of observational and experimental studies revealed that this widespread disturbance was most likely the result of increased acidic deposition due to the abundance of industrial activities in the eastern United States (Shortle and Smith 1988; DeHayes et al. 1999; Adams et al. 2012).

Indeed, experiments on red spruce trees have shown a variety of negative physiological responses to enhanced acidic deposition. Under high rates of atmospheric deposition, the accumulation of H^+ ions displaces base cations from the soil exchange complex resulting in more acidic soils (Fernandez et al. 2003). As base cations such as Ca^{2+} and Mg^{2+} are displaced, they become highly mobile in soils and can be easily leached away through ground water (Likens et al. 1996; Fernandez et al. 2003). Acidic deposition can also induce Ca^{2+} losses directly through foliar leaching. Experimental studies have demonstrated that leaves exposed to acidic mist have accelerated rates of Ca^{2+} leaching, which may exacerbate foliar calcium deficiencies due to soil leaching (Joslin et al. 1988; DeHayes et al. 1991; McLaughlin et al. 1996; DeHayes et al. 1999; Schaberg et al. 2000). Because Ca^{2+} is critically important in plant stress responses, foliar Ca^{2+} depletions may potentially disrupt the ability of red spruce to cope with excessively cold temperatures, freezing injury, and Al^{3+} toxicity and may lead to decreased C storage over time (Minocha et al. 1996, 1997; DeHayes et al. 1999; Hawley et al. 2006; Halman et al. 2008). In particular, freezing injury associated with acid deposition can result in severe foliar dieback that can ultimately lead to reduced crown volume and basal area in red spruce populations (Wilkinson 1990; Tobi et al. 1995).

Given the inherent difficulties of studying long-term effects of atmospheric deposition on forest productivity, a large majority of studies have relied on high-dose fertilizer experiments to simulate chronic deposition. While these studies have provided ample evidence that excessive N additions can often lead to severe growth declines in both temperate deciduous (Duchesne et al.

2002) and coniferous forests (Aber et al. 1993; McNulty and Aber 1993; Magill et al. 2004), there are limitations to this approach that may impede our understanding of atmospheric deposition and forest health. First, high-dose fertilization experiments conducted over short intervals do not mimic anthropogenic N deposition, which is a chronic, low-level process occurring over a large temporal and spatial scale. Furthermore, plant responses to experimental N additions can vary over the course of an experiment depending on the rate and total amount of fertilizer applied.

In contrast, I conducted this research project using a gradient-based approach in order to examine whether patterns of N availability, belowground C balance, and tree growth in red spruce forests were associated with historically high rates of N deposition for the Central Appalachian region. Unlike studies that rely on experimental N additions, this type of observational approach across a large spatial scale offers distinct advantages. First, since these forests have only experienced N inputs from atmospheric deposition, these sites may be more representative of natural stands compared to those that receive high-dose experimental inputs. In addition, high-elevation red spruce forests have been historically sensitive to atmospheric N deposition; thus, this research affords a unique opportunity to study the effects of deposition in forests that are particularly vulnerable and may be more likely to respond.

Though high N deposition was a severe environmental perturbation in the 1980s, the passage of the 1990 Clean Air Act has initiated significant declines in NO_x emissions beginning around 2000. Consequently, rates of atmospheric N deposition have also decreased over the last two decades. While numerous studies have examined the negative effects of increased N deposition on terrestrial ecosystems, very few have examined the potential legacy effects of historically high pollution. As such there remains many uncertainties as to how quickly ecosystem processes return to ambient conditions following years of N additions. Thus, the aim of this research project was to evaluate whether historically high rates of N deposition were continuing to have effects on C and N cycling patterns in high-elevation forests.

1.2. Study Area and Plot Descriptions.

This research project was conducted in seven high-elevation red spruce forests located in the Appalachian Mountains of West Virginia and Virginia, a region where atmospheric N deposition rates have historically been among the highest in the United States. I established these study sites in 2011 along a 200 km transect that encompasses a range of estimated N deposition values for the region. In the mid 1980s, estimates of wet-only N deposition ($NO_3^- + NH_4^+$) along this transect were between 8.1 and 2.0 kg N ha^{-1} y^{-1} (North to South) (National Atmospheric Deposition Program 2014). However, following the Clean Air Act of 1990, significant reductions in NO_x emissions have led to a 39-51% decrease in rainwater NO_3^- concentrations for this area (for comparison, rainwater NH_4^+ concentrations have remained relatively constant since the 1980s) (National Atmospheric Deposition Program 2016). As a result, this spatial gradient of atmospheric N deposition is currently less steep, with recent estimates that range between 6.6 and 3.5 kg N ha^{-1} y^{-1} (North to South). Despite these declines in N deposition, it remains an open question whether historically high rates of N deposition can have persistent impacts on forest health, particularly as N accumulates in ecosystem sinks. To account for potential legacy effects of historical deposition, I used interpolated data from the National Atmospheric Deposition Program (2014) to calculate cumulative N loading to each study site for the period between 1985 and 2012. Along the study transect, site-level estimates of wet-only ($NO_3^- + NH_4^+$) cumulative N deposition ranged between 117.5 and 206.9 kg N ha^{-1}. After taking into account N inputs from dry deposition ($HNO_3 + NO_3^- + NH_4^+$), I estimated cumulative total N deposition (wet + dry) to range from 326.1 to 399.6 kg N ha^{-1} for these sites.

In the Central and Southern Appalachian Mountains, red spruce forests are naturally restricted to high elevation areas; thus, all of the study sites are located above 900 m, with each having a SW aspect and slopes between 0 and 10%. These sites represent second growth forests (~65-154 years) with red spruce as a major, canopy-dominant species, and eastern hemlock (*Tsuga canadensis*), yellow birch (*Betula alleghaniensis* var. *alleganiensis*), red maple (*Acer rubrum*), and black cherry (*Prunus serotina* var. *serotina*) as common associates. In 2011 my colleagues and I estimated the year of stand establishment for these forests by crossdating a representative sample of red spruce tree cores taken from each site (Stokes and Smiley 1996). The variation in stand age for these sites ranged between 68-157 years, which was supported by independent estimates from the U.S. Forest Service (Kent Karriker, *personal communication*).

While soil types at each site vary slightly across the spatial gradient, they are all representative of red spruce forests, with high organic matter content, low pH, and low micronutrient supply. Since long-term climatological records for these sites are unavailable, I relied on digital grid estimates of point data to evaluate site-level differences in precipitation and temperature. Averaged over the period 1981-2010, annual precipitation for these sites varies between 1238 and 1591 mm, while mean annual temperature (MAT) ranges from 6.3 and 8.4°C across the study gradient (PRISM Climate Group, Oregon State University).

1.3. Study Objectives.

In this research project conducted in seven red spruce forests in the Central Appalachian Mountains, I tested three main objectives:

In *Chapter 2*, I examined whether the variation in N status at these sites was associated with historically high rates of N deposition. To characterize ecosystem N status, I used multiple indicators of N availability that incorporate the N cycling history of a forest stand over varying timescales, from seasons (e.g. net N transformations and resin ion-exchange) to years (e.g. foliar and soil $\delta^{15}N$ composition) to decades (e.g. soil C:N). I then used a model selection approach to compare the relative strength of various environmental controls on N status, including N deposition, climate (temperature and precipitation), stand age, and tree species composition.

In *Chapter 3*, I tested the hypothesis that belowground C balance would be related to differences in N status. Over a two-year interval, I took repeated measurements of soil CO_2 efflux and litterfall in order to obtain annual estimates of soil respiration and total belowground C allocation by plants. I then examined the primary environmental controls on these processes using the same model selection approach employed in *Chapter 2*.

In *Chapter 4*, I examined the growth of red spruce trees at each site to determine whether historically high rates of N deposition were continuing to have negative effects on red spruce aboveground growth. In addition, I measured the C balance of red spruce foliage and stems to determine whether the observed growth responses were reflected by differences in C uptake via net photosynthesis and C loss via dark respiration. These estimates of growth and C flux were then modeled against a suite of environmental factors (including N deposition) to determine the main controls on aboveground C balance.

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Chapter 2. Site-level importance of broadleaf deciduous trees outweighs the legacy of high nitrogen (N) deposition on ecosystem N status of Central Appalachian red spruce forests.

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2.1. Abstract.

Atmospheric nitrogen (N) deposition can influence forest ecosystem N status, and the resilience of forests to the effects of N deposition depends on a number of co-occurring environmental factors that regulate N retention or loss. In this study, I test the idea that N deposition may have important and long-lasting impacts on patterns of N cycling by using field and laboratory techniques to assess N status in seven high-elevation Central Appalachian red spruce (*Picea rubens* Sarg.) forests located at sites that historically received moderate to high inputs of N atmospheric deposition. During 2011 and 2012, I measured multiple indices of N availability (e.g. foliar/soil C:N and δ^{15}N, resin ion-exchange, and N transformation rates) that integrate N cycling over seasonal to decadal time scales. Using a model selection approach, I compared the strength of the association between various environmental factors and temporally-integrated indices of N status in a series of regression models. Site-level differences in the relative importance value of broadleaf deciduous (BD) trees consistently explained most of the observed variation in N status. Soil C:N was significantly lower for sites with greater BD importance (R^2 = 0.67-0.77), and there was a strong positive relationship between BD importance and soil δ^{15}N content (R^2 = 0.64-0.85). Despite a four-fold difference in historic deposition across the seven forest sites, I did not observe any significant relationships between site N status and N deposition. These findings suggest that potential legacy effects of N deposition were obscured by the influence of BD importance on N status at these sites. My results add strong support to the idea that predicting the resilience of forests to the effects of N deposition requires detailed knowledge on the contribution of tree species composition to soil N cycling and retention.

2.2. Introduction.

Forest ecosystems are the largest terrestrial carbon (C) sink, sequestering ~ 2.4 Pg C annually (Pan et al. 2011) and about 30% of C emissions from fossil fuel combustion (Le Quéré et al. 2013). For many forests in the northeastern United States, a key limitation to forest C storage is the forest nitrogen (N) status, or amount of plant-available N in soils (Vitousek and Howarth 1991; LeBauer and Treseder 2008; Nadelhoffer et al. 1985; Reich et al. 1997). However, despite the importance of N status as a potential constraint to ecosystem production, there are many research challenges associated with defining and measuring plant-available N as well as characterizing and distinguishing the numerous factors that have shown to impact N cycling in forest ecosystems (Gundersen et al. 1998; Ros et al. 2011). Thus, improving our understanding of the controls on soil N status will reduce uncertainty in current models of ecosystem dynamics.

The deposition of reactive N on terrestrial forests can have large, and potentially long-lasting, impacts on nutrient availability, plant productivity, and C storage. Since the enactment of the Clean Air Act in 1990, both pollutant emissions and subsequent rates of N deposition have declined for the Central Appalachian region (US Environmental Protection Agency 2015), which raises the question of how long the effects of chronic pollution will persist following reductions in N deposition. While a few studies have examined these 'legacy effects' (Boxman et al. 1995, 1998; Koopmans et al. 1995; Strengbom et al. 2001; Chen and Högberg 2006), there is little consensus on the time required for full ecosystem recovery. For example, some N addition experiments have reported elevated rates of N mineralization decades after N treatments had ceased (Vinton and Burke 1995; Chen and Högberg 2006). In contrast, throughfall manipulation studies found that net N mineralization rates, foliar N, and nitrate (NO_3^-) leaching recovered within only 2-4 years of N removal (Boxman et al. 1995, 1998; Koopmans et al. 1995). The long-term effects of N deposition may also be evident in forest ecosystems that have only been exposed to background levels of pollution. Despite widespread declines in pollutant emissions for the eastern United States, several studies conducted along ambient N deposition gradients in this region have observed strong relationships between estimates of N deposition and foliar and soil chemistry (Boggs et al. 2005; Pardo et al. 2007; McNeil et al. 2012).

Much of the uncertainty about the resilience of forests to chronic N deposition depends on how N cycling is altered by other environmental factors like in climate, disturbance history, and species composition (Aber et al. 2003; Phillips et al. 2013); thus, a better understanding of what factors control ecosystem N status should improve our ability to predict how forests recover from long-term pollution (Galloway et al. 2004). For instance, while foliar N concentration is often reported to increase in response to atmospheric N deposition (Pardo et al. 2006; McNeil et al. 2007; Crowley et al. 2012), studies conducted across broad spatial gradients of elevation and latitude have shown that foliar N concentrations can vary with respect to climatic differences as well (Aber et al. 2003), where cooler temperatures and shorter growing seasons are associated with enriched foliar N (Körner 1989; Yin 1992). Likewise, NO_3^- export to surface waters has been shown to increase with N fertilization (Magill et al. 2000; Lovett and Goodale 2011), but may also be related to differences in mean annual temperature (MAT) (Murdoch et al. 1998). In addition to climate, disturbances like forest harvest and wildfire can have both short- and long-term impacts on N status. For example, forest harvest can result in initial increases in NO_3^- export due to reduced plant uptake (Aber et al. 2002); however, over longer timescales (e.g. several decades after harvest), NO_3^- export may decline due to high N demand during forest regrowth (Goodale et al. 2000). Since NO_3^- leaching losses are generally higher for soils that have high nitrification rates and low soil C:N ratios (Gundersen et al. 1998), these N cycling patterns are also expected to be related to tree species controls on soil quality. Indeed, tree species can also affect N cycling in forest ecosystems primarily through the quality and decomposability of their aboveground litter, which ultimately leads to the development of distinct soil profiles that vary in their N cycling characteristics (Melillo et al. 1982; Stump and Binkley 1993; Lovett et al. 2004). This relationship between tree species and N status has been observed in a variety of forests in the northeastern United States, where differences in tree species composition were strongly correlated with interspecific variation in soil C and N pools as well as rates of N transformations and NO_3^- export (Finzi et al. 1998; Lovett et al. 2002, 2004).

Identifying which factors exert the greatest influence over soil N availability can also be difficult since various factors operate at different timescales and on different components of the N cycle. For example, additions of reactive N from fertilizers and atmospheric deposition can strongly influence soil N availability in the short-term as added N is rapidly (i.e. within minutes) immobilized by the soil microbial community, and later mineralized back into soil solution as plant available N (Zogg et al. 2000). On the other hand, chronic, low-doses of N deposition can have long-term (i.e. years to decades) effects on N availability as inorganic N is assimilated into aboveground biomass, and transported back to the forest floor via litterfall where it can then be incorporated into soil organic matter (SOM; Nadelhoffer et al. 1995; Zak et al. 2004). Consequently, long-term N status indicators like soil C:N ratios are often associated with differences in tree species composition and their associated litterfall characteristics (Lovett et al. 2004). Moreover, many studies have shown a negative correlation between soil C:N and short- to medium-term N status indicators including net N mineralization and nitrification (Ollinger et al. 2002), NO_3^- export (Lovett et al. 2002), and the fractionation of stable N isotopes ($^{15}N/^{14}N$) in vegetation and soil sinks (Pardo et al. 2007). On the other hand, climate variation may also affect forest N status over varying time frames via direct physical effects on biochemical processes like N transformations (Robinson 2002) or possibly through indirect effects on tree species composition. Thus, improving our understanding of how N is cycled through forest ecosystems requires a multifaceted approach to not only examine the numerous factors that can influence N availability, but also to examine indices of N availability that correspond to the timescale with which these factors are important.

In the Central Appalachian Mountains, high-elevation forests containing red spruce (*Picea rubens* Sarg.) have historically been sensitive to a wide range of natural and anthropogenic disturbances; thus, these forests provide an ideal system to examine the various controls on ecosystem N status. Beginning in the late 1800s, improved railroad technologies allowed the logging of these restricted high-elevation areas; as a result, extensive cutting and related slash fires reduced red spruce populations to their present extent of about 10,000 ha (Hopkins 1899; Lewis 1998). Elevated rates of acid precipitation have also had a severe impact on these red spruce ecosystems. Between the mid-1960s and mid-1980s, unexplained dieback and growth declines were reported in several red spruce stands throughout the Northeast (Siccama et al. 1982; Foster and Reiners 1983; Johnson and Siccama 1983; Scott et al. 1984; Johnson and McLaughlin 1986). Initial speculation about the cause of this decline included age-related senescence, insect outbreak, climate change, pollution, or a combination of these factors (Siccama et al. 1982). However, empirical evidence from controlled N addition experiments and from sampling along gradients of atmospheric N deposition revealed a strong linkage between N deposition and critical changes in forest floor and foliar N concentrations, suggesting that red spruce decline was likely caused by a combination of foliar nutrient imbalance (e.g. Ca/N and Mg/N; McNulty et al. 1991; McNulty et al. 2005), and reduced cold stress tolerance (DeHayes et al. 1999; Schaberg et al. 2002).

The objective of this study was to assess the extent to which N availability in red spruce forests in the Central Appalachian Mountains is associated with N deposition. Following the assumptions of the classic conceptual model of progressive N saturation (Aber et al. 1989, 1998), it is expected that inorganic N inputs from deposition would enhance the N status of red spruce ecosystems, particularly during periods of peak N deposition. However, recent evidence has challenged these assumptions, instead suggesting that long-term responses to N deposition are dependent on the factors that influence how N is retained by ecosystem sinks (Lovett and Goodale 2011). Thus, in the context of declining N deposition, it remains an open question whether historically high rates of N deposition can have persistent effects on enhanced N availability, or whether ecosystem N status is more strongly influenced by other environmental factors. I examined this question by assessing N status along a transect consisting of seven red spruce stands located in a region where high rates of N deposition have been declining since the Clean Air Act of 1990. During the mid-1980s, forests at the northern end of the transect received approximately four times greater wet-only N deposition than the southern end, with estimates ranging from 8.1 to 2.0 kg ha^{-1} yr^{-1} (North to South). Since 1990, reduced NO_x emissions have lowered the concentration of NO_3^- in wet deposition (kg ha^{-1} yr^{-1}) by 45-55% throughout the region (National Atmospheric Deposition Program 2016). Consequently, the N deposition gradient is weaker, with recent estimates of N deposition that range between 6.6 and 3.5 kg ha^{-1} yr^{-1} (North to South). In addition to the legacy effects of N deposition, these mixed red spruce stands differ in climate, stand age, and the relative importance of BD trees, providing a range of factors that need to be assessed when determining the environmental factors that most strongly influence the N status of these forests.

2.3. Methods.

Study site details

In June 2011, I established seven study sites (100-m diameter) containing red spruce as a major, canopy-dominant species. All sites are at high elevations within the central Appalachian Mountains, a region of moderate to high levels of historic atmospheric N deposition (Figure 2.1; Table 2.1). Since red spruce naturally occurs at high elevations along the central Appalachian Mountains, all study sites are above 900 m, each having a southwesterly aspect and slopes between 0 and 10%. All sites represent second growth forests with red spruce and eastern hemlock (*Tsuga canadensis*) as the commonly-occurring NE species. The selected sites also ranged in BD tree abundance which allowed me to assess the relative influence of BD species composition on N availability. The most common BD associates at my sites included yellow birch (*Betula alleghaniensis* var. *alleganiensis*), red maple (*Acer rubrum*), and black cherry (*Prunus serotina* var. *serotina*). I assessed the tree species composition at each site using the point-quarter sampling method (Pollard 1971) along three parallel 50-m transects (separated by 25 m) by sampling trees (> 10 cm DBH) at five randomly spaced points on each transect (*n* = 15 points). Relative importance values (RIV) for tree species found at each site and additional community characteristics of these red spruce stands are given in Table S2.1.

Soil types vary slightly between the study sites; however, they all have high organic matter content, low pH, and low micronutrient availability (Tables S2.2 and S2.3). The underlying geologic formations of these sites include Pennsylvanian Pottsville sandstone, Devonian Chemung shale, and Silurian-Devonian undivided shale (Table S2.4; WV Geological Survey).

Using standard tree core cross-dating techniques (Stokes and Smiley 1996), I estimated the year of stand establishment for each study site according to the age of the oldest red spruce tree that was sampled (Table 2.1). My tree-ring stand age dates for these sites range from 68 to 157 years, in agreement with independent estimates made by the U.S. Forest Service (Kent Karriker, *personal communication*). The variation in stand age at these sites is likely due to the timing of extensive spruce timber harvests and subsequent wildfires that occurred throughout the Central Appalachians during the late 1800s to early 1900s (Clarkson 1964; Lewis 1998; Nowacki and

Wendt 2010). It is also possible that these stand-resetting disturbances affected the present-day differences in species composition at my sites. However, there is not a significant co-variation among stand age and BD RIV at these sites, indicating that disturbance history and species composition could have independent effects on N availability.

Atmospheric N Deposition and Climate Variables

Historically, anthropogenic N inputs from wet deposition have been moderate to high for the study region, with wet deposition rates as high as ~9.3 kg N ha^{-1} yr^{-1} in the mid 1980s (National Atmospheric Deposition Program 2016). Following the 1990 Clean Air Act Amendments, both nitrogen oxide (NO_x) emissions, as well as subsequent deposition of atmospheric NO_3^-, have declined by ~45-55% (US Environmental Protection Agency 2015; National Atmospheric Deposition Program 2016). Nevertheless, decades of elevated N inputs could result in important legacy effects that still enhance N availability in these forests.

I used a highly-resolved and well-validated spatial model of atmospheric N deposition to estimate annual rates of inorganic N (NO_3^- + NH_4^+) inputs at each of my field sites for the period between 1985 and 2012 (National Atmospheric Deposition Program 2014). Sites were then selected based on estimates of accumulated wet-only N deposition so that they ranged in cumulative N inputs from 117.5 to 206.9 kg N ha^{-1} (Table 2.1). Taking into account the recent declines in NO_x emissions, average wet N deposition rates over the period 1985 to 2012 range from 4.2 to 7.4 kg N ha^{-1} yr^{-1}. To account for the influence of dry deposition (HNO_3 + NO_3^- + NH_4^+), I used a spatial model of total (wet + dry) N deposition rates for the period that data were available, between 2000-2012 (National Atmospheric Deposition Program 2014). I then calculated the proportion of wet deposition to total deposition on a pixel-wide basis for the mid-Atlantic region (West Virginia and surrounding states) to generate a set of site-specific correction factors, which were used to estimate total N deposition rates for the years 1985 to 1999. For my sites, average rates of total N deposition range from 11.6 to 14.3 kg N ha^{-1} yr^{-1} while cumulative total deposition loading ranged from 326.1 to 399.6 kg N ha^{-1} for the period between 1985 and 2012 (Table 2.1).

Since climatological data for these high-elevation sites are limited, I used interpolated PRISM climate data for each study site to estimate 30-year averages (1981-2010) of annual and growing season (May through September) temperature and precipitation (Table 2.1; PRISM Climate Group, Oregon State University). My study sites received between 1238 and 1591 mm of precipitation annually, and mean annual temperature (MAT) varies between 6.3 and 8.4°C.

Indices of soil N availability

I measured a suite of N availability indices that are thought to integrate over different temporal scales in their assessment of soil N status. Laboratory measurements of net N mineralization and net nitrification were used to estimate the current N status under controlled conditions; whereas, ion-exchange resins provided a snapshot of plant available N under current field conditions. I measured the natural abundance of ^{15}N as an indicator of N status that should integrate over several years (e.g. needle ^{15}N) or several decades (e.g. soil ^{15}N). Finally, soil C:N was used as an index of N availability that should integrate over the time period since the stand-resetting clear cutting and fires that occurred in the early 1900s. All sample measurements were taken from a single 30-m diameter sample plot at the center of each 100-m study site.

Two 28-day laboratory incubations were conducted in July and September 2012 to measure net rates of soil N transformations, using methods adapted from Rinkes et al. (2011). Each 30-m plot was divided into four quadrants and multiple soil cores (5-cm diameter) were removed from the top 5 cm of mineral soil until approximately 200 g of soil (wet weight) were collected from each quadrant in both sampling periods. Soil samples were returned to the laboratory within 24 h of collection and immediately homogenized by hand and coarse debris and roots were removed.

Approximately 28 g (wet weight) of homogenized soil was added to six glass jars and were incubated in the dark at 20°C for 0, 1, 2, 7, 14, or 28 d. Jars were loosely covered to allow gas exchange while reducing evaporation. Each week deionized water was added to compensate for evaporative water loss. The incubated soil samples were then collected after the appropriate incubation time to measure extractable NH_4^+ and NO_3^-.

Inorganic N content (NH_4^+ and NO_3^-) was determined using colorimetric microplate assays of soil extracts. Subsamples of soil (5 g wet weight) were extracted by adding 25 ml of 0.5 M K_2SO_4 and shaking the mixture on an orbital shaker for ~ 1 hr. Samples were then vacuum-filtered and extracts were frozen until further analysis. A modified Berthelot reaction (Rhine et al. 1998) was used to determine the NH_4^+ concentration of soil extracts, while NO_3^- concentrations were measured using a modified Griess reaction (Doane and Horwáth 2003). Both assays were conducted in 96-well microplates, and absorbance was measured using a Tecan Genios microplate reader (Tecan Group Ltd., Mannedorf, Switzerland) at 660 nm for NH_4^+ and 540 nm for NO_3^-. I calculated rates of net nitrification and net N mineralization as the change in NO_3^- and total inorganic N content (NH_4^+ and NO_3^-), respectively, that occurred after a given time interval.

To estimate plant available N *in situ*, I installed ion-exchange resins (Plant Root Simulator (PRS^{TM}) probes; Western Ag Innovations Inc., Saskatoon, SK) in each 30-m sample plot in July 2011. These probes consisted of either an anion or cation exchange resin housed within a plastic casing. At each site, between 16 and 20 anion and cation probes were randomly deployed by inserting each vertically starting at the surface of the O horizon (~ 12 cm depth). After 59 days, all probes were removed and cleaned with a coarse brush and deionized water to remove soil debris. Probes were kept on ice during transport and then immediately shipped to the Western Ag Innovations, Inc. laboratory in Saskatoon, SK. Both anion and cation exchange resins were analyzed for NO_3^-, NH_4^+, and total inorganic N and were expressed as soil N supply rates (μg 10 cm^{-2} burial $length^{-1}$).

During the 2011 growing season, I measured the N isotopic composition ($\delta^{15}N$) of red spruce needles and of organic and mineral soil. Red spruce needles were harvested from three randomly selected canopy trees near the center point of each 30-m study plot using a shotgun. To account for differences in leaf morphology through the canopy, I sampled needles at three general canopy heights: lower, middle, and upper. Foliar samples were dried for 48 h at 65°C and ground using a Wiley Mill (#40 mesh). All samples were analyzed for %N and $\delta^{15}N$ isotopic composition using a ThermoFisher Delta V+ Isotope Ratio Mass Spectrometer (Central Appalachians Stable Isotope Facility, University of Maryland Center for Environmental Science, Frostburg, MD).

Foliar %N and δ^{15}N were expressed as the average N content across all canopy heights for all trees sampled at a given site (*n* = 9 trees).

To determine soil isotopic N content and C:N ratio, I divided each 30-m sample plot into four quadrants from which I collected two cores (5-cm diameter) from the organic horizon (O_e and O_a) and two cores (2.2-cm diameter) from the upper 5 cm of the mineral horizon. Soil samples were immediately returned to the laboratory, dried for 48 h at 65°C, hand-homogenized to remove all debris, and ground to pass a #40 mesh using a Wiley Mill. Samples were analyzed for δ^{15}N isotopic composition using a ThermoFisher Delta V+ Isotope Ratio Mass Spectrometer (Central Appalachians Stable Isotope Facility, University of Maryland Center for Environmental Science, Frostburg, MD). A small subsample (~15 mg) of soil from each site was analyzed in triplicate for total soil C and N content by Dumas combustion using a NC 2500 elemental analyzer (Carlo Erba, Milan, Italy).

Statistical analysis

I compared several regression models to assess the relative strength of several environmental factors as predictors of soil N availability in mixed red spruce forests in the study region. In each model, I used an index of N availability (rates of net nitrification, net N mineralization, soil N supply rates, foliar %N and δ^{15}N, soil δ^{15}N, and soil C:N) as a response variable regressed against several environmental predictor variables that can influence plant-available N. The environmental variables I used were cumulative atmospheric N deposition for the period 1985-2012 (wet and total), mean temperature (annual and growing season, May through September), precipitation (annual and growing season), stand age, and relative importance of BD trees (hereafter referred to as BD RIV). Each regression model was then compared using Akaike's Information Criterion (AIC) to determine the likelihood of a particular model; those models that yielded the lowest AIC values were considered more likely and were therefore given greater statistical support. To account for low sample size (*n* = 7 study sites), I used corrected AIC (AIC_c) for all model comparisons (Burnham and Anderson 2002). Results of this analysis were expressed as ΔAIC_c, which is the difference between the AIC_c of the best model ($\Delta AIC_c = 0$) and competing models. In cases where the ΔAIC_c value between two competing models was less than two, I treated the models as indistinguishable in terms of their ability to predict a response in N availability. Relationships between measured rates of N cycling and field-based indices of N availability were conducted using bivariate regression models. All statistical analyses were performed using SAS-JMP 10.0 (SAS Institute, Cary, NC, USA).

2.4. Results.

Factors affecting N availability

Across all the potential factors I examined that could affect short- to long-term N availability, BD RIV was the parameter that most consistently explained differences in N status at my sites (Figure 2.2; Table 2.2). The foliar chemistry data for spruce trees showed a positive relationship between foliar N concentration and BD RIV ($p = 0.016$); likewise, I observed a positive trend relating the variation in foliar $\delta^{15}N$ to differences in BD importance ($p = 0.074$). BD RIV was also associated with medium- to long-term N availability at these sites, as illustrated by the positive relationships with soil $\delta^{15}N$ ($p = 0.003$-0.03) and negative relationships with soil C:N ($p = 0.009$-0.023). For each N availability index that was significantly related to BD importance, ΔAIC_c between the BD RIV model and the "next best" model ranged from 3.2 to 10.1 suggesting that BD RIV is a much better predictor of differences in N status at these sites compared with alternative models, including cumulative N deposition (Table 2.2).

In some instances, BD RIV was not the best model to explain the short-term variation in N cycling and availability. For example, stand age produced a better model fit than BD RIV for my estimates of both net N mineralization and NH_4^+ supply rate; however, neither of these were statistically significant and the models were indistinguishable from those with BD RIV as a predictor ($\Delta AIC_c = 0.7$-1.7). Similarly, a non-significant relationship was found relating net nitrification to annual precipitation, but this was largely influenced by the only two sites that had detectable rates of nitrification (McGowan Mountain and Cranberry Glades). Despite the lack of a significant response relating laboratory-measured N cycling rates and ion resin N supply rates to differences in BD importance, I nevertheless observed significantly higher rates of net nitrification, net N mineralization, and resin N supply above a threshold of ~40% BD RIV (Figure 2.2).

Comparison of N availability indices

I observed strong, positive correlations between field-based indices of soil N availability (ion resin N supply rates, $\delta^{15}N$, and soil C:N) and specific soil N cycling rates measured in laboratory incubations (Table S2.5). I observed significantly greater PRS-available N in sites where net N mineralization and nitrification rates were high ($p = 0.004$-0.03; Figure S2.1). However, this relationship is mostly driven by two sites (McGowan Mountain and Cranberry Glades) that had significantly higher N supply rates compared to all remaining sites ($p < 0.01$). Of the total inorganic N measured on PRS probes at these two sites, approximately 71% was in the form of NO_3^- rather than NH_4^+, suggesting that the higher rates of net nitrification at these two sites provided more plant-available NO_3^- to be captured by the soil resins. Rates of net mineralization rates were positively correlated with natural ^{15}N abundances in red spruce foliage and organic and mineral soil ($p = 0.003$-0.03; Figure 2.3A). I also observed strong negative relationships between net N mineralization rates and soil C:N in both the organic and mineral horizons ($p = 0.0003$-0.02; Figure 2.3B). Similar trends relating soil $\delta^{15}N$ and C:N to net nitrification rates were also observed; however, these relationships were not as pronounced ($p = 0.01$-0.1; Table S2.5).

2.5. Discussion.

The Central Appalachian Mountains have historically had moderate to high levels of N deposition; therefore, I anticipated that N deposition would still have a strong association with N availability for this region. I especially expected to see this impact of N deposition on measures of N availability that integrate over longer time periods predating the steep declines in N deposition (e.g. soil C:N). However, across the seven forest sites I examined, the relative importance of BD tree species was consistently better than N deposition and all other environmental variables in predicting variability in N status (Figure 2.2; Table 2.2). In addition, the current N status at my field sites (measured by soil N transformations) appears to be strongly associated with the decadal-scale development of distinct soil profiles, marked by differences in SOM quality (soil C:N) and an integrated history of N cycling (foliar and soil $\delta^{15}N$; Figure 2.3; Table S2.5). Together, my results suggest that variation in short-, medium-, and long-term soil N status is primarily due to the influence of forest species composition.

Tree species can have important effects on short-term and long-term soil N cycling due to differences in the quality of aboveground litter inputs, where low quality litter (e.g. high C:N or high lignin:N) decomposes more slowly and accumulates more in SOM than high quality litter (Melillo et al. 1982; Stump and Binkley 1993; Prescott et al. 1993; Prescott 1995). In turn, these differences in SOM quality are often tightly correlated with short- (e.g. rates of net N mineralization; Scott and Binkley 1997) and medium-term (e.g. $\delta^{15}N$ in vegetation and soil; Pardo et al. 2007) indices of N cycling across a variety of forest types. In a comparison of 30 diverse forested stands in New Hampshire, Ollinger et al. (2002) observed patterns of foliar and soil chemistry that were linked to differences in tree species composition. In general, stands dominated by BD species like sugar maple (*Acer saccharum*) or yellow birch (*Betula alleghaniensis*) had greater soil N availability (e.g. low soil C:N, high N transformation rates) compared to stands dominated by NE species like red spruce and eastern hemlock (*Tsuga canadensis*). My results are in agreement with many studies that show a positive correlation between rates of soil N transformations and increasing SOM quality (Pastor et al. 1984; Plymale et al. 1987; Finzi et al. 1998; Thomas and Prescott 2000). For my forest sites, approximately 67-94% of the variation in net N mineralization and net nitrification could be explained by soil C:N (coefficients of determination in Table S2.5). Moreover, net nitrification was only detected at sites where the C:N ratio of organic soil was below ~24, a critical threshold by which increased net nitrification is typically observed (McNulty et al. 1991; Lovett and Rueth 1999; Goodale and Aber 2001).

Natural abundances of stable N isotope ratios are often positively correlated with the N cycling history of a particular site (Högberg and Johannisson 1993; Garten and Van Miegroet 1994; Robinson 2001). As organic matter is decomposed by soil microbes, isotopic fractionation occurs whereby enzymatic processes favor the lighter ^{14}N isotope; as a result, the product of net N mineralization and net nitrification is isotopically-depleted relative to the ^{15}N-enriched substrate (Mariotti et al. 1981). This is especially important for sites with a more open N cycle, wherein the production and subsequent losses of isotopically-light NO_3^- will result in residual soil N pools that are enriched in $\delta^{15}N$ (Nadelhoffer and Fry 1994). Therefore, differences in foliar and soil $\delta^{15}N$ can be influenced by the factors that also promote increased net nitrification, including experimental N additions (Pardo et al. 1998), background rates of atmospheric N deposition (Emmett et al. 1998), and disturbance from harvesting (Pardo et al. 2002). However, a regional-scale analysis of foliar $\delta^{15}N$ showed the variation in $\delta^{15}N$ content was best explained by local soil conditions that regulate N availability (e.g. tree species effects on litter and SOM quality, soil C:N, and net nitrification/mineralization), not regional differences in N deposition (Pardo et al. 2006). My results support the findings of Pardo et al. (2006) where the $\delta^{15}N$ composition of soil (organic and mineral horizons) and red spruce foliage was positively correlated with net N mineralization and nitrification (Table S2.5). Likewise, the variation in $\delta^{15}N$ at my forest sites was best explained by differences in BD importance rather than N deposition (Table 2.2).

To address why I did not observe any effects of N deposition on N status, I present three possible explanations: 1) modeled values of N deposition were inaccurate; 2) the historic or current N deposition gradient was not sufficiently broad to elicit a measurable response; 3) effects of deposition are outweighed by effects of species composition on N availability. I find little support for the first two explanations. First, my N deposition estimates are derived from a robust and well-validated model constructed from long-term atmospheric deposition monitoring data throughout the region (National Atmospheric Deposition Program 2014). It is possible that my estimates of total (wet + dry) N deposition were less precise than my estimates of wet-only N deposition, primarily since the dataset used to derive estimates of dry deposition was more limited in scope (available data ranged between 2000-2012) and was interpolated over a coarser resolution (36 km grid) compared with my estimates of wet-only deposition (dataset ranged between 1985-2012; 2.6 km grid). Because of the uncertainty in dry deposition amounts at these high elevation sites, the actual magnitude of these deposition estimates may be imprecise. Nevertheless, the endpoints of this gradient are strongly grounded by two long-running atmospheric deposition monitoring stations, so the differences in historical N inputs at the high elevation sites between these stations should be proportionally consistent.

Second, although the current range of estimated N deposition rates is likely too narrow to be detected via changes in N status, I would expect that the four-fold difference (2.0-8.1 kg N ka^{-1} y^{-1}) in historical wet-only N deposition rates along this gradient is sufficiently broad to have had an influence on the N status of these sites at one point. Moreover, other studies in high-elevation red spruce forests have detected responses along smaller or at least comparable deposition gradients in the northern and southern Appalachian Mountains (McNulty et al. 1991; Boggs et al. 2005). These studies were conducted closer to the peak of deposition in the 1980s, so to test whether historical estimates of peak N deposition could potentially explain my results, I examined incremental changes in accumulated and average atmospheric N inputs for every year that data was available. Using the same model selection approach to determine potential drivers of N availability, I found that the variation in N availability indices was still best explained by either BD importance or stand age (ΔAICc > 3.1). Hence, my results are qualitatively unchanged because of the recent declines in N deposition to the region.

Given that the range of estimated N deposition rates is lower relative to the range of broadleaf abundance at these sites, the most plausible explanation for my results is that any potential long-term effects of atmospheric N deposition on N status were masked by differences in the abundance of BD species that produce high quality aboveground litter. This has important implications for studies seeking to assess the rate and patterns of ecosystem recovery from declines in atmospheric N deposition. For instance, Power et al. (2006) showed that microbial activity and microbial biomass N remained high 6-8 years after experimental N additions had ceased, suggesting that N-induced shifts to soil nutrient cycling may persist for several years after treatment. Likewise, some studies have observed elevated N mineralization rates in plots that had received experimental N inputs 12-14 years prior (Chen and Högberg 2006; Clark et al. 2009). By contrast, there is evidence from artificial roof experiments that the removal of N inputs resulted in rapidly (within 1 year) suppressed N mineralization rates via immobilization (Koopmans et al. 1995; Corre and Lamersdorf 2004). The variation in these ecosystem responses to reduced N fertilization likely depends on the abiotic and biotic factors that influence N cycling and retention. Thus, while my study cannot presently determine the degree to which these sites have recovered from historical deposition, my results have shown that tree species composition may be an overriding control on N status, regardless of the chosen metric of N status.

The influence that the composition of tree species can have on N status has been supported by numerous studies (Finzi et al. 1998; Lovett and Rueth 1999; Peterjohn et al. 1999; Christ et al. 2002; Lovett et al. 2004). For example, in a survey of 39 streams in the Catskill Mountains, Lovett et al. (2000) found 17-fold differences in stream-water NO_3^- concentrations despite similar topography, land use history, and atmospheric deposition rates across the study area. The authors hypothesized that differences in the relative abundance of certain tree species could be responsible for some of the variability that was observed. More specifically, watersheds with low stream-water NO_3^- concentrations tended to be dominated by either red oak (*Quercus rubra*) or American beech (*Fagus grandifolia*); whereas, oak was completely absent from watersheds with high stream-water NO_3^-. Since both oak and beech are characterized as having recalcitrant litter with high lignin:N ratios and low rates of net nitrification (Finzi et al. 1998), this could partially explain why stream-water NO_3^- concentrations were low in these watersheds. In general, species producing more recalcitrant litter (e.g. oaks, beech, and conifers) are associated with lower NO_3^- availability and losses than species that produce more labile litter (e.g. sugar maple and birch).

While my model comparison approach reveals valuable insight on the effects of BD tree abundance on N status, it does not allow me to explicitly examine the potential influence of individual tree species at my plots. Part of this limitation stems from the fact that many of the species that I surveyed are not present at all of the study sites. However, the most common BD species, yellow birch and red maple, were found in at least five of the seven sites. I analyzed for potential relationships between either yellow birch or red maple RIV and my indices of N availability, and I only found significant ($p < 0.05$) relationships between yellow birch RIV and red spruce foliar %N, $\delta^{15}N$ of organic soil, and C:N of organic soil. In contrast, no significant relationships were found between red maple RIV and indices of N availability; however, this was likely due to narrow range in red maple abundance (i.e. 0-19.9% RIV). While the scope of analysis is limited, it does offer support that yellow birch is an important contributor to enhanced soil N availability (Templer et al. 2003; Lovett et al. 2004; Christenson et al. 2009).

Most studies conducted along N deposition gradients (e.g. McNulty et al. 1991; Lovett and Rueth 1999; Boggs et al. 2005, 2007) do not explicitly include a gradient of BD abundance; as such, comparing the relative influence of plant functional type and atmospheric N deposition is not possible for these types of experiments. However, McNeil et al. (2008) did examine differences in N status along multiple environmental gradients (e.g. N deposition, species composition, climate, stand history, and geology) in Adirondack Park, New York. The results of this analysis showed that across a N deposition gradient of similar magnitude (i.e. ~4.5-7.5 kg N ha^{-1} yr^{-1}), the variation in canopy N and soil C:N was still largely associated with differences in species composition. However, when analyzed on an individual species basis, N deposition was a significant predictor for changes in foliar N, suggesting that deposition effects may be evident within the N status of individual tree species (McNeil et al. 2012). Moreover, a recent meta-analysis of 30 N addition experiments showed that mycorrhizal association was a strong predictor of forest responses to deposition (Midgley and Phillips 2014). The results of this study lend further support to the idea that species composition (and perhaps their mycorrhizal associations) remains an important control on ecosystem N status, even in studies where N is added in severe excess of background rates.

Given the association between tree species composition and patterns of N availability, it is expected that future changes in BD importance will also have a considerable impact on ecosystem N status, particularly if these BD species include those with easily mineralizable litter like maple or birch (Templer et al. 2003; Lovett et al. 2004). I can consider two possible scenarios: 1) a BD forest is converted to a spruce forest; and 2) a red spruce forest is gradually replaced by encroaching BD species. In a study conducted by Kelly et al. (2011), soil N cycling rates were compared between two adjacent watersheds in the Fernow Experimental Forest. Both watersheds originally contained a mix of BD species but were clearcut and managed with herbicide between 1964-1969; one of the watersheds (WS 6) was replanted with Norway spruce (*Picea abies*) while the other (WS 7) was allowed to regenerate as a BD forest. Despite similar aspect, topography, and climate between the two watersheds, the BD forest had nitrification rates that were nine times greater than the spruce forest (Kelly et al. 2011). Accordingly, stream NO_3^- export also significantly differed between the two watersheds, with mean annual rates that were two orders of magnitude higher in the BD forest compared to the spruce forest (Kelly 2010). Based on this evidence, I can assume that encroachment by BD tree species into spruce-fir forests of the Central Appalachians will also have significant consequences for C and N pools.

There is some evidence that red spruce forests have been pushed to higher elevations in response to climate warming (Hamburg and Cogbill 1988); as such, red spruce has been predicted to disappear from West Virginia under future climatic stress (Prasad et al. 2007; Iverson et al. 2008). In addition to the substantial release of C into the atmosphere from the decomposition of SOM (Lal 2005), the replacement of red spruce dominated forests by BD species (especially maple or birch) is likely to be accompanied by significant increases in N status, with possible adverse effects on N loading to surface waters.

Conclusions

In this study conducted in seven red spruce forests in the central Appalachian Mountains, I found strong co-variation between multiple indicators of N availability that integrate over seasonal to decadal time scales. To assess the relative importance of environmental factors causing these consistent site-level differences in N availability, I used a model selection approach. I found robust evidence that the site-level importance of BD tree species was a better predictor of N status than all other environmental factors, including historically moderate to high rates of atmospheric N deposition. Although red spruce trees are well represented at all sites, the increased importance of BD tree species, and their associated litterfall characteristics, had a significant positive effect on ecosystem N status. As such, any observable legacy effects on soil N availability from atmospheric N deposition was likely obscured by the influence of BD tree species; however, this does not preclude the potential importance of atmospheric N deposition on N availability in temperate forests of the Central Appalachian Mountains. Thus, this study adds strong support to the idea that predicting forest responses to changes in anthropogenic N inputs will require a strong understanding of the role of tree species as a primary control on ecosystem N status, particularly in forests with mixed species composition.

2.6. Tables and Figures.

Table 2.1. Site descriptions of the seven red spruce stands in the central Appalachian Mountains. Estimates of atmospheric N deposition rates were derived from National Atmospheric Deposition Program (2014). Annual and growing season (May through September) mean temperature (°C) and precipitation (mm) were derived from PRISM Climate Group dataset for 1981-2010 normals (available online at http://prism.oregonstate.edu; accessed 17 April 2015). Year of stand establishment ("Est. Year") was determined by the age of the oldest red spruce tree sampled using standard tree core cross-dating techniques. Relative importance of canopy broadleaf deciduous species ("BD RIV") was calculated using the point-quarter sampling method in May 2014.

Study Site	Abbrev.	Ave. N Dep. ($kg\ N\ ha^{-1}\ yr^{-1}$)		Cumulative N Dep. ($kg\ N\ ha^{-1}$)		Mean Temperature (°C)		Precipitation (mm)		Est. Year	BD RIV (%)
		Wet	Total (wet + dry)	Wet	Total (wet + dry)	Annual	Growing Season	Annual	Growing Season		
Flat Ridge	FLR	7.4	14.3	206.9	399.6	6.8	15.1	1469.3	675.5	1939	26.2
Gaudineer Knob	GKB	6.8	13.1	189.0	366.8	6.3	14.5	1420.1	687.2	1911	0.0
McGowan Mtn	MCG	6.7	13.6	188.5	380.3	7.9	16.2	1407.0	674.5	1858	49.8
Cranberry Glades	CGL	6.5	13.1	182.4	366.4	7.3	15.6	1591.2	736.0	1858	43.1
Upper Middle Mtn	UMM	6.2	13.3	173.6	372.4	7.1	15.2	1361.7	643.4	1947	26.2
Span Oak Run	SOR	6.1	13.1	171.5	366.4	7.6	15.6	1276.4	594.4	1885	37.6
Little Spruce Bog	LSB	4.2	11.6	117.5	326.1	8.4	16.5	1238.8	573.3	1865	32.6

Table 2.2. Comparison of corrected Akaike Information Criterion (ΔAIC_c) values for linear regression models of potential drivers of N availability and indices of N availability. ΔAIC values reflect the difference between the AIC of the most parsimonius model ($\Delta AIC = 0$) and competing models.

	ΔAIC_c										
Model	Nitr.	Min.	Total N	NH_4^+	NO_3^-	$\%N_f$	$\delta^{15}N_f$	$\delta^{15}N_{Org}$	$\delta^{15}N_{Min}$	$C:N_{Org}$	$C:N_{Min}$
Cumulative N Deposition (wet)	5.4	4.7	4.3	5.0	4.5	8.6	4.6	13.0	7.0	10.3	7.8
Cumulative N Deposition (wet + dry)	5.6	4.8	4.5	5.3	4.6	8.0	4.8	13.0	7.0	10.3	7.8
Mean Annual Temperature	5.6	4.2	4.2	4.9	4.4	7.0	4.5	10.8	5.2	7.4	5.9
Mean Growing Season Temperature	5.3	3.7	3.4	4.3	3.6	5.9	4.2	10.1	4.7	5.8	5.0
Annual Precipitation	0	2.1	2.0	2.6	2.2	8.7	3.6	13.0	7.0	9.7	7.3
Growing Season Precipitation	1.1	2.6	1.9	2.4	2.1	8.8	3.9	13.0	7.1	9.8	7.4
Stand Age	2.9	0	1.1	0	2.0	7.4	2.1	10.1	4.3	3.2	3.3
BD Relative Importance (RIV)	3.6	0.7	0	1.7	0	0	0	0	0	0	0
R^2 (best model)	0.55	0.49	0.50	0.53	0.51	0.72	0.50	0.85	0.64	0.77	0.68
p (best model)	0.055	0.078	0.077	0.062	0.072	0.016	0.074	0.003	0.030	0.009	0.023

Nitr., net nitrification; Min., net N mineralization; Total N/NH_4^+/NO_3^-, PRS^{TM} Total N/NH_4^+/NO_3^- supply rate; $\%N_f$, foliar %N; $\delta^{15}N_f$, foliar $\delta^{15}N$; $\delta^{15}N_{Org}$, organic soil $\delta^{15}N$; $\delta^{15}N_{Min}$, mineral soil $\delta^{15}N$; $C:N_{Org}$, organic soil C:N; $C:N_{Min}$, mineral soil C:N.

Figure 2.1. Map of the seven red spruce stands in the central Appalachian Mountains. Arranged by highest to lowest estimates of N deposition, the seven study sites are: Flat Ridge (FLR), Gaudineer Knob (GKB), McGowan Mountain (MCG), Cranberry Glades (CGL), Upper Middle Mountain (UMM), Span Oak Run (SOR), and Little Spruce Bog (LSB).

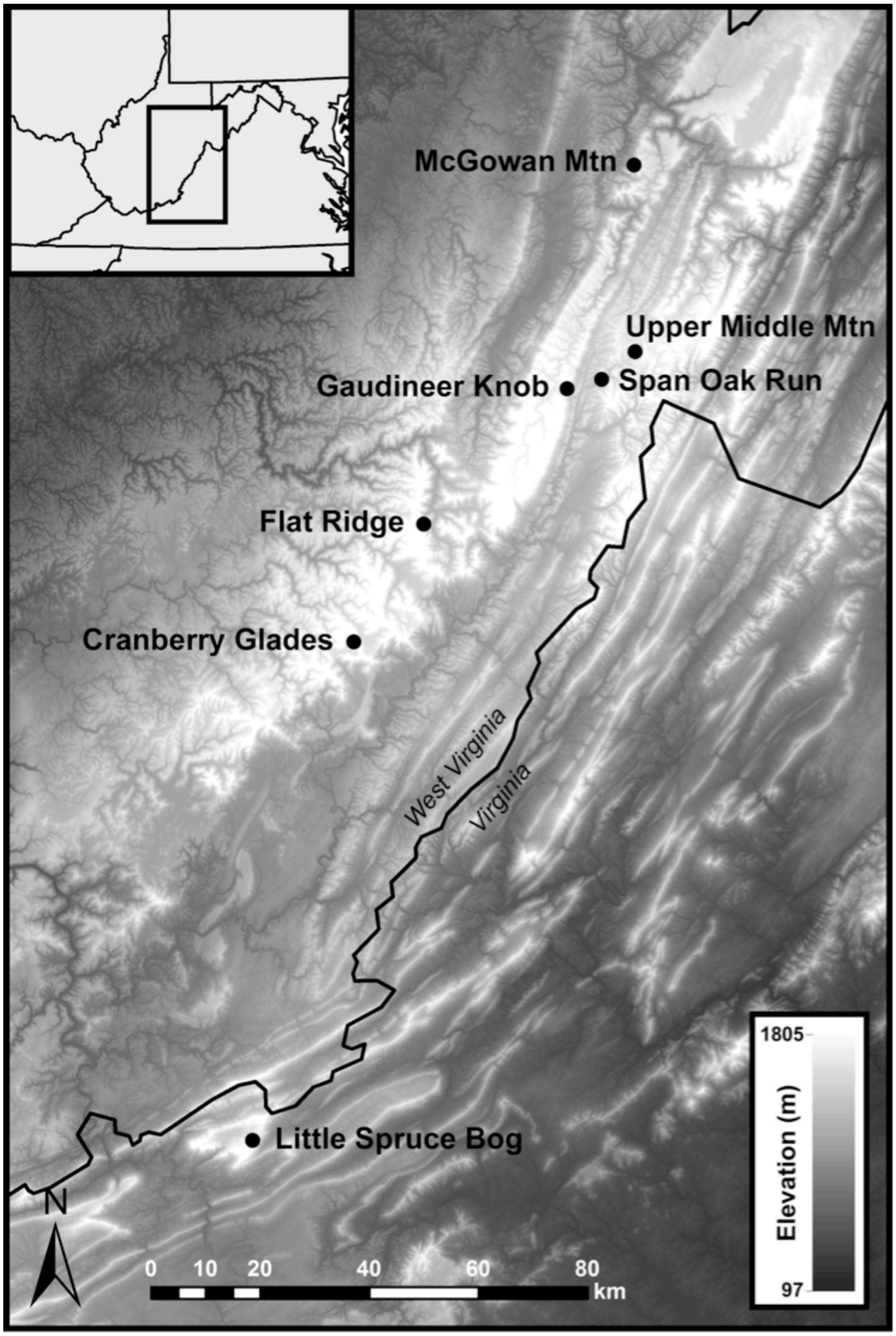


Figure 2.2. Regressions of mean net N transformation rates (± std. error bars; $n = 2$), mean total dissolved inorganic N (DIN) supply rates (measured using PRS^{TM} probes across a 59-d burial period; ± std. error bars; $n = 5$), soil and foliar $\delta^{15}N$ composition ($n = 1$), and mean soil C:N (± std. error bars; $n = 3$) versus cumulative inorganic N in wet deposition (A, C, E, G) and relative importance values of all broadleaf deciduous (BD) species (B, D, F, H) for each site along the study gradient ($n = 7$). *n.s.*, not significant ($p < 0.10$), *, $p < 0.05$, **, $p < 0.01$.

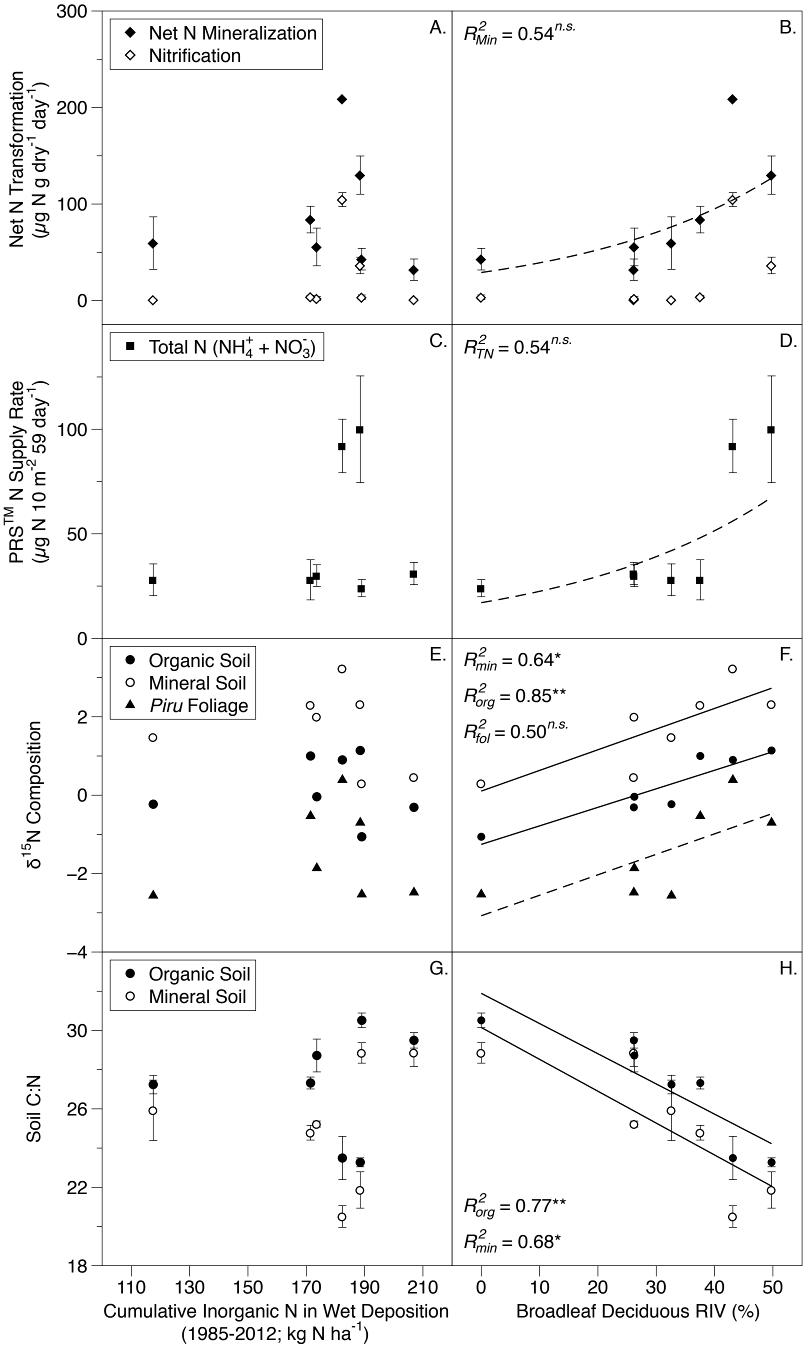
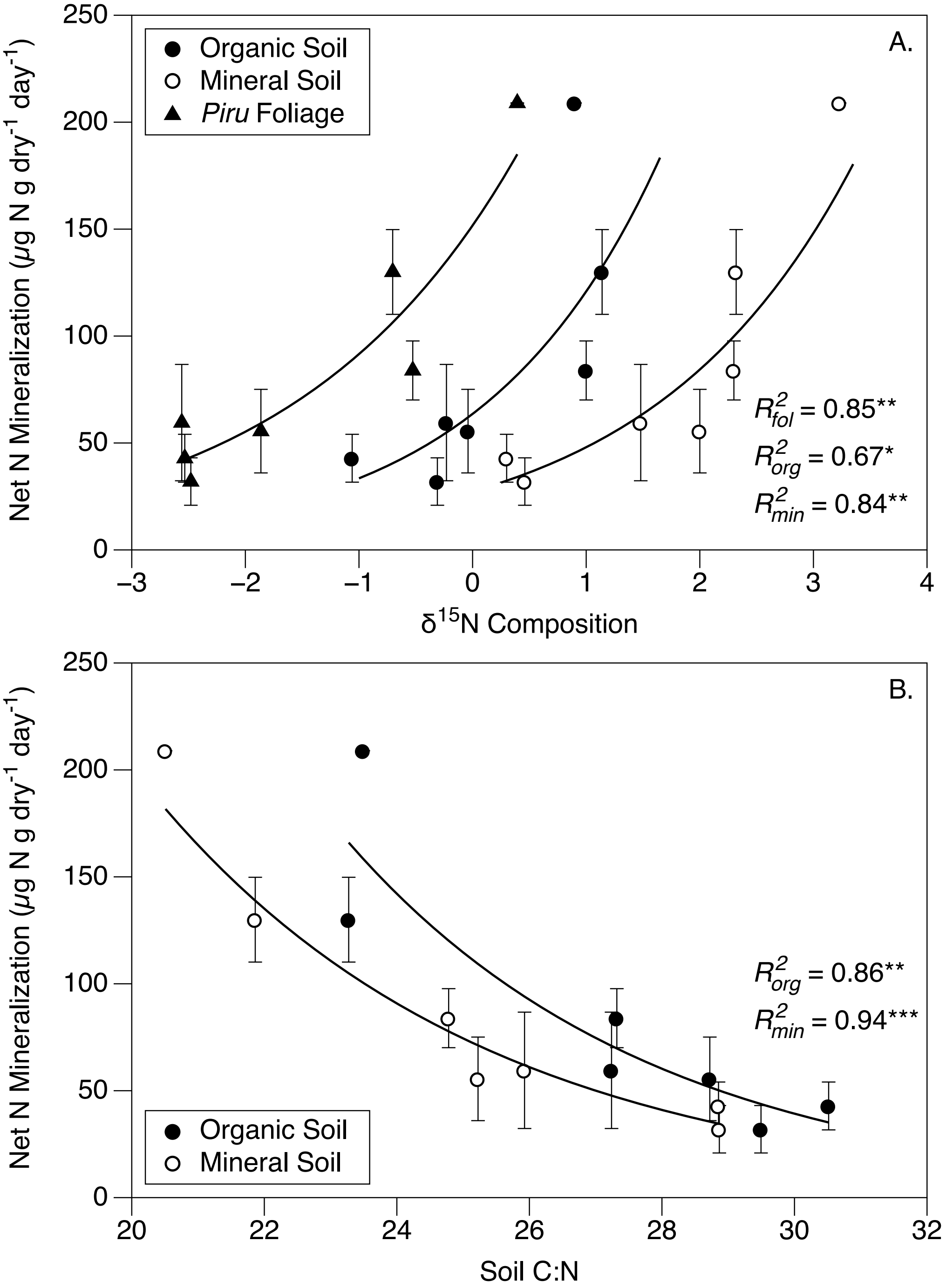


Figure 2.3. Bivariate regressions of mean net N mineralization rates (± std. error bars; $n = 2$) versus δ^{15}N composition of red spruce leaves, organic and mineral soil (A), and soil C:N content in both the organic and mineral soil horizons (B) for each study site along a gradient of atmospheric N deposition ($n = 7$). Mineralization rates were fit against N availability indices using an exponential function. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.



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Chapter 3. Annual soil respiration and belowground carbon allocation in high-elevation red spruce forests are linked to broadleaf controls on N status

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3.1. Abstract.

Soils can be important sinks or sources for atmospheric CO_2; however, quantifying the major fluxes of belowground carbon (C) balance remains a major source of uncertainty in attempts to develop precise global C budgets. Two fluxes are primarily responsible for soil net C storage: soil respiration and total belowground C allocation (TBCA). Many factors influence these two processes including climate, species composition, gross primary productivity (GPP), and substrate availability. Atmospheric inputs of reactive nitrogen (N) may also influence both soil respiration and TBCA through mechanisms such as enhanced plant productivity, shifts in biomass allocation, increased stabilization of organic matter, and alterations in microbial enzyme synthesis. In this study, I examined annual rates of soil respiration and TBCA in seven high-elevation red spruce communities that vary in tree species composition and N status. Since these forests are also located in a region where atmospheric N deposition has been historically high, I tested whether patterns of soil respiration and TBCA were more strongly associated with the potential legacy effects of N deposition or with the N status of these sites. I used repeated field measurements of soil respiration and TBCA at each of the seven sites to estimate annual belowground C balance in 2011 and 2012. Annual soil respiration estimates varied between 577-863 g C m^{-2} y^{-1} in 2011 and between 823-1384 g m^{-2} y^{-1} in 2012; likewise, TBCA varied between 511-745 g C m^{-2} y^{-1} in 2011 and between 734-1267 g m^{-2} y^{-1} in 2012. I attributed the 42-70% increase in soil respiration between 2011 and 2012 and TBCA to the warmer conditions in 2012. Contrary to my expectations, no significant relationships between modeled N deposition and annual estimates of soil respiration and TBCA were observed. Instead, I found that these processes were positively correlated with site-level differences in both N status and tree species composition. In particular, I found that the $\delta^{15}N$ content of organic soil was consistently a strong predictor of both soil respiration (2011: $R^2 = 0.84$; 2012: $R^2 = 0.95$) and TBCA (2011: $R^2 = 0.86$; 2012: $R^2 = 0.96$). I also found that the relative importance of broadleaf deciduous trees was a strong predictor of soil respiration (2011: $R^2 = 0.81$; 2012: $R^2 = 0.91$) and TBCA (2011: $R^2 = 0.83$; 2012: $R^2 = 0.81$). The result that soil respiration and TBCA were positively correlated with N status was surprising given that belowground C allocation tends to increase when resources (like N availability) are limited. On the other hand, this study does not account for the proportion of GPP that was allocated belowground; thus, it remains an open question whether the relative balance of TBCA to GPP was actually negatively associated with increasing soil fertility.

3.2. Introduction.

As Earth's climate continues to change in response to increasing atmospheric CO_2 concentrations, a greater understanding of the mechanisms that underlie the global carbon (C) balance is necessary. For forest ecosystems, two primary processes determine net C storage: C assimilated through gross primary production (GPP), and C lost through ecosystem respiration. Over the last several decades, improved experimental and modeling techniques have produced reliable aboveground estimates of forest C sequestration, with aboveground net primary productivity (ANPP) accounting for a significant proportion (approximately 30%) of forest GPP (Waring et al. 1998; Litton et al. 2007). However, our ability to estimate whole ecosystem C storage is limited by a number of uncertainties associated with quantifying belowground C balance. From a technical standpoint, belowground C fluxes are among the most difficult to measure, despite the numerous methodological approaches that have been developed over recent years. Furthermore, the various environmental controls on belowground C balance remain poorly understood, which complicates our ability to develop robust C budget models that are applicable to a broad range of forest environments. Resolving these issues is of critical importance in efforts to better predict the strength of forest C sinks under future environmental change.

The major fluxes that constitute belowground C balance include respiration by roots and soil microbes (the latter of which is related to the decomposition of above- and belowground detritus), and allocation of C to the production of coarse and fine roots, as well as rhizosphere exudates, and mycorrhizal symbionts. Total soil respiration, or the sum of autotrophic (root) plus heterotrophic (microbial) respiration, is by far the most important of these belowground fluxes that accounts for ~60-80% of respiration for forest ecosystems (Law et al. 1999; Janssens et al. 2001). On a global scale, soil respiration is the greatest source of C emissions from terrestrial ecosystems, releasing ~80-98 Pg C annually (Raich et al. 2002; Bond-Lamberty and Thomson 2010), which is approximately 10 times the current rate of global fossil fuel C emissions (Boden et al. 2010). Given that soils make up the largest terrestrial pool of organic C (Batjes 2014), there is growing concern that positive feedbacks between soil respiration and environmental stress could augment C emissions from anthropogenic sources, and enhance warming (Jenkinson et al. 1991; Davidson and Janssens 2006).

The importance of such a positive feedback mechanism depends, in part, on the temperature sensitivity of soil CO_2 efflux. And indeed, many studies have shown that rates of soil respiration are positively correlated with soil temperature, with the temperature response of respiration most commonly following an exponential Q_{10} or Arrhenius-type curve (Singh and Gupta 1977; Raich and Schlesinger 1992; Lloyd and Taylor 1994; Kicklighter et al. 1994; Peterjohn et al. 1994). While soil temperature can reliably explain a large portion of the variance in soil respiration (Raich and Schlesinger 1992; Fang and Moncrieff 2001), other factors can have a strong influence on respiration as well, including: soil moisture (Howard and Howard 1993; Davidson et al. 1998; Qi and Xu 2001; Gaumont-Guay et al. 2006), photosynthetically assimilated C (Högberg et al. 2001), microbial biomass (Scott-Denton et al. 2003), and the decomposability of litter (Taylor et al. 1989).

Since soil respiration includes the activity of autotrophs as well as heterotrophs, it is also highly dependent on how plant processes allocate C to belowground structures. Total belowground C allocation (TBCA) includes all of the photosynthate that is partitioned to production and respiration of roots and mycorrhizae, as well as root exudation, making it an integral component of forest C budgets (Giardina and Ryan 2002; Litton et al. 2007). However, despite accounting for a significant fraction of GPP (~40%; Litton et al. 2007), TBCA remains one of the least understood components of forest C balance due to difficulties with measuring root and mycorrhizal activity *in situ* (Hanson et al. 2000). To overcome these methodological constraints, Raich and Nadelhoffer (1989) developed a C mass balance approach to estimate TBCA as:

$$TBCA = F_s + F_E - F_A + \Delta[C_S + C_R + C_L]/\Delta t \quad (1)$$

where F_S is soil respiration, F_E is C export (e.g. erosion and leaching), F_A is litterfall C, and C_S, C_R, and C_L are the change in C stocks of mineral soil, root biomass, and surface litter, respectively over time *t.* Thus, TBCA is the net annual sum of soil C release ($F_S + F_E$) minus C inputs (F_A) plus changes in C stocks ($\Delta[C_S + C_R + C_L]$). If we assume that the annual change in soil, root, and litter C stocks is negligible and that C losses via export are small (which is to be expected in mature, undisturbed forests; Nadelhoffer et al. 1998), then we can rearrange *Equation 1* as:

$$TBCA = F_S - F_A \tag{2}$$

where litterfall (F_A) and soil respiration (F_S) are the predominant fluxes of C into and out of soil, and the difference between them provides an estimate of the remaining C that was allocated belowground. Provided that these steady-state assumptions are met, this conservation of mass approach provides a reliable, yet simple method to estimate a major component of forest C balance.

For many forests of the world, productivity is strongly co-limited by the availability of inorganic N to support growth (Vitousek and Howarth 1991; Elser et al. 2007; LeBauer and Treseder 2008); consequently, most tree species are expected to allocate a greater proportion of GPP belowground in order to increase nutrient uptake in these N-limited ecosystems (Chapin 1991; Phillips and Fahey 2008). One method to test this hypothesis is by comparing forest C balance between stands that differ in N availability. Recent N addition studies have shown that the fraction of GPP that is allocated belowground (TBCA/GPP ratio) typically declines as a function of soil fertility (Ryan et al. 1996, 2004). Likewise, Bae et al. (2015) showed that TBCA was inversely related to N status in forest sites that differ in native fertility. However, TBCA has also been shown to increase along gradients of N availability that are associated with differences in tree species composition (Nadelhoffer et al. 1985). Whether TBCA increases or decreases in response to changes in N status likely depends on the N sensitivity of the components of belowground C flux.

Changes in N status via experimental fertilization or atmospheric N deposition can also have a direct impact on belowground C cycling through altered rates of litter decomposition and soil respiration (Aber et al. 1998). Like TBCA, soil respiration rates often decline in response to experimental N additions for a variety of forest types (Söderstrom et al. 1983; Haynes and Gower 1995; Bowden et al. 2004; Burton et al. 2004); however, some neutral (Kane et al. 2003;

Allison et al. 2008) and positive responses have also been reported (Craine et al. 2001; Yan et al. 2010). A variety of mechanisms may explain these N-associated effects on soil C cycling. In N-enriched soils, the reduction in belowground C allocation to roots and mycorrhizal root symbionts can directly inhibit rates of rhizosphere respiration (Phillips and Fahey 2007). Shifts in TBCA may also limit exudation of organic compounds that would otherwise be used as a C source for soil microbial decomposition (Högberg and Högberg 2002). In addition, chronic atmospheric N inputs can also reduce soil respiration through changes in saprotrophic community structure and function (Janssens et al. 2010). Ågren et al. (2001) suggested that shifts in microbial community composition toward more efficient decomposers (i.e. low N demand, high C assimilation per unit CO_2 lost) could partially explain why N additions decrease organic matter decomposition. Studies have also reported N-induced changes in the activity of enzymes directly involved in decomposition, such that cellulose-degrading enzymes are up-regulated (Carreiro et al. 2000; Sjöberg et al. 2004) while enzymes involved in lignin-degradation are inhibited (DeForest et al. 2004). It is likely that chronic N additions lead to changes in the activity of both autotrophic and heterotrophic organisms, often with a net result of reduced decomposition, soil CO_2 efflux, and TBCA.

The objective of this study was to examine whether belowground C balance was associated with differences in N status in seven high-elevation red spruce (*Picea rubens*) forests in the Central Appalachian Mountains. I conducted this study as part of a larger body of research that examined the long-term effects of chronic N deposition on N and C cycling of these red spruce stands. In a previous study (Smith et al. 2016), I tested whether the site-level variation in N status among these seven study sites was associated with the legacy of historically high rates of N deposition in the region. The results of that study revealed that differences in N status were more associated with the abundance of broadleaf deciduous (BD) tree species than the cumulative N inputs from deposition. This suggests that the variation in N availability appears to be independent of the N deposition history of a particular site. Despite this decoupling of N status from atmospheric N inputs, the long-term effects of N deposition may still be manifested through alterations in belowground C storage and flux, since red spruce ecosystems are widely regarded as one of the more sensitive forest types to N deposition (Siccama et al. 1982; Scott et al. 1984; Schaberg et al. 2002; Thomas et al. 2010). In this study, I tested two hypotheses. First, I hypothesized that TBCA and soil respiration would be strongly associated with site-level differences in N status, which I have previously characterized using multiple indices of N status (see Smith et al. 2016). However, since both negative (e.g. Bae et al. 2015) and positive (Nadelhoffer et al. 1985) responses have been observed, this remains a major source of uncertainty in our current understanding of how resource limitation affects C allocation strategies. Alternatively, I hypothesized that TBCA and soil respiration would be negatively related to the historically high rates of atmospheric N inputs that these stands have received. Given that soil respiration is typically (but not always) suppressed in response to N deposition, this too remains an open question whether the cumulative, long-term effects of N deposition are manifested via reductions in soil C release and belowground C allocation. To test these hypotheses, I took repeated measures of soil CO_2 efflux and litterfall C to estimate annual rates of soil respiration and TBCA at each of the seven red spruce stands over a two-year interval. I then used a model selection approach to evaluate which environmental factors (i.e. N status, N deposition, climate, stand age, and broadleaf abundance) could best explain the observed differences in soil respiration and TBCA.

3.3. Methods.

Study area and site descriptions

This study was performed in seven high-elevation study sites ($n = 7$, 100-m diameter) containing red spruce in the Central Appalachian Mountains. I established these sites in June 2011 along a transect of moderate to high N deposition as part of a larger study to examine the long-term influences of enhanced N deposition on ecosystem N status and C balance. In the mid-1980s, peak estimates of wet-only N deposition ($NH_4^+ + NO_3^-$) in these high-elevation areas ranged from 2.0 to 8.1 kg N ha^{-1} y^{-1} (South to North). Today, the magnitude of this gradient is less steep, with recent estimates that range from 3.5 to 6.6 kg N ha^{-1} y^{-1} (South to North). I attribute the weakening of this gradient to recent declines (45–55%) in rainwater NO_3^- concentrations following the Clean Air Act in 1990 (in contrast, rainwater NH_4^+ has remained relatively constant or has slightly increased in some areas).

I estimated N deposition rates for each study site using a spatially-interpolated model of wet-only N deposition for the period 1985 to 2012 (National Atmospheric Deposition Program 2014). To evaluate the cumulative long-term effects of N deposition for these study sites, I iteratively calculated the sum of annual estimates for the range of data that was available. Along this spatial gradient, cumulative wet-only N inputs ranged from 117.5 to 206.9 kg N ha^{-1} between 1985 and 2012. Using a separate dataset from the National Atmospheric Deposition Program (2014) to account for the contribution of dry deposition (HNO_3 + NO_3^- + NH_4^+), I found that cumulative total (wet + dry) N deposition ranged from 326.1 to 399.6 kg N ha^{-1} for the period 1985 to 2012.

All seven stands are located above 900 m in elevation and are typical of second growth red spruce communities in the Central Appalachian Mountains, with red spruce, yellow birch, eastern hemlock, and red maple as the primary canopy-dominant species. Soil types vary in taxonomic classification across all study sites, but generally have similar soil drainage characteristics from moderately well-drained to well-drained. Along the spatial gradient, mean annual temperature (MAT) varies between 6.3 and 8.4°C, while annual precipitation is between 1238 and 1591 mm (PRISM Climate Group, Oregon State University). Complete descriptions of N deposition, community composition, climate, and soil type for these stands can be found in Smith et al. (2016).

Estimates of N status

Site-level differences in N status were estimated at each study site using multiple indices that integrate the N cycle over different time scales following the methods described in Smith et al. (2016). These indices included: 1) laboratory incubations of soil samples to capture current rates of net nitrification and N mineralization under controlled conditions; 2) ion-exchange resins to measure soil N supply rates under current field conditions; 3) stable ^{15}N isotopic composition of soils (both the organic and mineral horizons), and red spruce foliar samples, which provided a historically integrated index of soil N dynamics (Shearer et al. 1974; Garten and Van Miegroet 1994; Nadelhoffer and Fry 1994); and 4) soil C:N ratio for the organic and mineral horizons, which provided an long-term index of N availability and soil organic matter quality.

Measurements of soil respiration and temperature

Preliminary soil respiration measurements began in March and April 2011 for four of the study sites (McGowan Mountain, Gaudineer Knob, Cranberry Glades, and Little Spruce Bog). Three additional stands (Flat Ridge, Upper Middle Mountain, and Span Oak Run) were established in June 2011 at which point measurements were conducted across the entire spatial gradient. Prior to initial measurements, 16 polyvinyl chloride (PVC) collars (4 cm height, 10 cm inside diameter) were permanently installed at random locations within each study site. After clearing aboveground litter and debris from the soil surface, I inserted each collar approximately 2-3 cm into the soil profile using care to limit subsurface root damage. Collars were installed at least two weeks prior to measurements of soil respiration to prevent abnormally high CO_2 fluxes as a result of soil disturbance.

Soil respiration was measured using a closed chamber, soil CO_2 flux system attached to a portable infrared gas analyzer (LI-8100 with LI-8100-102 chamber, Li-Cor Inc., Lincoln, NE, USA). Measurements of soil respiration were made weekly across the entire gradient from June through the end of July, and monthly from September through November. Despite increasing evidence that winter soil respiration should be accounted for in subalpine forests (Mast et al. 1998; McDowell et al. 2000), a persistent snowpack and limited access to field sites throughout the winter and early spring prevented consistent sampling during those months, although two measurement campaigns were conducted during March and April 2011.

On a given sampling campaign, soil respiration was measured once at each of the 16 collars in each study site. After connecting the LI-8100-102 chamber to a PVC collar, a single measurement cycle was conducted over a 90 s period during which the chamber opened and closed automatically. Rates of soil CO_2 efflux were calculated as the exponential increase in chamber CO_2 concentration over the measurement interval.

During each respiration measurement I recorded soil temperatures (5 cm depth) directly adjacent to each collar using a digital stainless-steel thermometer. In addition, hourly air and soil (5 cm depth) temperatures were measured continuously at each site using dataloggers (three dataloggers per study site, two soil, one air; HOBO Pendant Temperature Data Logger, Onset

Computer Corporation, Bourne, MA, USA) between January 2011 and December 2012. Occasional gaps in this dataset were filled by building multiple linear regression models to predict hourly temperature for any particular site. The general form of this model is given as:

$$T_{\mathrm{a,s}} = a + b_1 T_1 + b_2 T_2 + \cdots + b_p T_p \qquad (3)$$

where $T_{\mathrm{a,s}}$ is the missing hourly temperature value (air or soil, respectively) for a given site, a is the intercept, b is the regression coefficient, and T is the observed hourly temperature (air or soil) for an adjacent study site (up to six additional sites). Stepwise regression was used to determine which sites should be included in the model to best predict the missing temperature term. I found that when temperature data was unavailable, this method produced a more unbiased estimate of hourly temperature compared to other gap-filling methods like simple linear interpolation between points.

I used my continuous air temperature data from each site to estimate cumulative growing degree days, calculated as the total number of days throughout the year (2011 and 2012) where daily mean air temperature was > 5°C (Figure 3.1a). Similarly, I estimated cumulative precipitation as the sum of total daily rainfall measurements for both 2011 and 2012 (Figure 3.1b). Since precipitation data were unavailable for my study sites, I relied on an open-access spatial dataset to estimate daily and monthly precipitation for each site (Livneh et al. 2015) (Figure 3.4c).

Litterfall C

I collected total aboveground litterfall (leaves plus woody tissue < 1 cm in diameter) in eight litter baskets (0.42 m^2 ground area) at each site. Litter samples were periodically collected in June, September, and October, beginning in June 2011 and concluding in June 2013. On each sample date I returned litter samples to the laboratory where they were hand-sorted into leaf and wood litter (including twigs, branches, bark, and reproductive tissue) for red spruce, other needleleaf evergreen species (e.g. hemlock and pine), and all broadleaf deciduous tree species (e.g. yellow birch and red maple). Sorted samples were then dried at 65°C for 48 hrs and weighed to estimate dry biomass of each litter and plant type.

Samples from each category (leaves and woody tissue for red spruce, other conifers, and all broadleaf deciduous trees) were pooled for a particular site and year. Pooled samples were ground to pass a #40 mesh using a Wiley Mill and a small subsample of each was analyzed for total C content using a NC 2500 elemental analyzer (Carlo Erba, Milan, Italy). I found that regardless of litter type and tree species, the C content of all samples was approximately 50%. This value was applied to all litterfall biomass estimates to calculate annual litterfall C (g C m^{-2} y^{-1}) for each site.

Data analysis

For each study site, I used hourly soil temperature data to estimate annual soil respiration based on the relationship between observed rates of soil CO_2 efflux and discrete soil temperature measurements. Though the temperature response of soil respiration is often modeled using an exponential Q_{10} function (Raich and Schlesinger 1992; Raich and Potter 1995), strong evidence has shown that the temperature sensitivity of respiration is higher at low temperatures (Kirschbaum 1995; Lloyd and Taylor 1994). For this reason, I chose the Lloyd and Taylor (1994) model to estimate annual soil respiration since it allows the temperature response function to vary with changes in measurement temperature. Similar to an Arrhenius function, the Lloyd and Taylor (1994) model is given as:

$$R_s = Ae^{-E_0/(T-T_0)} \tag{4}$$

where R_s is the rate of soil respiration (mg C m^{-2} hr^{-1}) at temperature T (K), E_0 is a fitted parameter similar to a temperature-dependent activation energy, and T_0 (K) is a fitted temperature parameter between 0 K and T. A is a site-specific model parameter calculated as the mean of observed soil respiration rates (mg C m^{-2} hr^{-1}). Similar to methods described in Savage and Davidson (2001), I multiplied the A value by 1000 in order to parameterize E_0 to a value within the range of expected activation energies. For each site, I used nonlinear least squares regression to estimate model parameters using the Gauss-Newton algorithm with SAS-JMP Pro 10.0 software (SAS Institute Inc., Cary, NC). Using this relationship, I calculated hourly estimates of total soil CO_2 efflux and integrated these values for the period from 1 January 2011 to 31 December 2012 to determine annual rates of soil respiration for each site.

During preliminary data analysis I anticipated that site-specific temperature responses of soil respiration would vary between 2011 and 2012. To test whether the temperature response of respiration depended on sample year (and study site), I used a general linear model with two fixed nominal variables (sample year and study site) and one continuous variable (soil temperature), and included all interactions. To meet the assumptions of normality for this model, all soil respiration data were transformed using the Box-Cox power transformation. To examine how the temperature response of soil respiration varied by year (and site), I estimated the temperature response function Q_{10} as:

$$Q_{10} = \left(\frac{R_S}{R_0}\right)^{10/(T-T_0)} \tag{5}$$

where R_s is the rate of soil respiration (mg C m^{-2} hr^{-1}) at temperature T (°C), R_0 is the rate of soil respiration (mg C m^{-2} hr^{-1}) at temperature T_0 (°C), and Q_{10} is a unitless scalar that describes the factor by which soil respiration is increased over the range from T_0 to T. However, since the Lloyd and Taylor (1994) temperature response function (see *Equation 4*) allows Q_{10} to vary with respect to measurement temperature, I calculated Q_{10} values between 10-15°C, the interval at which ~60% of my soil temperature measurements were taken.

Although my field measurements of litterfall and soil respiration occurred simultaneously during the summer and fall of both 2011 and 2012, my modeled estimates of annual soil respiration (January 2011 through December 2012) were not aligned with the timing of litterfall sampling (June 2011 through May 2013). To account for this discrepancy, I first assumed that the largest differences in litterfall rates between years would occur during the growing season and early fall (June through October); whereas, I assumed that litterfall rates during the winter and spring (November through May) would be similar between years. Operating under these assumptions, I calculated daily "winter" litterfall for both 2011 and 2012 as the total amount of litterfall that occurred between November and May divided by the number of days in this time interval. These estimates were used to calculate the average daily "winter" litterfall rates between 2011 and 2012. Next, I applied this metric to back-calculate total litterfall that occurred prior to sampling in 2011 (January through May) and to account for litterfall that occurred from November through

December 2012. These calculations allowed me to express annual soil respiration and litterfall over the same time frame for both study years.

I used a conservation of mass approach to estimate total belowground carbon allocation (TBCA; g C m^{-2} y^{-1}) at each of my study sites. Following Raich and Nadelhoffer (1989), I calculated TBCA as the difference between annual estimates of soil respiration and litterfall C (see *Equation 2*).

To assess potential controls of temperature response functions, annual soil respiration, litterfall C, and TBCA, I performed regression analyses relating estimates of each response variable to abiotic and biotic environmental factors for each site. The environmental factors considered included site-specific N deposition estimates (cumulative wet and total deposition), indices of N status (rates of net nitrification, net N mineralization, ion-resin N supply rates, foliar and soil δ^{15}N, and soil C:N), stand age, the relative importance of canopy broadleaf deciduous trees (hereafter referred to as BD RIV), mean temperature (annual and growing season, May through September), and precipitation (annual and growing season). I then compared the likelihood of competing regression models using corrected Akaike's Information Criterion (AIC_c). In comparing two or more models, those with the lowest AIC_c value were considered to be better predictors of changes in soil respiration, litterfall, and TBCA. Units were expressed as ΔAIC_c, which indicates the difference between the model with the strongest statistical support and competing models. Thus, the most parsimonious model will have ΔAIC_c equal to 0, while weaker models will have ΔAIC_c greater than 0. All statistical analyses were performed with SAS-JMP Pro 10.0 software (SAS Institute Inc., Cary, NC).

3.4. Results.

Seasonal variation of soil respiration

Throughout the two-year study period, soil respiration for all sites varied as a function of changes in soil temperature (Figure 3.2). The range of measured soil respiration values was between 0.17 and 13.53 μmol m^{-2} s^{-1} while discrete soil temperature measurements were between 0.9 and 19.3°C. Use of the Lloyd and Taylor (1994) function produced good fits (R^2 = 0.49-

0.71) for models that included all mean observed soil respiration rates for 2011 and 2012 (Table 3.1). However, in comparing observations for 2011 and 2012, I found strong differences in respiration rates despite minor differences in soil temperature (Table 3.2). Across all sites, June soil respiration was 31-48% greater in 2012 compared to 2011; whereas, soil temperature was 0.76-0.01% lower. Likewise, in July I observed respiration rates that were 26-103% greater in 2012 than 2011, while the difference in soil temperature between years was between -0.13 and 0.61%. Since the large differences in observed respiration rates between study years were likely not due to changes in soil temperature alone, I modeled the temperature response of respiration for 2011 and 2012 separately. In order to ensure that both models operated similarly at low temperatures for both years, I included some 2011 observations ($n \leq 3$) when low temperature data was not available for the 2012 year. Separating the overall fit into two independent models greatly improved model fits for both years (R^2 = 0.72-0.96).

Given the higher rates of soil respiration observed in 2012, I found that the temperature sensitivity of soil respiration was also significantly higher in 2012 ($p = 0.04$). In addition, I found that the between-year variation in the response of soil respiration to changes in soil temperature was different among sites ($p = 0.04$), with some sites (e.g. McGowan Mountain and Cranberry Glades) exhibiting markedly higher temperature sensitivities between 2011 and 2012.

I found only minor site-level differences in estimated Q_{10} values for my 2011 data, with estimates ranging between 2.51 and 3.02 (Table 3.3). In contrast, my 2012 data revealed much stronger site-level variation with values that ranged between 2.33 and 4.67. I also observed that the greatest percent change in Q_{10} between 2011 and 2012 occurred at sites that were previously characterized as having greater N availability as well as a higher abundance of broadleaf associates (e.g. Cranberry Glades, 72% increase; McGowan Mountain, 68% increase). In comparison, sites that were dominated primarily by needleleaf evergreen species exhibited only minor interannual differences in their temperature response functions (e.g. Flat Ridge, 22% decrease; Gaudineer Knob, 9% increase). I conducted simple bivariate regressions to examine the environmental factors (e.g. N status, N deposition, climate, stand age, and broadleaf abundance) that best predicted the interannual variation in temperature response functions across study sites. Due to the lack of site-level differences in Q_{10} for 2011, I found no predictor variables that could significantly explain the variation in Q_{10} (Table 3.4). For my 2012 data, however, I found that Q_{10} of soil respiration was best predicted by site-level differences in stand age ($p = 0.01$, $R^2 = 0.76$) (Figure 3.3a), soil C:N in the organic ($p = 0.01$, $R^2 = 0.74$) (Figure 3.3c) and mineral horizons ($p = 0.02$, $R^2 = 0.70$), and mean temperature (annual, $p = 0.03$, $R^2 = 0.64$; growing season, $p = 0.02$, $R^2 = 0.72$). I also found a weak positive trend relating Q_{10} values with differences in BD RIV for 2012 ($p = 0.06$, $R^2 = 0.53$) but not 2011 ($p = 0.44$, $R^2 = 0.12$) (Figure 3.3b).

Model estimates compared to observed

For each site, hourly estimates of soil respiration were calculated by applying continuous measurements of soil temperature to *Equation* 4. I compared the seasonal variation in simulated daytime (0700-1900 h) soil respiration to my observed values of mean soil respiration measured in the field and found that across all sites, my modeled estimates strongly predicted my observed data for both years ($R^2 = 0.89$ for 2011 and $R^2 = 0.86$ for 2012) (Figure 3.4a). Likewise, I found strong agreement between my continuous measurements of mean daytime soil temperature and discrete soil temperature measurements ($R^2 = 0.96$ for 2011 and $R^2 = 0.93$ for 2012) (Figure 3.4b).

Annual estimates of soil respiration, litterfall C, and belowground C allocation

Along the study gradient, modeled estimates of annual soil respiration varied between 577-863 g C m^{-2} y^{-1} for 2011 and between 823-1384 g C m^{-2} y^{-1} for 2012 (Table 3.3). Total soil respiration during the growing season (May 15 through September 15) accounted for the majority of annual soil CO_2 efflux for both years (~55-57% in 2011 and ~51-64% in 2012) (Figure 3.4a). The between-year variation in my estimates of litterfall C was not as pronounced as soil respiration, with litterfall rates ranging from 65-118 g C m^{-2} y^{-1} in 2011 and 87-117 g C m^{-2} y^{-1} in 2012 (Table 3.3). I did find that the site-level variation in litterfall estimates was a good predictor of annual soil respiration rates for 2011 ($p = 0.057$) (Figure 3.5) but not 2012 ($p = 0.15$). Because of this positive relationship, my mass balance estimates of TBCA primarily reflected the spatial variation in soil respiration, with estimates that ranged from 511-745 g C m^{-2} y^{-1} in 2011 and 734-1267 g C m^{-2} y^{-1} in 2012 (Table 3.3).

Neither cumulative wet deposition nor total deposition could adequately explain the variation in either annual soil respiration or TBCA for my study sites ($p > 0.76$) (Table 3.4; Figure 3.6b). Instead, soil respiration and TBCA for both 2011 and 2012 were strongly associated with both N status and BD RIV at these sites (Table 3.4). For my 2011 estimates, annual soil respiration and TBCA were significantly related to the $\delta^{15}N$ content of organic soil ($p < 0.004$) (Figure 3.6a). I also observed slight trends relating 2011 soil respiration and TBCA to organic soil C:N ($p < 0.06$) (Figure 3.6c) and mineral soil $\delta^{15}N$ ($p < 0.10$). Likewise, in 2012, I found that annual soil respiration and TBCA were strongly associated with differences in the $\delta^{15}N$ content of organic ($p < 0.0002$) (Figure 3.6a) and mineral soil ($p < 0.04$), and of red spruce leaves ($p < 0.03$). My 2012 estimates of soil respiration and TBCA were also related to organic ($p < 0.05$) (Figure 3.6c) and mineral soil C:N ($p < 0.06$). Despite the positive trend between soil respiration and litterfall (Figure 3.5), I did not observe any significant relationships between litterfall and any of my environmental predictor variables. For both 2011 and 2012, annual soil respiration and TBCA were positively associated with differences in canopy BD RIV (Table 3.4), with BD RIV explaining ~81% of the variability in annual soil respiration and TBCA for 2011 and 2012 ($p < 0.006$, respectively) (Figure 3.6d).

The results from my model selection approach suggest that for 2011, differences in soil respiration among sites were best explained by organic soil $\delta^{15}N$ content ($\Delta AIC_c = 0$) (Table 3.4) and the relative importance of BD species ($\Delta AIC_c = 1.0$). For 2012, the single best predictor of changes in annual soil respiration was organic soil $\delta^{15}N$ ($\Delta AIC_c = 0$), although I did observe significant relationships between soil respiration and the relative importance of BD species ($\Delta AIC_c = 9.3$), foliar and mineral soil $\delta^{15}N$ content ($\Delta AIC_c = 13.6$ and 14.7, respectively), and organic soil C:N ($\Delta AIC_c = 14.9$). Of all of the models included in this approach, however, N deposition was the least likely to explain differences in respiration for both years ($\Delta AIC_c = 12.7$-20.9).

My model selection results for TBCA were nearly identical to those for soil respiration (Table 3.4), with organic soil $\delta^{15}N$ explaining 86% of the variation in TBCA for 2011 ($\Delta AIC_c = 0$), and 96% of the variation in TBCA for 2012 ($\Delta AIC_c = 0$).

3.5. Discussion.

In this study, I found that soil respiration was strongly associated with seasonal changes in soil temperature, but also varied with respect to study year and study site. In comparing the variation of soil respiration between study years, I found that respiration rates were significantly higher in 2012 compared to 2011 (Table 3.2), but the magnitude of this interannual variation was dependent on site. Likewise, some sites exhibited markedly different temperature responses of soil respiration between study years, and these differences seem to be related to site-level variation in stand history, tree species composition, and soil organic matter quality (Figure 3.3). In addition, my use of a C mass balance approach to estimate annual belowground C allocation revealed similar results as were observed with soil respiration, where the spatial variation in TBCA was positively related to tree species effects on the N status of these sites (Table 3.4; Figure 3.4). These results partially supported the hypothesis that spatial patterns of belowground C balance would be correlated with differences in N status; however, my study was not in agreement with the generally observed trend that soil C release and belowground allocation typically decline as soil fertility increases (Gower et al. 1994; Litton et al. 2007; Phillips and Fahey 2007; Janssen et al. 2010; Bae et al. 2015). Finally, I did not find any supporting evidence to suggest that the high rates of N deposition that these sites have historically received had any impact on current patterns of soil respiration and TBCA.

Seasonal and interannual variability of soil respiration

I examined a number of factors that could explain the seasonal and interannual variation of soil respiration. For both years, soil temperature was a strong predictor of the seasonal variability of soil respiration (Figure 3.2); however, I found that the higher rates of respiration in 2012 could not be explained by differences in soil temperature alone (Table 3.2; Figure 3.4). Given that absolute rates of soil respiration are not only related to seasonal changes in temperature but also soil moisture (Davidson et al. 1998; Gaumont-Guay et al. 2006), photosynthesis (Högberg et al. 2001), and litter quality (Taylor et al. 1989), I hypothesized that the higher rates of respiration in 2012 may have been driven by indirect effects of early growing season climate and site-level differences in tree species composition and soil organic matter quality. Prior to initial sample measurements during the 2012 field season (DOY 157), my sites experienced 8-21 more growing-degree days (sum of days with mean air temperature > 5°C starting on January 1 of a given year) in 2012 compared to the same date in 2011 (Figure 3.1a). This increased period of warming is important for forest C dynamics since the number of cumulative growing-degree days can alter plant phenology and stimulate plant productivity (Cleland et al. 2007; Piao et al. 2007). Thus, the interannual differences in the early growing season temperatures I observed may have facilitated earlier leafout in 2012, potentially resulting in increased canopy photosynthesis, which, in turn, could provide more photosynthetically assimilated C to be allocated to root and rhizosphere respiration (Högberg et al. 2001; Savage and Davidson 2001). In addition, I would expect this phenological "priming" effect on soil respiration to be more pronounced in mixed forests that contain a greater abundance of high-productivity tree species (Raich and Tufekciogul 2000; Yuste et al. 2004; Khomik et al. 2006). My results tend to support this idea, where sites with greater broadleaf abundance (e.g. McGowan Mountain and Cranberry Glades) exhibited much greater interannual variability in soil respiration than sites that were dominated by needleleaf evergreen species (e.g. Flat Ridge and Gaudineer Knob) (Figure 3.2). Since the allocation of recent photosynthate to root respiration has been shown to account for ~52-56% of total soil respiration (Högberg et al. 2001), I suggest that species-specific differences in C uptake and belowground C allocation are likely responsible for much of the spatial and temporal heterogeneity in observed respiration rates that could not be ascribed to interannual temperature variation alone.

Differences in tree species composition (and in particular, different leaf strategies among species) can influence the apparent temperature sensitivity of soil respiration (Q_{10}) as well. In a study that compared soil respiration rates under different tree species in a mixed temperate forest, Yuste et al. (2004) showed that seasonal Q_{10} values were significantly higher under deciduous canopies compared with evergreen canopies. These results suggest that under similar climate regimes, broadleaf deciduous trees allocate comparatively more photosynthate to root activity (growth and respiration) than their needleleaf evergreen counterparts (Steele et al. 1997); thus, species-level differences in phenology and root C allocation patterns are expected to modulate the temperature response of soil respiration, resulting in a positive relationship between Q_{10} values and the deciduousness of a particular forest. My results generally agree with this finding, where estimated Q_{10} values were positively associated with differences in broadleaf abundance for 2012, but not 2011 (Table 3.4; Figure 3.3).

The interannual variability of soil respiration at my sites may also have been related to differences in precipitation between 2011 and 2012. At the start of the growing season in 2012 (April-June), precipitation patterns were characteristic of a waning La Niña system that brought variable drought conditions across most of the United States including the Central Appalachians (NOAA 2012). As a result, cumulative precipitation prior to June sampling was 38-185 mm lower (137 mm average) in 2012 compared to 2011 (Figures 3.1b and 3.4c). This evidence (in combination with the interannual differences in cumulative degree days) suggests that these study sites likely experienced a relatively cool, moist spring in 2011 and a warm, dry spring in 2012 (Figures 3.1 and 3.4), which could alter both the timing and magnitude of peak respiration rates during the growing season (Savage and Davidson 2001; Scott-Denton et al. 2003). For example, in a comparison of interannual soil respiration rates between two New England forests, Savage and Davidson (2001) found that when springtime soil conditions were warm and moist, soil respiration increased earlier in the spring but also peaked earlier in the summer. However, when spring conditions were warm and dry, increases in soil respiration were delayed until ~mid-June. Although warmer temperatures usually result in elevated rates of total soil respiration (owing to Michaelis-Menten enzyme kinetics), the temperature stimulation of soil respiration can be depressed under conditions of low soil moisture, obscuring the apparent temperature sensitivity of respiration (Savage and Davidson 2001; Scott-Denton et al. 2003). Thus, while the warmer temperatures of 2012 may have initiated earlier leafout and greater productivity, the confounding effects of low soil moisture during the early spring may have suppressed peak soil respiration rates until mid- to late-June.

Finally, the 26-103% higher rates of soil respiration in July 2012 (Table 3.2) may have been related to a destructive derecho (straight-line wind storm) that tracked across the Midwestern United States and through most of West Virginia on June 29, 2012 (DOY 181). Maximum wind speeds for this event were as high as 87 mph, which resulted in multiple tip-ups and canopy damage at most of my study sites. Studies on the effects of wind-throw on forest C balance show that soil disturbance and increased decay of canopy deadwood can enhance ecosystem respiration for several weeks or months after disturbance (Knohl et al. 2002; Lindroth et al. 2009). Thus, I hypothesize that the variation in growing-degree days and cumulative precipitation during the early growing season, coupled with severe weather in late June could partially explain the higher observed respiration rates throughout summer 2012 (Table 3.2).

Spatial controls on belowground C cycling

My analysis of the components of the TBCA budget revealed similar results as studies that have also used a C mass balance approach to estimate belowground C allocation (e.g. Raich and Nadelhoffer 1989; Davidson et al. 2002). These studies show that annual soil respiration tends to be positively related to litterfall C inputs across a broad range of forest types, with estimates of TBCA that are about 4x higher than litterfall on average. I examined whether my annual estimates of soil respiration and litterfall agreed with this trend by plotting my values against a similar dataset from Davidson et al. (2002) (Figure 3.5). For my 2011 data, annual litterfall was a strong predictor for the spatial variation in soil respiration ($p = 0.057$) with a slope (slope = 3.4) that was similar to what Davidson et al. (2002) reported for forests worldwide (slope = 3.6). In contrast, I did not find a significant relationship between soil respiration and litterfall for my 2012 data ($p = 0.15$). In general, my results tended to be more biased toward higher rates of soil respiration, although this was more apparent in 2012 than 2011.

My model comparison approach revealed that annual estimates of soil respiration and TBCA were most strongly associated with differences in tree species composition and N status, but were unrelated to N deposition (Table 3.4). This result was not entirely surprising since it is well established that rates of decomposition and soil CO_2 efflux are positively correlated with the higher litter quality of many broadleaf species including common red spruce associates like maple and birch (Melillo et al. 1982; Taylor et al. 1989; Raich and Tufekciogul, 2000). In addition, previous work in these seven red spruce stands showed that these differences in tree species composition led to distinct profiles of N status at each site (Smith et al. 2016); thus, I would expect that these patterns of belowground C balance would be related to N availability as well as broadleaf importance. Indeed, I found a strong negative correlation between the C:N ratio of organic soil and my annual estimates of soil respiration and TBCA ($R^2 = 0.53$-0.60); likewise, soil respiration and TBCA were positively correlated with organic soil $\delta^{15}N$ content (R^2

= 0.84-0.96). Since soil C:N is primarily controlled by the quantity and quality of aboveground litter inputs, it is likely that both decomposition and total soil respiration would be suppressed in soils enriched in organic matter that is highly recalcitrant to microbial decay (i.e high C:N). Moreover, changes in the $\delta^{15}N$ composition of foliar and soil pools have been linked to the activity of soil microbes and are well-correlated with rates of N transformations (e.g. nitrification and mineralization) in soil (Mariotti et al. 1981; Pardo et al. 2006; Kahmen et al. 2008). Given that absolute rates of soil CO_2 efflux are due in part to the activity of soil heterotrophs, total soil respiration may reflect rates of microbial decomposition as well as the availability of inorganic N.

On the other hand, total soil respiration also includes the respiratory fluxes of autotrophs, so I might expect rates of soil CO_2 efflux to decrease with greater N supply. For example, when N is limiting, plants allocate more C to nutrient acquisition pathways (i.e. root production, rhizosphere exudation, mycorrhizal symbiosis) at the expense of higher respiratory costs (Haynes and Gower 1995). My results were in contrast with this conceptual model, with TBCA increasing with respect to site-level differences in N status (Table 3.4; Figure 3.6). Although these results were unexpected, other studies have also shown variable responses in the relationship between belowground allocation and N status (Raich and Nadelhoffer 1989; Ryan et al. 1996; Raich 1998; Giardina et al. 2003). For example, Raich (1998) examined C allocation patterns in three Hawaiian rainforests that differed in soil fertility and productivity. The results of that study showed that TBCA increased with both N mineralization and ANPP; however, the fraction of ANPP that was allocated belowground decreased with N status and productivity. In contrast, Ryan et al. (1996) showed that experimental N additions to *Pinus radiata* stands reduced both TBCA as well as the fraction of GPP that was allocated belowground (i.e. TBCA/GPP ratio). A similar N fertilization study in *Eucalyptus saligna* stands showed that TBCA was not significantly different between fertilized and control plots, but the TBCA/GPP ratio did decline under high N (Giardina et al. 2003). The general consensus from these studies is that while absolute rates of TBCA may increase, decrease, or remain unchanged in relation to N status, the proportion of GPP that is allocated belowground typically declines when N availability is high. Thus, while my study showed a positive relationship between TBCA and N status, I cannot presently examine the relationship between soil fertility and the relative C contribution of GPP to TBCA, since my study did not account for aboveground production at my sites.

While my study did show that soil respiration and TBCA were related to differences in species composition and N status at my sites, the hypothesis that belowground C balance would be associated with historically high rates of atmospheric N deposition was not supported. This may be due to several reasons: 1) the estimates of N deposition for my sites were inaccurate; 2) the estimates of deposition are accurate, but the gradient was not broad enough to detect a response; or 3) the magnitude and range of my deposition estimates were sufficiently large, but broadleaf controls on litter C inputs and decomposition overwhelmed any effects of deposition. Although I did not measure rates of N deposition at my sites, my estimates were derived from a well-validated dataset of long-term deposition monitoring for this region (National Atmospheric Deposition Program 2014). A second concern is whether my selection of study sites represent a broad enough range of N deposition rates (4.2 to 7.4 kg N ha^{-1} y^{-1}) to detect a significant response. While the range of estimated N deposition for my sites is more narrow than similar studies in New England (Aber et al. 2003; Boggs et al. 2007; McNeil et al. 2008) and in the Southern Appalachians (Boggs et al. 2005), there remains some uncertainty as to whether the legacy effects of pollution can contribute to current rates of C flux. As such, I feel that the two-fold difference in cumulative wet deposition inputs from 1985 to 2012 (117.5 to 206.9 kg N ha^{-1}) is sufficiently broad enough to still influence soil C cycling. Thus, it appears to me that any potential influence of N deposition on annual soil respiration and TBCA for my sites is minimal compared to tree species effects on litter quality, rates of decomposition, and N availability.

Given the importance of tree species as a potential control on N status and belowground C cycling, the results of this study has broad implications for my understanding of climate change and terrestrial C storage. For example, if broadleaf deciduous species that produce labile litter replace needleleaf evergreen forests as temperatures increase, then it seems likely that soil respiration will become more sensitive to climatic stressors like drought and severe storms, both of which are predicted to increase in frequency and severity under anthropogenic global warming (IPCC 2014). Furthermore, it is predicted that even under the most conservative climate change scenarios, red spruce forests in the Central Appalachians are likely to be replaced by low-elevation broadleaf deciduous communities migrating to higher elevations (Byers et al. 2010). As a result, I expect a strong decline in the strength of soil C sinks as the broadleaf ecotone shifts upward, owing to higher rates of soil CO_2 efflux coupled with potential declines in TBCA relative to GPP.

In conclusion I showed that site-level differences in tree species composition (and their associated influences on soil organic matter quality and N status) exerted a strong spatial and temporal control on soil respiration and TBCA in high-elevation red spruce forests; however, I found no evidence that historically high rates of N deposition were influencing current patterns of belowground C cycling. While my results do not support the idea that belowground C allocation decreases as a function of soil fertility, I nevertheless showed that soil C dynamics are positively associated with differences in N status, suggesting that the variation in leaf habit in mixed forests can affect both nutrient availability as well as soil C loss, with possible large implications for future C storage.

3.6. Tables and Figures.

Table 3.1. Model parameter estimates of Lloyd and Taylor (1994) function (*Equation* 4) applied to empirical observations of soil temperature (5 cm) and soil respiration. Models were generated for the entire dataset (“All Pts”), and for 2011 and 2012 observations separately. Note: For the 2012 model, I included some 2011 observations (campaigns shown in parentheses) when low temperature data was not available for 2012.

Site	Model	Data Range Included in Model	A, g C m^{-2} hr^{-1}	E_0, K	T_0, K	n	R^2
FLR	2011	060811 to 111211	114.4	436.3	222.4	9	0.77
	2012	060612 to 101312 (111211)	144.3	553.0	204.8	8	0.72
	All Pts	060811 to 101312	134.0	579.5	201.7	16	0.49
GKB	2011	043011 to 111311	86.9	504.4	211.6	10	0.89
	2012	060712 to 101312 (043011, 111311)	106.6	447.7	218.9	8	0.88
	All Pts	043011 to 101312	102.6	536.4	207.1	16	0.64
MCG	2011	043011 to 111311	127.7	477.0	216.7	10	0.95
	2012	060712 to 101312 (043011, 111311)	185.9	312.3	240.3	9	0.94
	All Pts	043011 to 101312	166.5	347.9	235.8	17	0.60
CGL	2011	032411 to 111211	99.7	451.6	218.4	11	0.86
	2012	060612 to 101312 (032411, 050111, 111211)	137.9	278.2	243.1	10	0.95
	All Pts	032411 to 101312	129.3	368.7	230.9	18	0.58
UMM	2011	060811 to 111311	108.2	500.8	213.8	9	0.85
	2012	060712 to 101312 (111311)	136.6	390.7	228.5	7	0.95
	All Pts	060811 to 101312	126.0	477.7	217.1	15	0.58
SOR	2011	060811 to 111311	132.2	491.2	215.2	9	0.77
	2012	060712 to 101312 (111311)	179.7	371.5	231.5	7	0.91
	All Pts	060811 to 101312	159.6	448.4	221.3	15	0.50
LSB	2011	032411 to 111211	108.3	436.0	222.8	11	0.91
	2012	061312 to 101312 (032411, 050111, 111211)	137.7	311.0	240.5	9	0.96
	All Pts	032411 to 101312	133.6	393.5	229.4	17	0.71

Table 3.2. Monthly comparisons of discrete field measurements (i.e. soil respiration and soil temperature), continuously logged temperature data, and estimated total precipitation between the summer months of 2011 and 2012 (calculated as the average of $n = 7$ sites). Monthly means were compared by year using one-way ANOVA; asterisks denote values that were significantly different in 2012. Note: No field campaigns were conducted during August 2011.

		Observed Data		Continuous Data		Estimated
Month	Year	Soil Respiration (mg C m^{-2} hr^{-1})	Soil Temp (°C)	Air Temp (°C)	Soil Temp (°C)	Total Precip (mm)
June	2011	116.4	13.3	16.9	13.4	103.6
	2012	159.6*	12.0*	15.5*	13.2	73.9*
July	2011	146.7	15.1	19.3	15.7	114.7
	2012	238.7*	15.5*	19.3	16.2	168.6
Aug	2011	-	-	17.6	15.6	98.9
	2012	268.7	14.3	16.8*	15.5	84.6
Sept	2011	138.6	12.7	14.0	13.9	151.4
	2012	185.1	13.1	13.5	13.9	151.0

Note: Precipitation data estimated from Livneh et al. *(2015).*

Table 3.3. Estimates of annual soil respiration, the temperature response function (Q_{10}) of soil respiration over the soil temperature interval 10-15°C, annual litterfall C, and total belowground C allocation (TBCA) for 2011 and 2012.

	Soil respiration ($g\ C\ m^{-2}\ y^{-1}$)		Q_{10} (10-15°C)		Litterfall C ($g\ C\ m^{-2}\ y^{-1}$)		TBCA ($g\ C\ m^{-2}\ y^{-1}$)	
Site	2011	2012	2011	2012	2011	2012	2011	2012
FLR	702.6	1060.7	2.98	2.33	80.0	96.6	622.7	964.2
GKB	576.8	823.5	2.51	2.73	65.4	89.6	511.4	733.9
MCG	855.5	1336.2	2.73	4.58	118.3	114.7	737.2	1221.5
CGL	761.3	1246.6	2.72	4.67	70.5	86.8	690.8	1159.7
UMM	674.4	1032.8	2.64	3.31	103.8	104.1	570.6	928.6
SOR	862.5	1384.0	2.69	3.56	117.4	117.2	745.1	1266.9
LSB	746.5	1057.6	3.02	4.61	99.6	106.4	646.9	951.1

Table 3.4. Coefficients of determination (R^2) for linear regressions of soil respiration, Q_{10} (over the soil temperature interval 10-15°C), and total belowground C allocation (TBCA) versus site-level predictor variables. ΔAIC_c for each column of data are shown in parentheses, with $\Delta AIC_c = 0$ for the most parsimonious model. Values shown in bold are significant ($p < 0.05$).

	Soil respiration ($g\ C\ m^{-2}\ y^{-1}$)		Q_{10} (10-15°C)		TBCA ($g\ C\ m^{-2}\ y^{-1}$)	
Model	2011	2012	2011	2012	2011	2012
N status indices						
Net nitrification	0.07 (12.3)	0.16 (19.6)	0.02 (2.6)	0.34 (7.0)	0.15 (12.5)	0.20 (20.4)
Net N mineralization	0.23 (10.9)	0.36 (17.7)	0.02 (2.5)	0.53 (4.7)	0.33 (10.8)	0.40 (18.3)
Resin – Total N	0.25 (10.8)	0.33 (18.1)	0.01 (2.7)	0.44 (6.0)	0.32 (10.9)	0.35 (18.9)
Resin – NH_4^+	0.25 (10.8)	0.33 (18.0)	0.03 (2.5)	0.45 (5.8)	0.33 (10.8)	0.36 (18.8)
Resin – NO_3^-	0.24 (10.9)	0.31 (18.2)	0.01 (2.7)	0.41 (6.3)	0.30 (11.1)	0.34 (19.1)
Foliar $\delta^{15}N$	0.43 (8.8)	**0.64 (13.6)**	0.08 (2.2)	0.33 (7.2)	0.52 (8.5)	**0.68 (14.0)**
Organic soil $\delta^{15}N$	**0.84 (0)**	**0.95 (0)**	0.01 (2.7)	0.40 (6.4)	**0.86 (0)**	**0.96 (0)**
Mineral soil $\delta^{15}N$	0.45 (8.6)	**0.59 (14.7)**	0.01 (2.6)	**0.59 (3.7)**	0.48 (9.0)	**0.61 (15.4)**
Organic soil C:N	0.53 (7.5)	**0.58 (14.9)**	0.01 (2.7)	**0.74 (0.5)**	**0.60 (7.2)**	**0.60 (15.6)**
Mineral soil C:N	0.43 (8.8)	0.54 (15.4)	0.01 (2.6)	**0.70 (1.5)**	0.48 (9.1)	0.56 (16.1)
Environmental factors						
Cumulative N Deposition (wet only)	0.02 (12.7)	0.01 (20.9)	0.14 (1.6)	0.30 (7.4)	0.01 (13.6)	0.01 (21.9)
Cumulative N Deposition (wet + dry)	0.01 (12.8)	0.01 (20.8)	0.03 (2.5)	0.32 (7.2)	0.01 (13.6)	0.01 (21.8)
Mean Annual Temperature	0.51 (7.8)	0.32 (18.2)	0.32 (0.1)	**0.64 (2.8)**	0.45 (9.4)	0.30 (19.5)
Mean Growing Season Temperature	0.54 (7.3)	0.36 (17.7)	0.32 (0)	**0.72 (1.2)**	0.51 (8.7)	0.35 (19.0)
Annual Precipitation	0.04 (12.5)	0.01 (20.9)	0.04 (2.4)	0.01 (10)	0.01 (13.6)	0.01 (21.9)
Growing Season Precipitation	0.07 (12.3)	0.01 (20.8)	0.14 (1.7)	0.01 (9.9)	0.03 (13.4)	0.01 (21.9)
Stand Age	0.38 (9.5)	0.32 (18.2)	0.01 (2.6)	**0.76 (0)**	0.45 (9.4)	0.33 (19.2)
BD Relative Importance	**0.81 (1.0)**	**0.81 (9.3)**	0.12 (1.8)	0.53 (4.7)	**0.83 (1.4)**	**0.81 (10.2)**

Figure 3.1. Comparison of (a) cumulative effective degree days and (b) cumulative precipitation between 2011 (solid line) and 2012 (dotted line), averaged across all study sites (shaded area depicts standard error of the mean for *n* = 7 sites). Cumulative effective degree days were iteratively calculated as the number of days in which the average daily air temperature was > 5°C. Similarly, cumulative precipitation was calculated as the sum of total daily precipitation throughout the year. The two vertical dashed lines represent the first (DOY 157) and last (DOY 287) sampling campaigns that were conducted across all sites for both 2011 and 2012.

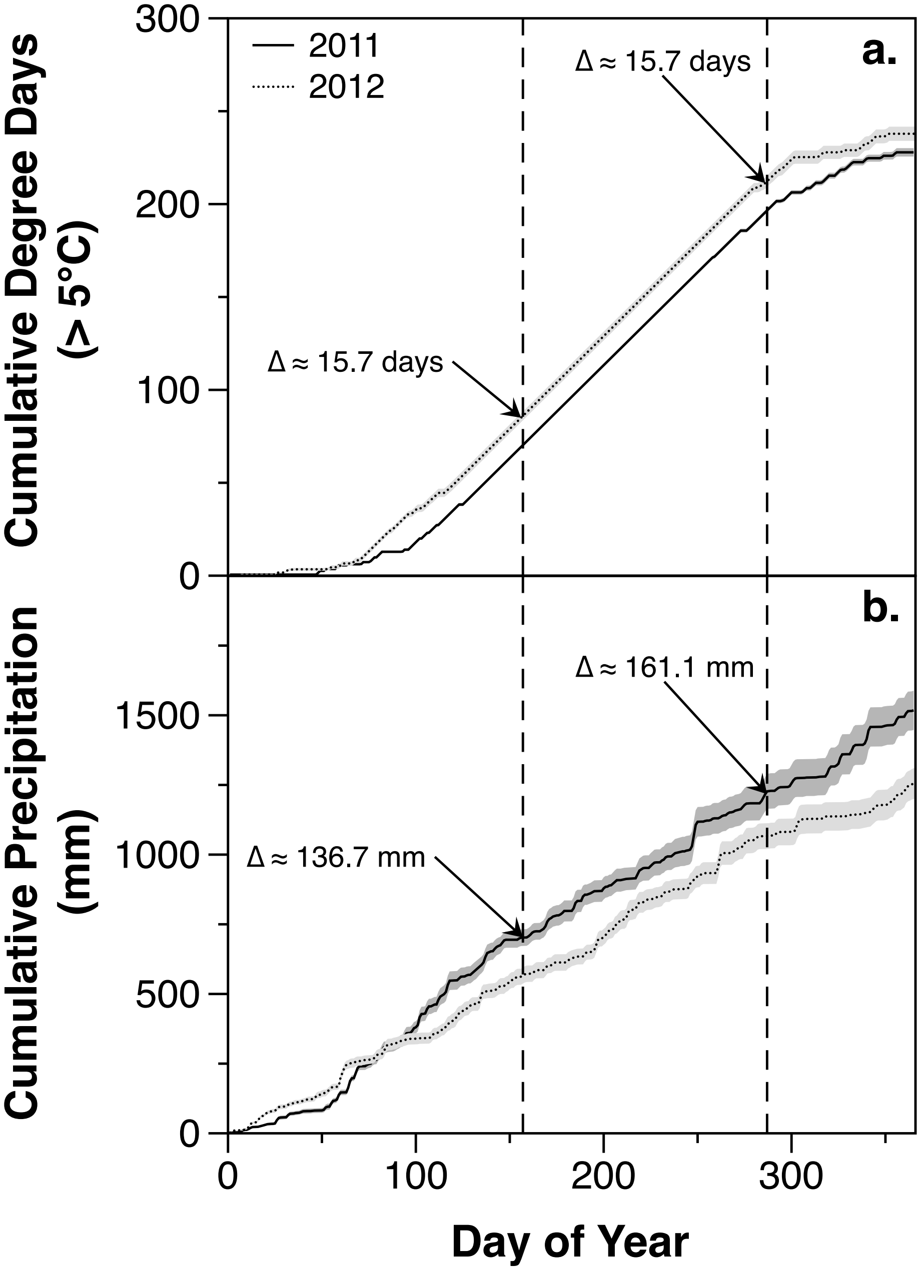


Figure 3.2. Site-specific relationships between observed mean daily soil respiration and soil temperature at 5-cm depth. Data for 2011 (closed circles) and 2012 (open circles) were fit separately using a modified Arrhenius-type function (Lloyd and Taylor, 1994). I found that the temperature response of soil respiration was significantly different between 2011 and 2012 (p = 0.04) and that the between-year variation in this response was dependent on study site (p = 0.04). All model parameters and goodness of fit are listed in Table 3.1.

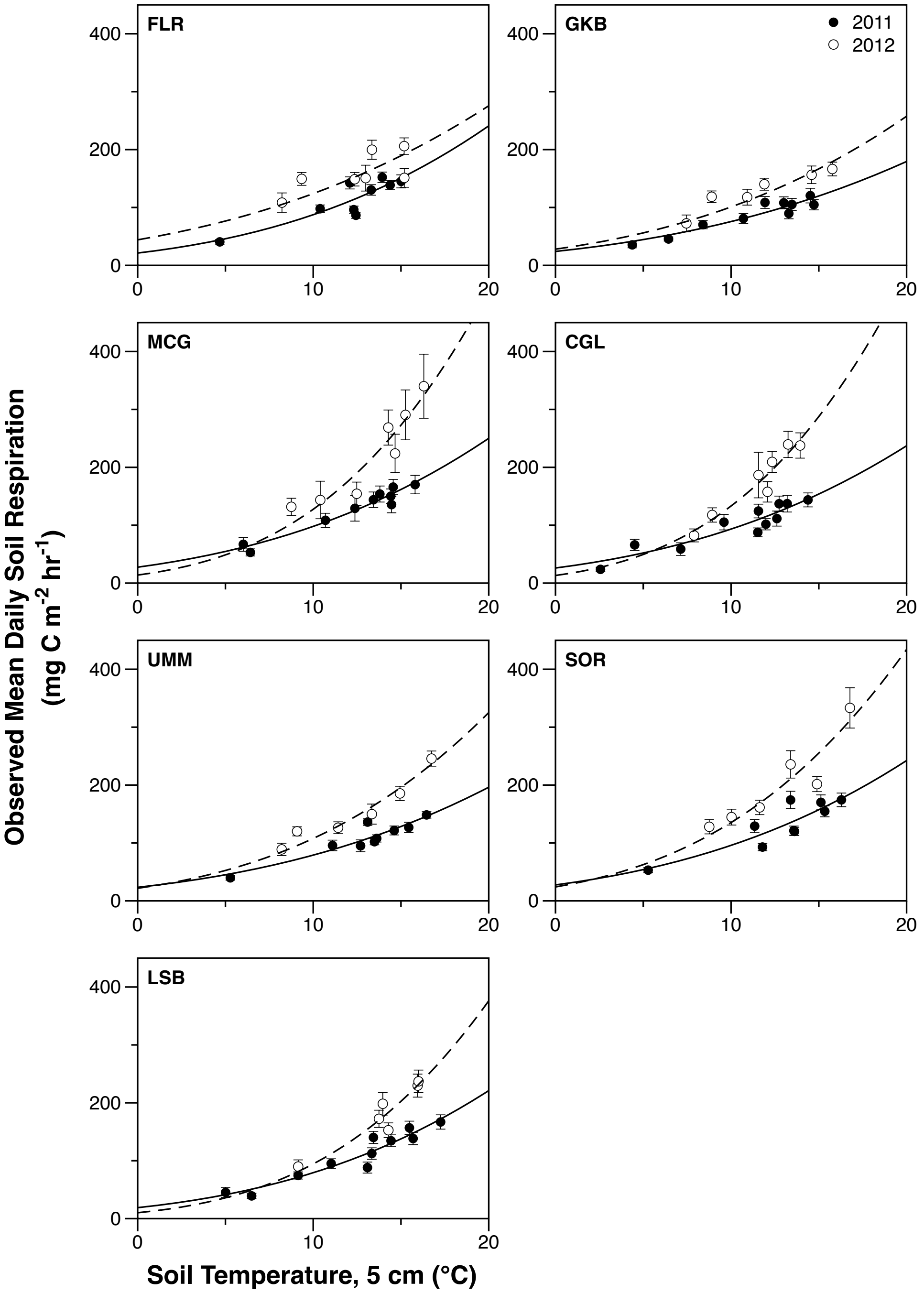


Figure 3.3. Relationships between the temperature coefficient (Q_{10}) of soil respiration and (a) stand age, (b) broadleaf deciduous relative importance values (RIV), and (c) organic soil C:N for the two study years, 2011 and 2012. All Q_{10} values were estimated over the soil temperature interval, 10-15°C.

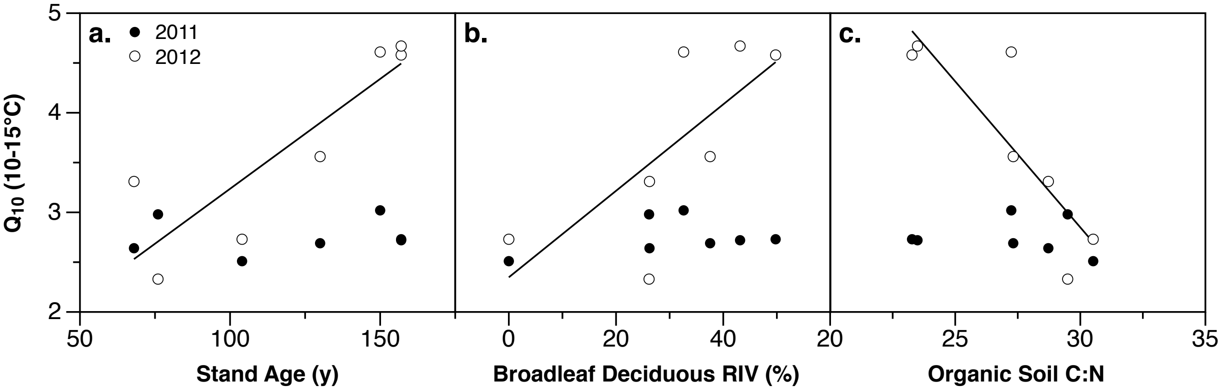


Figure 3.4. Comparisons of soil respiration and climatic conditions between the two study years (2011 and 2012): (a) data points are daily mean observed soil respiration (*R_s*) for all study sites (2011, closed circles; 2012 open circles) while the daily trend line is modeled mean daytime (0700-1900 hrs) soil respiration averaged across $n = 7$ sites (2011, solid line; 2012, dotted line); (b) data points are daily mean observed soil temperature at 5-cm depth (*T_s*) for all sites (2011, closed circles; 2012 open circles) while the daily trend line is datalogged mean daytime (0700-1900 hrs) soil temperature averaged across all sites (2011, solid line; 2012, dotted line); (c) mean cumulative monthly precipitation (*Ppt*) (± standard error bars) averaged across all sites (2011, grey bars; 2012, white bars). Note: Site-level precipitation data estimated from Livneh et al. (2015).

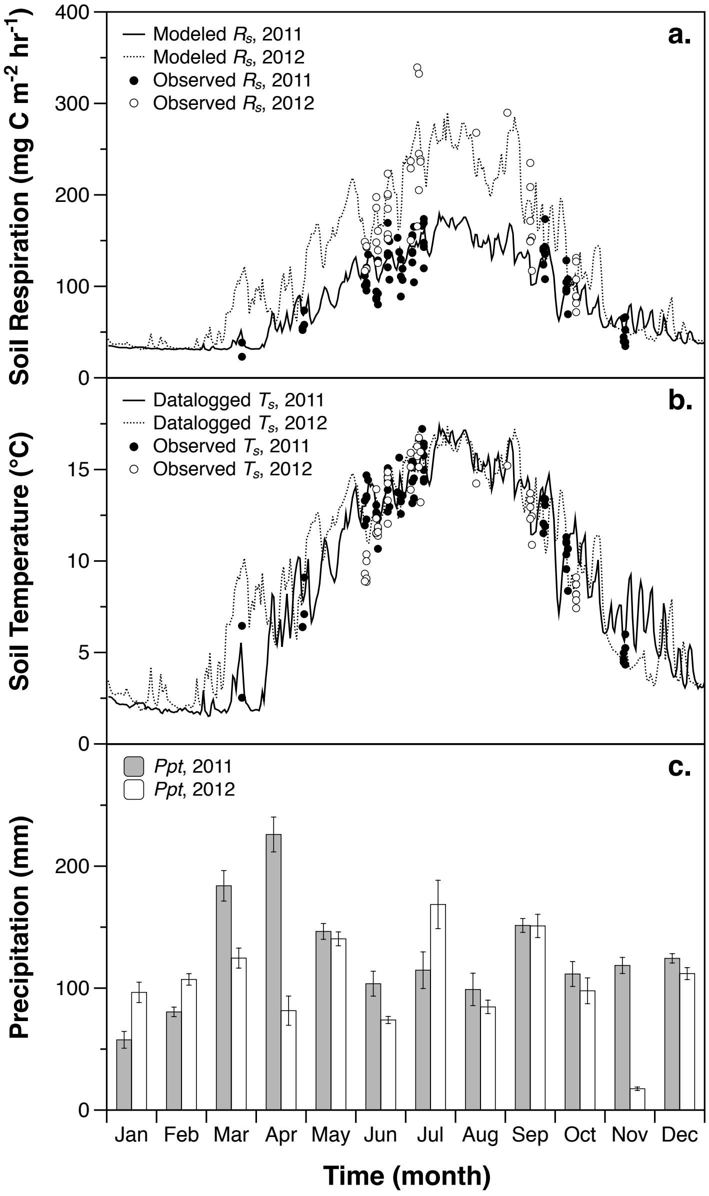


Figure 3.5. Relationships between annual soil respiration and annual litterfall for this study (2011: closed circles, 2012: open circles) and for a recently published meta-analysis (plus signs) (Davidson et al. 2002). Linear regression of these variables showed a slight trend for 2011 data ($p = 0.057$, $R^2 = 0.54$), but was not significant for 2012 data ($p = 0.15$, $R^2 = 0.36$).

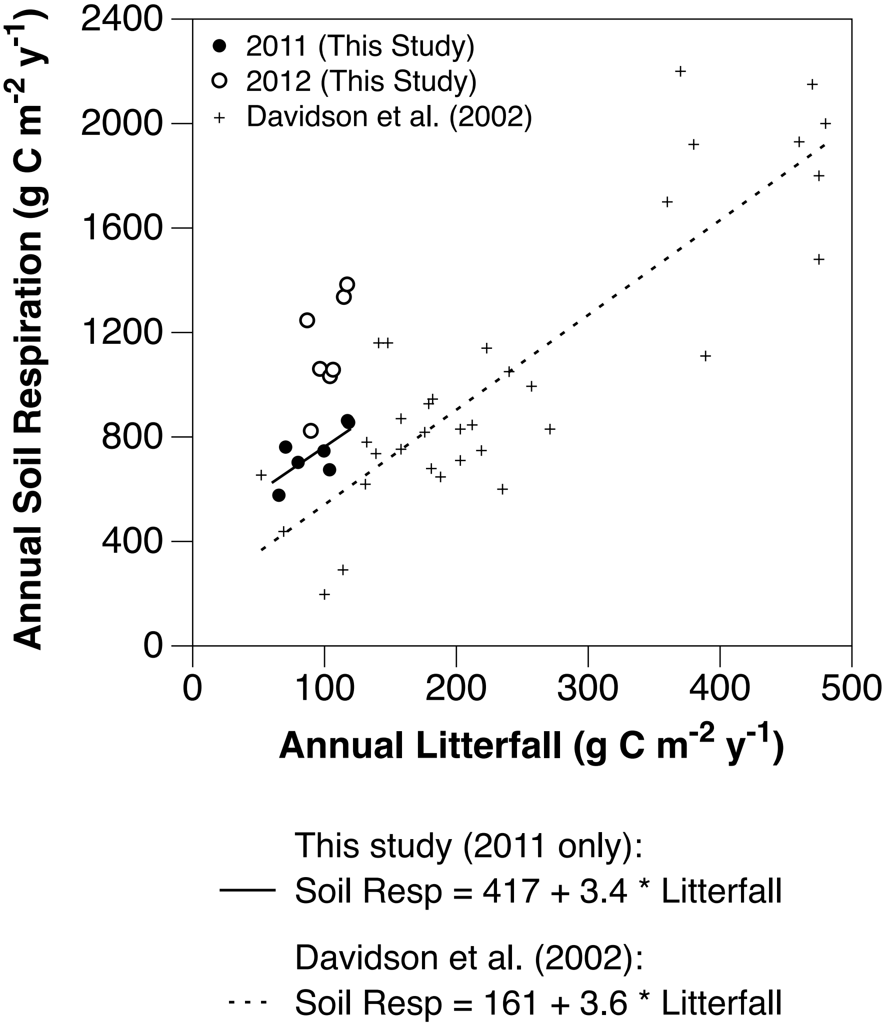
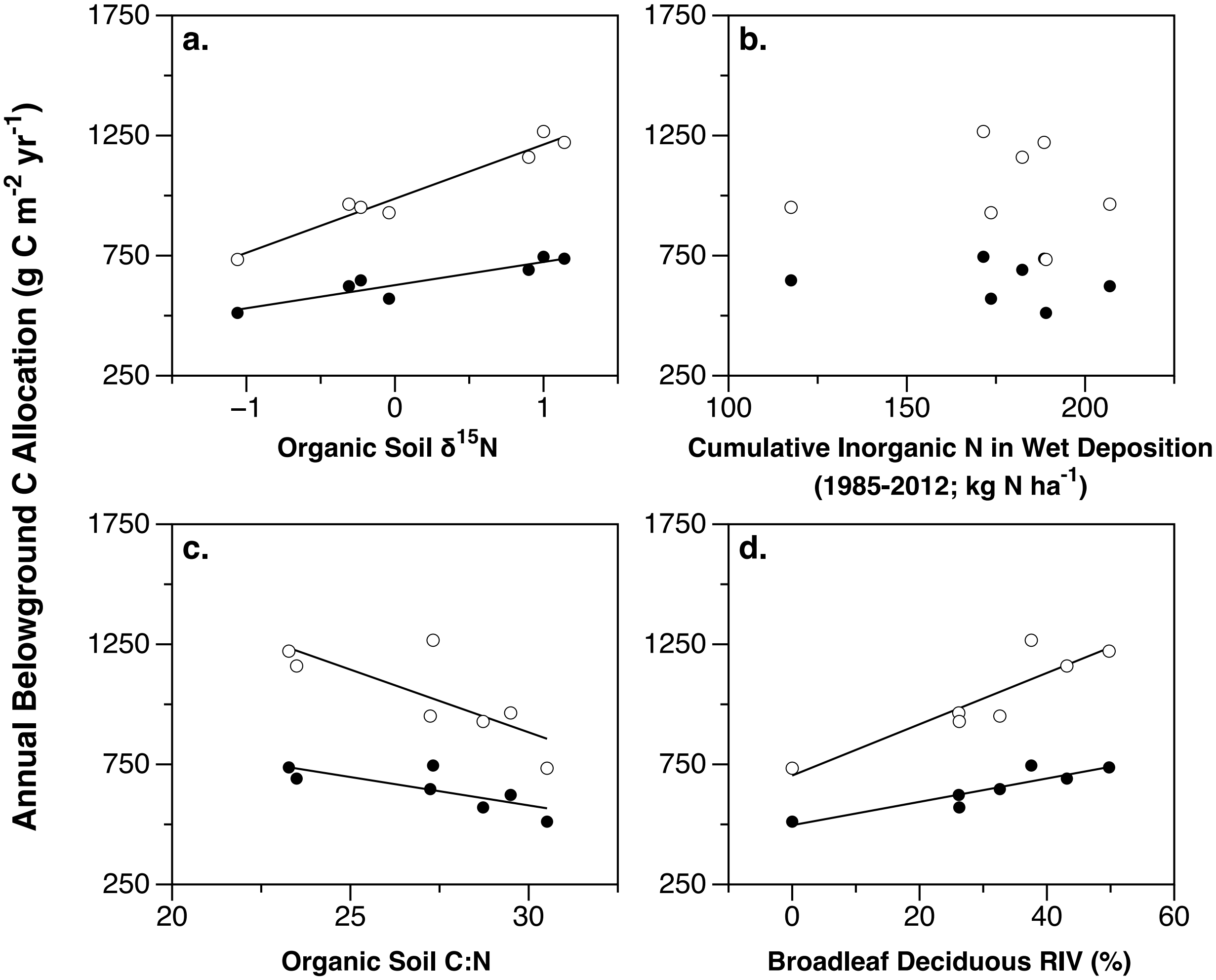


Figure 3.6. Relationships between 2011 and 2012 estimates of total belowground C allocation (TBCA) and (a) δ^{15}N content of organic soil, (b) cumulative inorganic N in wet deposition (1985-2012), (c) C:N ratio of organic soil, and (d) relative importance values of all canopy broadleaf deciduous species.



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Chapter 4. Aboveground growth of red spruce trees at seven forest locations in the Central Appalachian Mountains with differing N availabilities.

4.1. Abstract.

Chronic nitrogen (N) additions via atmospheric deposition can have important and often long-lasting effects on carbon (C) and N cycling in forest ecosystems. In this study, I used a biometric-based approach to examine the growth of red spruce trees located in a region of historically high rates of atmospheric N deposition. Previous research in these stands has shown that differences in N status among sites were associated with the abundance of broadleaf deciduous trees, but were unrelated to estimates of N deposition. Thus, a primary aim of this project was to assess whether red spruce growth was more strongly related to the legacy effects of N deposition, the current differences in N status, or the influence of other co-varying environmental factors like climate and stand age. I also took repeated measures of foliar net photosynthesis and dark respiration, and red spruce wood CO_2 efflux to determine whether observed growth responses could be explained by differences in C balance of aboveground biomass. During 2011 and 2012, I observed significant between-site variation in the growth of red spruce trees ($p < 0.0001$), but surprisingly, these growth responses were not correlated with either N status or N deposition estimates. Instead, I found that red spruce growth patterns were positively correlated with mean temperature ($R^2 = 0.92$ and 0.95, annual and growing season, respectively) and stand age ($R^2 = 0.58$). I also found a slight trend where red spruce aboveground production was negatively associated with the ratio of foliar dark respiration to net photosynthesis ($R^2 = 0.54$); in addition, red spruce wood biomass increment was positively correlated with wood CO_2 efflux expressed on a stem surface area basis ($R^2 = 0.77$), but not on a wood biomass basis ($R^2 = 0.01$). These findings suggest that, despite site-level differences in N status and historical N deposition, the observed red spruce growth patterns appear to be driven by local climatic conditions that favor a positive balance between C uptake via photosynthesis and C loss via increased respiration. Thus, red spruce growth is more constrained by cooler temperatures than by N-limitation.

4.2. Introduction.

Forest ecosystems play an important role in the global carbon (C) cycle by sequestering an estimated 2.4 Pg C annually (Pan et al. 2011) and roughly 30% of anthropogenic C emissions (Le Quéré et al. 2013). However, while studies have suggested that forests may continue to be a substantial sink for increases in atmospheric CO_2 (Nabuurs et al. 2003; Pan et al. 2011), the efficiency of these C sinks may decline in response to a multitude of environmental stressors, including climate change, drought, and land use change (Falkowski et al. 2000; Hurtt et al. 2002; Friedlingstein et al. 2006). Understanding the impacts of environmental change on forest C sequestration is of vital importance to improving terrestrial biosphere models. However, our ability to predict future C storage by forests is constrained by our limited knowledge on how environmental factors influence the processes that underlie tree growth in forest ecosystems.

For many forests, the availability of N to support aboveground production produces a large constraint on C sink strength (Nadelhoffer et al. 1985; Vitousek and Howarth 1991; LeBauer and Treseder 2008). Indeed, several lines of evidence have shown that N additions via fertilization or atmospheric N deposition often stimulate productivity in ecosystems that are strongly co-limited by N availability (Miller 1981; Ågren 1983; Bonan 1990; Elser et al. 2007). For example, as N supply increases, trees are expected to shift biomass allocation from belowground to the production of new foliage and wood biomass above ground (Giardina et al. 2003). In addition to enhanced foliar biomass and canopy leaf area, foliar N content may also increase (Aber et al 1989, 1998), and is likely to be accompanied by greater rates of photosynthesis and respiration (Evans 1989; Mitchell and Hinckley 1993).

While N deposition can enhance forest productivity by satisfying plant nutritional demands (Hyvönen et al. 2008; Nave et al. 2009), adverse effects due to chronic N saturation have also been observed (McNulty et al. 1996; Peterjohn et al. 1996; Wallace et al. 2007). The widely-accepted paradigm of N saturation suggests that when the sustained supply of inorganic N exceeds biological demand, critical changes in ecosystem N cycling begin to occur (Aber et al. 1989, 1998). Among these changes are increased NO_3^- leaching into surface waters, higher rates of net nitrification, and increased soil acidification (Fenn et al. 1998; Driscoll et al. 2003). These symptoms of progressive N saturation have been observed in a variety of controlled and observational studies (McNulty et al. 1991; McNulty et al. 1996; Magill et al. 2004), but there are often numerous exceptions due to confounding effects of soil type, species composition, and climate (Nadelhoffer et al. 1999; Aber et al. 2003; Emmett 2007). To resolve some of these inconsistencies, Lovett and Goodale (2011) offered an alternative conceptual model of N saturation in forest ecosystems, which posited that forest responses to N deposition depends on whether added N can be efficiently retained in vegetation and soil. However, predicting the efficiency of these N sinks is complicated since a number of factors influence how N is retained, including tree species composition, resource limitation, and stand age (Lovett and Goodale 2011). Thus, our limited understanding of how N availability and other environmental factors regulate forest C sequestration remains an area of uncertainty in current attempts to estimate C budgets for terrestrial ecosystems.

Red spruce forests in the northeastern United States are an ideal model system to examine the influence of N status on forest production, since they are among the most sensitive ecosystems to the acidic effects of atmospheric N deposition (Adams et al. 2012). Beginning in the 1960s and continuing through much of the 1980s, multiple red spruce stands exhibited symptoms of declining growth, including reduced crown volume and basal area, needle chlorosis, and mortality that were associated with high rates of acidic deposition in the region (Siccama et al. 1982; Foster and Reiners 1983; Scott et al. 1984; Johnson and McLaughlin 1986). The mechanism that initiated this decline was primarily related to acid-induced imbalances of key foliar nutrients (e.g. Ca/N and Mg/N; McNulty et al. 1991; McNulty et al. 2005) and associated reductions in cold stress tolerance of red spruce foliage (DeHayes et al. 1999; Schaberg et al. 2002). Following the enactment of the Clean Air Act in 1990, rates of N deposition have declined throughout the Central Appalachian Mountains (US Environmental Protection Agency 2015); consequently, many of the red spruce stands that were negatively affected by N deposition may be in a stage of recovery (Rollins et al. 2010). Although this evidence is encouraging, it nevertheless remains an open question whether historically high rates of N deposition are continuing to affect the N status and tree growth in these sensitive ecosystems.

This study builds on previous work conducted in high-elevation red spruce forests located in a region that once received some of the highest rates of atmospheric N deposition in the United States. Previously, I examined whether decades of atmospheric N deposition were responsible for site-level differences in N status among seven red spruce study sites in the Central Appalachian region (Smith et al. 2016). The results of this study showed that although these sites had distinctly different levels of N availability, these differences were not currently associated with historical or current N deposition. Instead, I found that the abundance of broadleaf deciduous (BD) tree species were a better predictor of N status, primarily due to differences in the quality of aboveground litter inputs among different species at each site. I also tested a similar hypothesis, that historically high rates of N deposition might be responsible for changes in soil CO_2 efflux and total belowground C allocation (TBCA) (Smith et al. *in preparation*). I found that both soil respiration and TBCA were positively associated with increases in BD tree abundance, suggesting that species differences in leaf habit and litter quality translated into higher rates of soil CO_2 efflux (and lower soil C accumulation) for sites where BD species were more prevalent.

In the current study, my objectives were to estimate aboveground growth of red spruce trees and to examine whether tree growth was positively correlated with differences in N status among the seven study sites. To test this hypothesis, I used a biometric approach to estimate aboveground growth over two years from repeated measures of basal area increment and litter production at each location. I also examined net photosynthesis and autotrophic respiration of leaves and stems at each site to determine whether red spruce growth responses could be explained by either enhanced C uptake through photosynthesis or increased respiratory C costs for the construction and maintenance of leaf and wood biomass. I then tested these response variables against a range of site level environmental predictors (including N status, N deposition, climate, and stand age) to evaluate which factors were most strongly related to observed growth responses. Although previous work in these stands revealed little evidence that historical N deposition was responsible for current patterns of N availability and soil C flux, I included this parameter in my models to evaluate whether the long-term effects of deposition were more evident in aboveground tree growth responses than in soil processes.

4.3. Methods.

Study site details

High-elevation study sites (n = 7, 100-m diameter) containing red spruce as a major, canopy-dominant species were established in June 2011. These locations were originally selected based on their position along a transect of historically high rates of atmospheric N deposition for the region. At its peak in 1985, wet deposition of atmospheric N ($NO_3^- + NH_4^+$) was 4x greater at the northernmost stand of the transect (McGowan Mountain) than the southernmost stand (Little Spruce Bog), with estimates ranging from 8.1 to 2.0 kg N ha^{-1} y^{-1} (North to South). Since the Clean Air Act, however, the concentration of NO_3^- in rainwater has declined by 39-51% along this transect, while NH_4^+ rainwater concentrations have remained relatively constant (National Atmospheric Deposition Program 2016). Because of these steep declines in rainwater concentrations of NO_3^-, the gradient of wet deposition has been diminished, with recent estimates of N deposition that range between 6.6 and 3.5 kg N ha^{-1} y^{-1} (North to South).

I used a robust spatial model of atmospheric N deposition for the study region to estimate annual N deposition rates for each field site (National Atmospheric Deposition Program 2014). Using the range of data that was available, I obtained estimates of cumulative wet-only N inputs that ranged from 117.5 to 206.9 kg N ha^{-1} for the period 1985 to 2012. After accounting for the influence of dry deposition ($HNO_3 + NO_3^- + NH_4^+$), I calculated cumulative total deposition loading for the same time period, with estimates that ranged from 326.1 to 399.6 kg N ha^{-1} yr^{-1} (Smith et al. 2016).

All of my red spruce study sites are located at high elevations above 900 m in the Central Appalachian Mountains, with each site having a SW aspect and slopes between 0 and 10%. These sites are representative of second growth forests, with red spruce and eastern hemlock (*Tsuga canadensis*) as the commonly-occurring needleleaf evergreen species; the most common broadleaf deciduous (BD) associates at my sites were yellow birch (*Betula alleghaniensis* var. *alleganiensis*), red maple (*Acer rubrum*), and black cherry (*Prunus serotina* var. *serotina*).

Given the lack of long-term climate monitoring at my field sites, I used a spatially-interpolated climate model to estimate 30-year averages (1981-2010) of annual and growing season (May through September) temperature and precipitation (PRISM Climate Group, Oregon State University). Across the study region, mean annual temperatures range from 6.3 to 8.4°C while annual precipitation ranges from 1238 to 1591 mm. Additional details of estimated N deposition, community composition, climate, and soil type for these red spruce stands are described in Smith et al (2016).

Red spruce aboveground production

In June 2011, I installed stainless steel dendrometer bands at 1.4 m above ground level on sixteen randomly selected red spruce trees at each study site to estimate annual rates of red spruce aboveground production (Keeland and Sharitz 1993). I recorded circumference measurements for each tree at the end of the growing season (September) and again the following June to estimate tree growth over the entire year (e.g June 2011 to June 2012). These measurements were then repeated until June 2013 to determine growth over a two-year interval. I calculated tree size on the basis of basal area (BA; cm^2) and estimated tree growth as average basal area increment (BAI; cm^2 $tree^{-1}$ y^{-1}) over the two-year time interval using:

$$BAI = \pi(R_t^2 - R_{t-n}^2) \tag{1}$$

where R is the tree radius at a given time t, and n is the time interval of interest.

I also expressed annual tree growth measurements in terms of aboveground biomass C increment (*BCI*) on a per tree basis, which I estimated as the annual change in aboveground biomass C (kg C $tree^{-1}$ y^{-1}). Total aboveground biomass for a given time point was calculated by applying dendrometer estimates of tree diameter to published allometric equations for aboveground red spruce biomass (Whittaker et al. 1974; Siccama et al. 1994), using the assumption that the C content of aboveground biomass was 50%.

In addition to the development of new wood biomass, aboveground net primary productivity (*ANPP*) also includes the loss of C via canopy litterfall. To account for this important component of ANPP, I also measured the contribution of red spruce fine litterfall (leaves plus woody tissue < 1 cm in diameter) to total litterfall of all overstory trees for each study site. In June 2011, I deployed eight litter traps (0.42 m^2 ground area) at each forest location and periodically collected all fine litterfall over a two-year time interval. Litter samples were returned to the laboratory where they were hand-sorted into foliage and woody debris (twigs, branches, bark, and reproductive tissue). To examine litterfall production of red spruce trees only, I separated red spruce litter from the larger sample. These samples were then dried at 65°C for a minimum of 48 h and weighed to determine dry biomass expressed on a ground area basis (kg m^{-2}).

After pooling red spruce litter samples of each type (foliage and wood) for every site, I took a small finely ground subsample to analyze for total C content using a NC 2500 elemental analyzer (Carlo Erba, Milan, Italy). The C concentration of both foliage and woody tissue was found to be approximately 50% across all sites and this value was used to convert all biomass estimates to a C basis. I estimated annual litterfall C (*LFC*) on a per tree basis by dividing the contribution of litterfall from red spruce trees only (kg C m^{-2} y^{-1}) by site-level estimates of red spruce tree density (trees ha^{-1}). In doing so, I was able to estimate total aboveground tree growth (biomass C increment plus litterfall C) per red spruce tree at each site. Although this is not a true measure of *ANPP*, it nevertheless allows us to estimate the productivity of only red spruce trees in sites that vary in tree species composition. I hereafter refer to this estimate of total aboveground growth of red spruce trees as *ABG.*

Ecophysiological measurements

Foliar gas exchange

I randomly selected three canopy-dominant red spruce trees from each study site to measure light-saturated photosynthesis (A_{net}) and foliar dark respiration (R_{dark}) in July and August 2012. From each tree, I collected two small branch samples from the upper canopy using a shotgun ($n = 6$ foliage samples per site per measurement period; $n_{\text{tot}} = 12$ foliar gas exchange samples per site). After harvests, sample stems were immediately inserted into a water pick to facilitate normal stomatal function during measurements of foliar gas exchange. Net photosynthesis was measured using an open-flow infrared gas analyzer (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) after equilibration for 5-10 minutes at a CO_2 concentration of 380 μmol mol^{-1}. Leaf temperature was maintained at 25°C and relative humidity was maintained at approximately 50-60% by controlling airflow through a Li-COR Drierite scrub. Light levels were 1500 μmol m^{-2} s^{-1} net photosynthetic photon flux density (PPFD), which was predetermined to be saturating light for photosynthesis.

Foliar dark respiration (R_{dark}; μmol CO_2 m^{-2} s^{-1}) was measured between 2200 and 0300 h on the same foliage samples used for photosynthesis measurements. Needles were enclosed in the LI-6400XT sample chamber and were allowed to acclimate to the chamber environment for 5-10 minutes. Instantaneous rates of R_{dark} were logged every 20 seconds for 5 minutes, with chamber conditions kept constant at 400 μmol mol^{-1} CO_2 and at 20°C, in the dark. I estimated R_{dark} for each sample as the average of instantaneous measurements collected over the 5-minute interval and standardized to 25°C using the equation:

$$R_{25} = R_{dark} Q_{10}^{(25-T)/10} \tag{2}$$

where R_{25} is the estimated rate of foliar dark respiration at 25°C, R_{dark} is the observed rate of dark respiration at leaf temperature T, and Q_{10} of 2 and is the parameter that describes the temperature sensitivity of respiration.

Following measurements of dark respiration, needles contained within the leaf cuvette were removed from the stem and returned to lab to determine total projected leaf area of fresh biomass (ImageJ, v. 1.33u; National Institute of Health, Bethesda, MD, USA). Foliar samples were then placed in a drying oven at 65°C for 48 hrs to determine dry biomass, and leaf mass to area ratio (LMA). Foliar gas exchange measurements were expressed in units of projected needle area (μmol CO_2 m^{-2} s^{-1}) and needle biomass (μmol CO_2 kg^{-1} s^{-1}).

Wood CO_2 efflux

I measured wood CO_2 efflux (R_{wood}) on eight randomly selected canopy-dominant red spruce trees at each study site over the course of three separate field campaigns in June, September, and October 2012 ($n = 8$ wood CO_2 efflux samples per site per campaign; n_{tot} = 24 wood CO_2 efflux samples per site). All measurements were conducted at night between 2200 and 0400 hr in order to limit CO_2 fluxes from any potential bark photosynthesis (– flux) and from the diffusion of xylem-transported CO_2 (+ flux; Cernusak and Marshall 2000; McGuire and Teskey 2004; Bowman et al. 2005; Teskey et al. 2008). I modified the horizontally-oriented soil chamber technique (Xu et al. 2000) to measure R_{wood} using a LI-8100 Automated Soil CO_2 Flux System (with LI-8100-102 chamber, Li-Cor Inc., Lincoln, NE, USA). Each tree was fitted with a PVC collar (4 cm height, 10 cm open diameter) at breast height (1.3 m) and fastened using gas-tight putty to prevent air leaks. During sampling, the LI-8100-102 survey chamber was attached to the PVC collar and automatically opened and closed to achieve ambient CO_2 concentrations. R_{wood} for each tree was estimated as the average rate of stem CO_2 flux recorded over a 90 second interval. Stem temperature was measured simultaneously with R_{wood} by inserting a temperature probe approximately 1 cm past the cambium (3 cm total depth).

I expressed R_{wood} on the basis of stem surface area and wood biomass. Stem surface area enclosed by the chamber was estimated using the equation:

$$S = \frac{\pi^2}{720} D_c D_t \arcsin\left(\frac{D_c}{D_t}\right) \tag{3}$$

where S is the surface area of the stem enclosed by the chamber, D_c is the diameter of the chamber, and D_t is the diameter of the tree at chamber placement. To approximate wood volume sampled by the survey chamber, I multiplied the volume of the underlying wood cylinder (height equal to chamber height and radius equal to ½ *DBH*) by the ratio of the stem surface area enclosed by the chamber (S) to the total surface area of the wood cylinder. Wood volume was then converted to biomass using the density of red spruce wood (Whittaker et al. 1974). I expressed R_{wood} as the rate of CO_2 efflux per unit stem surface area (µmol CO_2 m^{-2} s^{-1}) or per unit wood biomass (µmol CO_2 kg^{-1} s^{-1}) after standardization to 25°C using a Q_{10} of 2.

Statistical analysis

I used one-way analysis of variance (ANOVA) and Tukey-Kramer HSD tests to examine site-level differences in mean red spruce *BAI* and *BCI* (averaged over the two-year study interval), as well as mean estimates of foliar and wood gas exchange (standardized to 25°C). To test whether red spruce growth was associated with site-level differences in N status, I performed a series of bivariate regressions with red spruce *BAI*, *BCI*, litterfall, and *ABG* as response variables and multiple indices of N status (see Smith et al. 2016 for full details) as predictor variables. I also tested whether red spruce growth was related to additional environmental parameters by regressing my estimates of *BAI* and *ABG* against cumulative atmospheric N deposition for the period 1985-2012 (wet and total deposition), mean temperature (annual and growing season May - September) and precipitation (annual and growing season) (PRISM Climate Group, Oregon State University), stand age, and the relative importance of BD tree species (hereafter referred to as BD RIV) (Smith et al. 2016).

To examine whether red spruce growth differences could be explained by the variation in aboveground gas exchange estimates, I performed similar analyses by regressing red spruce *BAI* and *ABG* against mean estimates of A_{net}, R_{dark}, and R_{wood}. Finally, I used linear regression to determine whether red spruce carbon balance varied independently from growth based on site-level differences in N status, N deposition, and climate. All analyses were performed using SAS-JMP 10.0 software (SAS Institute, Cary, NC, USA).

4.4. Results.

Red spruce growth

Mean *BAI* and *BCI* were not significantly different between the two years of study ($p = 0.45$); as such, all data related to red spruce growth have been averaged across the two-year interval. I found differences in mean red spruce *BAI* and *BCI* across the seven study sites ($p < 0.0001$; Figure 4.1), with average *BAI* ranging from 7.6 (Gaudineer Knob) to 25.2 $cm^2\ y^{-1}$ (Little Spruce Bog). Expressed in units of biomass C, the average annual *BCI* of red spruce trees ranged between 1.5 (Gaudineer Knob) and 5.4 kg C y^{-1} (Little Spruce Bog). Contrary to my initial hypothesis, the variation in red spruce tree growth could not be explained by differences in N status among sites (Table 4.1). Red spruce *BAI* was weakly associated with differences in mean growing season temperature ($p = 0.048$) and mean annual temperature ($p = 0.061$). Additionally, I observed a slight trend, whereby red spruce *BAI* was negatively associated with modelled estimates of cumulative inorganic N in wet deposition ($p = 0.099$); however, this was largely driven by only one site (Little Spruce Bog) that had the lowest rates of N deposition and highest growth. Therefore, I treat this relationship as being controlled by one outlier point as opposed to a meaningful trend. I found strong support that *ABG* (expressed on a per red spruce tree basis) was positively correlated with differences in temperature (annual and growing season) and stand age across sites (Table 4.1; Figure 4.2). Although site-level differences in N status indices could not explain the variation in *ABG*, I did observe a weakly positive correlation between *ABG* and BD RIV ($p = 0.06$), which was previously found to be associated with higher soil N availability (Smith et al. 2016).

Foliar gas exchange

I observed significant differences in mean A_{net} (expressed in units of leaf biomass at 25°C) between sites that ranged from 7.4 to 15.9 μmol CO_2 kg^{-1} s^{-1} ($p = 0.04$; Figure 4.3). Foliar R_{dark} ranged between 1.7 and 3.1 μmol CO_2 kg^{-1} s^{-1} but was not significantly different across the seven study sites ($p = 0.07$; Figure 4.3). I found that canopy-related differences in *LMA* could explain 19% of the observed variation in R_{dark}, but not A_{net} (Figure 4.4).

I found little evidence that the differences in mean A_{net} and R_{dark} were related to N status (Table 4.1); however, there was a trend where A_{net} was lower for sites with high soil organic C:N ($p = 0.07$) and low BD RIV ($p = 0.03$). I also used average rates of A_{net} and R_{dark} to examine whether the balance between daytime net foliar C uptake and C efflux at night (expressed as the ratio of $R_{\mathrm{dark}} / A_{\mathrm{net}}$) was related to any of the environmental factors that I included in my model analysis. My results showed no significant relationships between N status and $R_{\mathrm{dark}} / A_{\mathrm{net}}$ (Table 4.1). Mean A_{net} was strongly associated with differences in temperature (annual and growing season) among sites ($p = 0.01$-0.03); whereas, none of the environmental predictors could explain the variation in mean R_{dark}. Given the strong positive correlation between mean annual temperature and A_{net} coupled with the lack of variation in R_{dark}, I observed a slight negative trend relating the $R_{\mathrm{dark}} / A_{\mathrm{net}}$ ratio to differences in annual temperature ($p = 0.04$) and growing season temperatures ($p = 0.05$).

Wood CO_2 efflux

Mean stem CO_2 efflux per unit wood biomass at 25°C ranged from 0.12 to 0.15 μmol CO_2 kg^{-1} s^{-1} and did not significantly differ across sites ($p = 0.35$; Figure 4.3). I observed consistent evidence that stem CO_2 efflux per unit biomass was negatively associated with most (but not all) indices of site N status, particularly the $\delta^{15}N$ content of red spruce needles ($p = 0.007$; Table 4.1). When expressed on a per stem surface area basis, however, none of these relationships were present.

Relationships between red spruce growth and carbon balance

Red spruce growth differences were most consistently explained by site-level differences in mean A_{net} (Table 4.2). A_{net} expressed on a leaf biomass basis explained approximately 52% of the increases in both red spruce *BAI* and *BCI* (both $p = 0.07$). When A_{net} was expressed on a leaf area basis, mean A_{net} explained up to 63% of the variability in *BAI* and *BCI* ($p = 0.03$). I found a strong positive relationship between A_{net} and *ABG*, with A_{net} (area- and biomass-based) predicting up to 76% of the variability in aboveground production ($p = 0.01$; Figure 4.5a).

None of the red spruce growth parameters were associated with the variation in mean foliar R_{dark} ($p > 0.34$; Figure 4.5b); however, I observed a negative trend relating *ABG* to the R_{dark} / A_{net} ratio ($p = 0.06$; Figure 4.5c). Similarly, there was not a significant relationship between red spruce growth and mean rates of stem CO_2 efflux (R_{wood}) expressed in units of wood biomass ($p > 0.95$). However, when expressed per unit stem surface area, R_{wood} was positively associated with site-level variation in red spruce *BAI* and *BCI* ($p = 0.01$) but not *ABG* ($p = 0.17$).

4.5. Discussion.

Despite a strong N availability gradient across the seven red spruce locations (Smith et al. 2016), the hypotheses that site N status would be an important driver of red spruce aboveground growth and C sequestration was not supported in this study of red spruce tree growth conducted over two years. Given the site-level variation in both N availability and historical N deposition, I found it surprising that red spruce growth varied independently of the N cycling history of these forest sites, since many studies across diverse ecosystems have shown a clear linkage between plant productivity and N status (Pastor et al. 1984; Newman et al. 2006; Hyvönen et al. 2008; LeBauer and Treseder 2008). On the other hand, tree growth responses to enhanced N availability are species-specific (Boggs et al. 2005; Bedison and McNeil 2009; Thomas et al. 2010) and a long-term forest fertilization study has shown that the growth of sugar maple, but not red spruce, was stimulated with N additions (Elvir et al. 2003). Here, I found that that the observed differences in red spruce growth were more strongly related to site-level differences in mean annual temperature (Figure 4.2a), as well as the variation in stand age from 68 to 157 years (Figure 4.2b), than to N availability gradient across the seven forest locations.

The variation in red spruce growth and its underlying physiological processes were more associated with local differences in climate rather than N status, where higher mean annual temperatures appeared to be optimal for aboveground growth (Figure 4.2), and is consistent with our current understanding of climatic controls on forest productivity. For my sites, approximately 53% of the variation in red spruce *BCI* was explained by mean annual temperature (Table 4.1). I also found that the strength of this relationship was improved once I accounted for C losses through aboveground litterfall, with mean annual temperature explaining 92% of the variation in *ABG* (Figure 4.2). These results support field studies conducted along latitudinal and elevation gradients that have shown that forest *ANPP* is strongly correlated with differences in mean annual temperature (Raich et al. 1997; Curtis et al. 2002). Likewise, a meta-analysis of the environmental controls of productivity in boreal forests showed that *ANPP* increased linearly with mean annual temperature across a broad range of boreal forest types (Gower et al. 2001). A number of dendroecological studies have also examined the sensitivity of red spruce growth to interannual climate variation, where favorable springtime conditions during the early growing season were correlated with maximum latewood density (i.e. an indicator increased cambial activity) (Conkey 1986), while unfavorable conditions (e.g. above-average August and below-average December temperatures) during the year prior to ring formation were associated with synchronous red spruce growth declines across much of the northeastern United States (Johnson et al. 1988; Cook and Johnson 1989). This negative association with prior-year climate was especially evident during the mid 1960s through the mid 1980s, a period in which high rates of acidic deposition likely weakened red spruce trees to extreme climate stress (DeHayes et al. 1999). Thus at regional and global scales, temperature is often found to be among the important factors influencing tree growth and forest productivity, especially in high-elevation needleleaf evergreen ecosystems (Churkina and Running 1998).

The widespread association between temperature and forest productivity may be partially attributed to the temperature dependence of both foliar net photosynthesis and dark respiration, since the greatest red spruce growth responses were associated with a low R_{dark} / A_{net} ratio ($p = 0.06$; Figure 4.5c). In this study, net photosynthesis of red spruce trees measured at a standard temperature of 25°C was positively correlated with differences in mean annual temperature at my study sites (Table 4.1), suggesting that the photosynthetic temperature response of red spruce was locally-adapted to the temperature regime of their native habitat, as has been found in many plants (Berry and Björkman 1980). In contrast, there was little variation in foliar dark respiration across the seven study sites ($p = 0.07$; Figure 4.3) and, as a result, there was a trend where the R_{dark} / A_{net} ratio was negatively related to site-level differences in temperature ($p < 0.07$). Based on these data, I hypothesize that unlike net photosynthesis, foliar dark respiration was less thermally acclimated to the local climate at each stand. In addition, the negative relationship between red spruce *ABG* and foliar R_{dark} / A_{net} ratio suggests that tree growth was lower in locations where photosynthetic C gains were more significantly offset by C losses through foliar respiration.

In comparison to my results for foliar C gas exchange, I observed more within-site variation in wood CO_2 efflux than the variation between sites ($p = 0.35$; Figure 4.3). In addition, the hypothesis that red spruce tree growth differences could be explained by differences in wood CO_2 efflux was only partially supported. When expressed on the basis of stem surface area, wood CO_2 efflux was positively correlated with red spruce *BAI* and *BCI*, but not *ABG* (Table 4.2); however, when expressed in units of wood biomass, none of these relationships were present. This was likely due to the fact that wood CO_2 efflux sampled on the stem surface of a large tree will have proportionally greater underlying sapwood volume compared to a similar measurement made on a smaller tree. As a result, we would expect that as the volume of wood biomass increases, it will incur greater respiratory costs through the construction and maintenance of woody tissue (Levy and Jarvis 1998; Asao et al. 2015); whereas, wood CO_2 efflux per unit wood biomass appears to be somewhat conserved. My results are generally in agreement with other studies that show a positive relationship between tree growth and wood CO_2 efflux per unit surface area (Ryan et al. 1994; Zha et al. 2004).

The results of this study have broad implications for predicting red spruce responses to future environmental change. Given their historical sensitivity to climate stress (Hamburg and Cogbill 1988), the high-elevation red spruce forests of Central Appalachian Mountains have been predicted to diminish in land area due to pressures from anthropogenic climate change (Prasad et al. 2007; Iverson et al. 2008; Byers et al. 2010). However, my study showed that red spruce production was positively related to site-level differences in temperature, suggesting that red spruce may be suited to a relatively broad range of thermal environments. On the other hand, climate change is also likely to promote the encroachment of BD trees into these high-elevation habitats (Davis and Shaw 2001). I expect that shifts in species composition will also be accompanied by increased N availability and soil C release via enhanced litter decomposition, as evidenced in previous work at these stands. Since red spruce growth appeared to vary independently of N status, it may simply be a “passive observer” to changes in N availability and could be outcompeted by its faster-growing, nitrophillic counterparts. Thus, the positive relationships between red spruce growth and temperature observed in this study should be interpreted with caution, since most climate change scenarios appear to favor the growth of BD species over needleleaf evergreens like red spruce (Hansen et al. 2001; Iverson and Prasad 2001; Aitken et al. 2008).

Conclusions

This study examined two years of red spruce tree growth in seven forest locations that differ in N status. I found that growth responses were more strongly associated with the local temperature regime particular site and stand age than to soil N availability. I also observed evidence that these growth patterns could be partially explained by the relative balance of C uptake via foliar net photosynthesis and C losses via autotrophic respiration. This could suggest that for these sites, red spruce growth was more temperature-dependent than N-limited, and supports the view that climate is an important driving force for differences in tree growth across broad spatial gradients. This study provides important baseline data that can be re-examined in future studies to further test hypotheses about the effects of N availability on the productivity of red spruce forests, as well as the effects of continuing changes in environmental factors, such as air pollution and climate.

4.6. Tables and Figures.

Table 4.1. Coefficients of determination (R^2) for linear regressions of red spruce growth (expressed on a per red spruce tree basis and averaged over a two-year interval, 2011-2013) and gas exchange (expressed in units of biomass and standardized to 25°C) versus site-level predictor variables. *BAI*, basal area increment; *BCI*, biomass C increment; *ABG*, total aboveground growth; A_{net}, light-saturating photosynthesis; R_{dark}, foliar dark respiration; R_{wood}, wood CO_2 efflux. †, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$.

	Red spruce growth			Red spruce gas exchange (at 25°C)			
Model	*BAI* ($cm^2\ y^{-1}$)	*BCI* ($kg\ C\ y^{-1}$)	*ABG* ($kg\ C\ y^{-1}$)	A_{net} ($\mu mol\ kg^{-1}\ s^{-1}$)	R_{dark} ($\mu mol\ kg^{-1}\ s^{-1}$)	R_{dark} / A_{net}	R_{wood} ($\mu mol\ kg^{-1}\ s^{-1}$)
N status indices							
Net nitrification	0.08	0.10	0.02	0.10	0.16	0.01	$0.56^{\dagger}$
Net N mineralization	0.08	0.10	0.10	0.16	0.06	0.03	0.71*
Resin – Total N	0.04	0.06	0.16	0.38	0.01	0.23	$0.53^{\dagger}$
Resin – NH_4^+	0.03	0.04	0.14	0.25	0.01	0.10	0.74*
Resin – NO_3^-	0.04	0.06	0.16	0.41	0.01	0.27	$0.45^{\dagger}$
Foliar $\delta^{15}N$	0.01	0.01	0.05	0.06	0.04	0.02	0.79**
Organic soil $\delta^{15}N$	0.02	0.02	0.27	0.29	0.01	0.24	$0.54^{\dagger}$
Mineral soil $\delta^{15}N$	0.07	0.08	0.17	0.17	0.01	0.15	0.44
Organic soil C:N	0.17	0.19	0.42	$0.53^{\dagger}$	0.01	0.34	$0.57^{\dagger}$
Mineral soil C:N	0.09	0.10	0.23	0.29	0.01	0.23	$0.54^{\dagger}$
Environmental factors							
Cumulative N Deposition (wet only)	$0.45^{\dagger}$	0.42	0.37	0.08	0.01	0.09	0.05
Cumulative N Deposition (wet + dry)	0.35	0.34	0.25	0.02	0.01	0.01	0.01
Mean Annual Temperature	$0.54^{\dagger}$	$0.53^{\dagger}$	0.92***	0.63*	0.03	$0.56^{\dagger}$	0.02
Mean Growing Season Temperature	0.58*	0.57*	0.95***	0.75*	0.02	0.61*	0.06
Annual Precipitation	0.01	0.01	0.15	0.01	0.20	0.07	0.16
Growing Season Precipitation	0.05	0.04	0.19	0.01	0.07	0.07	0.17
Stand Age	0.27	0.29	0.58*	0.33	0.01	0.11	$0.55^{\dagger}$
BD Relative Importance	0.20	0.22	$0.53^{\dagger}$	0.66*	0.01	$0.50^{\dagger}$	0.28

Table 4.2. Coefficients of determination (R^2) for linear regressions of red spruce gas exchange (expressed on the basis of either surface area or biomass) and red spruce growth parameters. *BAI*, basal area increment; *BCI*, biomass C increment; *ABG*, total aboveground growth; A_{net}, light-saturating photosynthesis; R_{dark}, foliar dark respiration; R_{wood}, wood CO_2 efflux. †, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$.

Model	*BAI* ($cm^2\ y^{-1}$)	*BCI* ($kg\ C\ y^{-1}$)	*ABG* ($kg\ C\ y^{-1}$)
Red spruce gas exchange			
A_{net} (area, $\mu mol\ m^{-2}\ s^{-1}$)	0.62*	0.63*	0.76*
A_{net} (mass, $\mu mol\ kg^{-1}\ s^{-1}$)	$0.52^\dagger$	$0.52^\dagger$	0.75*
R_{dark} (area, $\mu mol\ m^{-2}\ s^{-1}$)	0.15	0.18	0.01
R_{dark} (mass, $\mu mol\ kg^{-1}\ s^{-1}$)	0.10	0.12	0.02
R_{dark} / A_{net} (area)	0.14	0.12	$0.45^\dagger$
R_{dark} / A_{net} (mass)	0.20	0.18	$0.54^\dagger$
R_{wood} (area, $\mu mol\ m^{-2}\ s^{-1}$)	0.76*	0.77**	0.34
R_{wood} (mass, $\mu mol\ kg^{-1}\ s^{-1}$)	0.01	0.01	0.07

Figure 4.1. Box plot of red spruce basal area increment (*BAI*, cm^2 y^{-1}) averaged across the two-year study period (June 2011 through June 2013). Solid squares show the mean of n = 16 trees at each study site, and sites that are connected by different letters had significantly different mean *BAI* values (Tukey-Kramer HSD). Circles represent outliers.

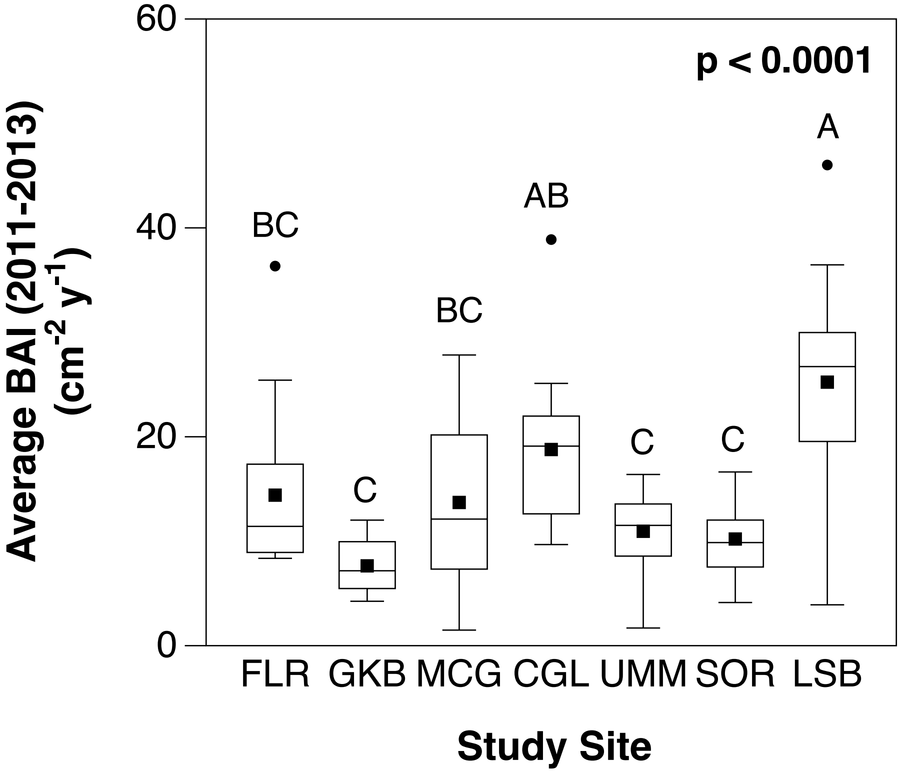


Figure 4.2. Linear regressions of red spruce total aboveground growth per unit tree (*ABG*; kg C y^{-1}) versus site-level differences in (a) mean annual temperature (°C) and (b) stand age (y). In a bivariate regression of mean annual temperature and stand age, we found a slight positive trend between these two variables, but no significant relationship was identified ($p = 0.099$, $R^2 = 0.45$; *data not shown*). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$.

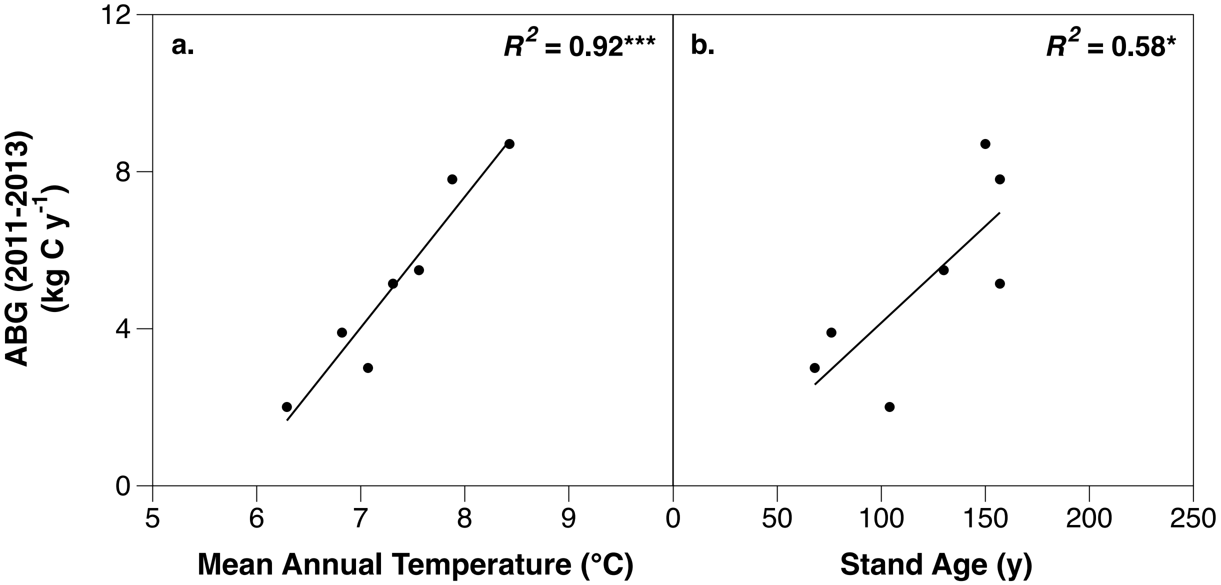


Figure 4.3. Box plots of red spruce gas exchange data expressed on the basis of biomass and standardized to 25°C (μmol kg^{-1} s^{-1}). Solid squares represent mean values for each study site, and sites that are connected by different letters had significantly different mean gas exchange values (Tukey-Kramer HSD). Circles represent outliers. A_{net}, light-saturating photosynthesis; R_{dark}, foliar dark respiration; R_{wood}, wood CO_2 efflux.

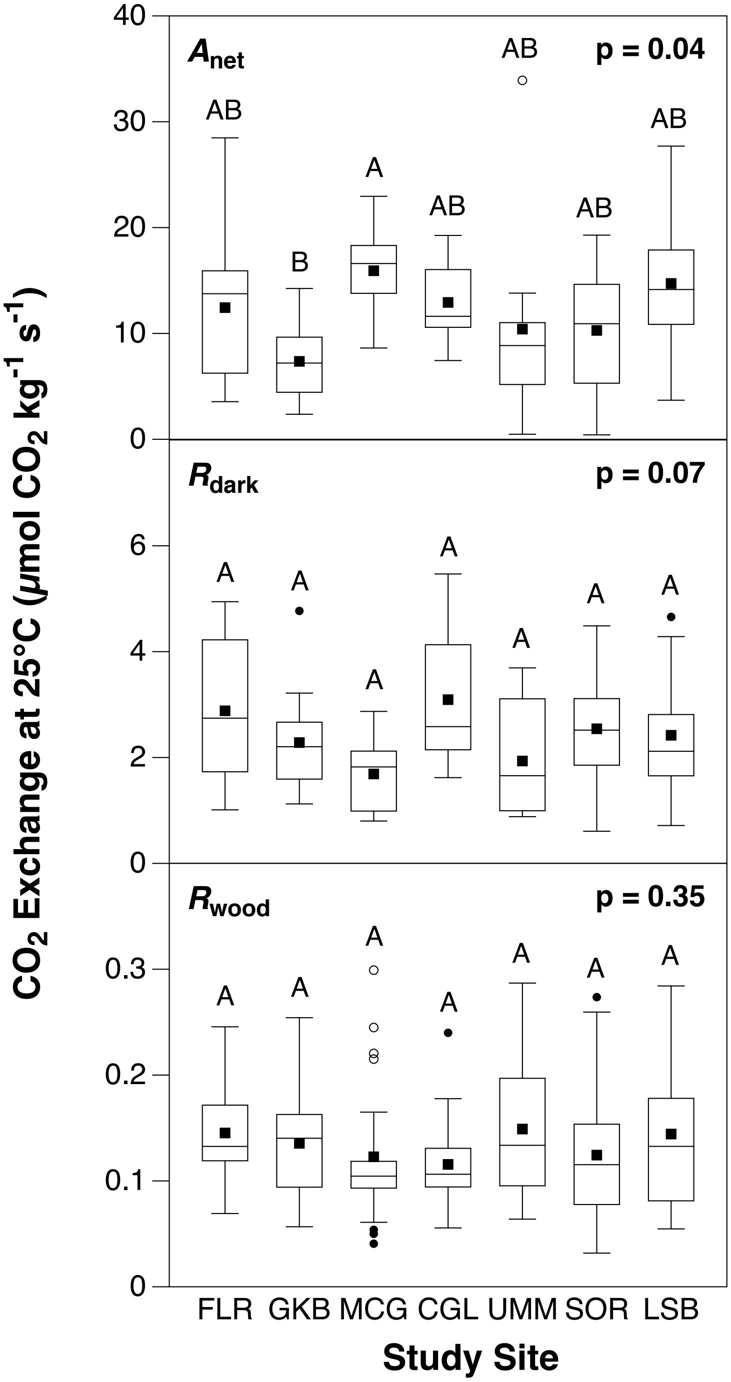


Figure 4.4. Relationships between leaf mass per area (*LMA*, g m^{-2}) and (a) light-saturating photosynthesis (A_{net}, µmol CO$_2$ m^{-2} s^{-1}) and (b) foliar dark respiration (R_{dark}, µmol CO$_2$ m^{-2} s^{-1}). Points represent individual measurements of foliar gas exchange collected during the 2012 growing season. ***, $p < 0.0001$.

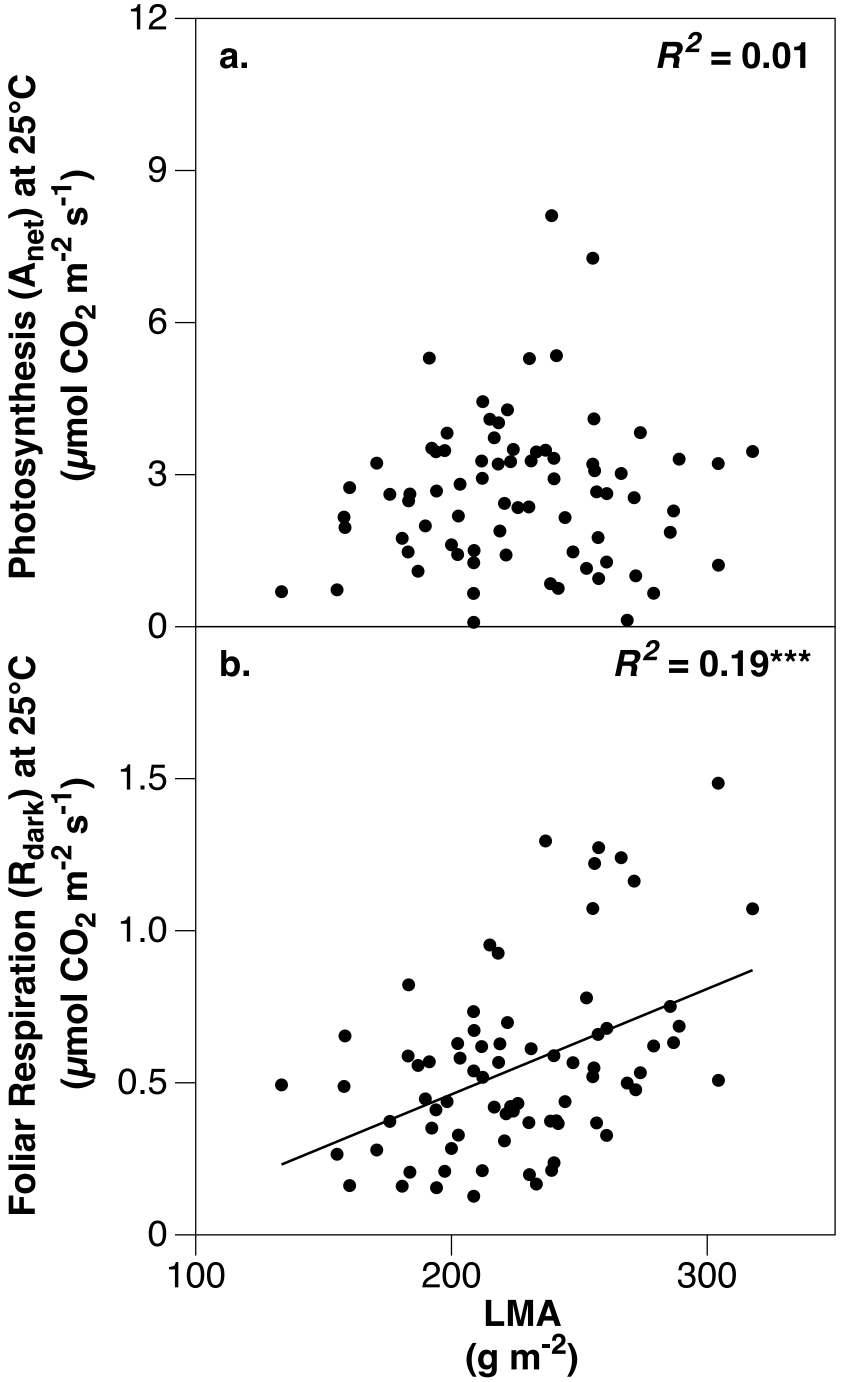
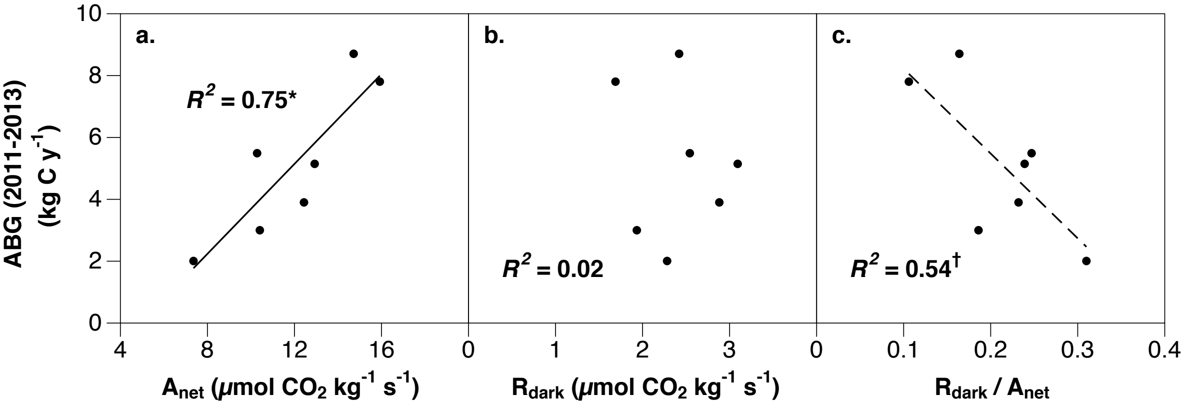


Figure 4.5. Linear regression of red spruce total aboveground growth (*ABG*; kg C y^{-1}) versus site-level differences in (a) light-saturating photosynthesis (A_{net}), (b) foliar dark respiration (R_{dark}), and (c) the ratio of dark respiration to net photosynthesis (R_{dark} / A_{net}). All gas exchange data standardized to 25°C and expressed on the basis of biomass (µmol CO_2 kg^{-1} s^{-1}). †, $p < 0.1$; *, $p < 0.05$.



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Chapter 5. General conclusions about the controls on N status and C balance in Central Appalachian red spruce forests.

In this project I examined the environmental controls on the N status and C balance of seven high-elevation red spruce forests that are located in a region where rates of atmospheric N deposition have historically been moderate to high. Given that red spruce ecosystems have been particularly sensitive to N deposition in the past, the overall aim of this research was to test whether these historically elevated rates of N deposition were having persistent effects on patterns of 1) N availability, 2) belowground C balance, and 3) tree growth.

In *Chapter 2*, I tested the hypothesis that patterns of N status at these sites were associated with the long-term effects of N deposition. I characterized the N status of these forests by measuring multiple indices of N availability (e.g. N transformation rates, resin ion-exchange, and foliar/soil δ^{15}N and C:N) that integrate the N cycling history of a particular site over short- to long-term timescales (from seasons to decades). Next, I used a statistical model selection approach to evaluate which environmental factors were most strongly correlated with observed differences in N availability along the study gradient. The environmental predictor variables that were used in this model comparison included estimates of cumulative N deposition (wet-only and total) for the period 1985-2012, mean temperature and precipitation (annual and growing season) for the period 1981-2010, stand age, and the relative importance values (RIV) for all canopy broadleaf deciduous (BD) trees. Contrary to my initial hypothesis, I did not observe any evidence that the N status of these seven red spruce forests was correlated with historical estimates of N deposition. Instead, I found that the variation in N status was most consistently related to differences in the abundance of BD tree species at each site. For example, I found that BD RIV could explain more than 50% of the variation in N status indices that integrate over short- (e.g. ion-resin N supply), medium- (e.g. δ^{15}N content of foliage and soil), and long-term timescales (e.g. soil C:N) (Figure 2.2). Overall, this evidence suggests that any potential legacy effects of historically high atmospheric N deposition on N status were overwhelmed by the influence of tree species composition and their associated litterfall characteristics.

In *Chapter 3* of this research project, I examined the variation in annual soil respiration and total belowground C allocation (TBCA) at these red spruce stands to determine whether these processes were associated with site-level differences in N cycling history. Our current understanding of how N influences belowground C balance suggests that both soil respiration and TBCA will be inversely related to soil fertility; however, N deposition has also been shown to inhibit respiration and TBCA via altered plant biomass allocation and shifts in saprotrophic community structure and function. Given the finding that N status of these forests is primarily related to BD abundance rather than historically high atmospheric N inputs, I tested multiple alternative hypotheses: 1) that soil CO_2 efflux and TBCA would be lowest at sites that have high N availability (and high BD abundance), 2) that these processes would instead be suppressed in sites that had previously received the highest rates of N deposition, or 3) that belowground C balance is more strongly driven by other environmental factors like climate or stand age. In 2011 and 2012 I took repeated measures of soil CO_2 efflux and litterfall at each of the seven study sites and scaled these measurements to evaluate site-level variation in annual belowground C flux. Using a C mass balance approach, I estimated TBCA as the net sum of C release via soil respiration minus C inputs via litterfall. Similar to the results of *Chapter 2*, I found that the variation in soil respiration and TBCA over the two-year study interval was more strongly associated with differences in BD RIV ($R^2 = 0.81$) than any other environmental factor, including N deposition ($R^2 < 0.09$) (Figure 3.5). Contrary to my initial hypothesis, however, I found that soil respiration and TBCA were positively (not negatively) correlated with increasing N availability. This result was unexpected since plants tend to allocate a greater proportion of gross primary production (GPP) to belowground structures when resources (like N supply) are low. On the other hand, I did not account for GPP in these stands, so it remains an open question whether the ratio of TBCA/GPP was in fact negatively correlated with N status. Despite this limitation in my methodology, I found consistent evidence that differences in tree species affected not only N status but also belowground C cycling at these forest sites, suggesting that potential shifts in community composition may result in enhanced soil C efflux as well as increased C allocation belowground.

Finally, in *Chapter 4* I used a biometric-based approach to estimate annual rates of red spruce growth along the study transect, and examined whether these growth responses could be explained by differences in C balance of foliage and woody tissue. Since red spruce is among the more sensitive species to acid deposition, I hypothesized that the long-term impacts of N deposition may result in reduced aboveground growth by red spruce trees, despite the evidence that N deposition has declined over recent years. However, given the finding that BD tree species had an overriding control on N availability and belowground C balance, I also examined whether these red spruce growth responses were instead linked to species composition, or whether another environmental factor (e.g. climate or stand age) had a more important role. In 2011 and 2012, I measured red spruce growth at each site by applying allometric relationships to dendrometer-based measurements of tree diameter. I also estimated the contribution of red spruce fine litterfall (leaves plus woody tissue < 1 cm in diameter) to total litterfall of all overstory trees by separating red spruce litter from the larger sample of canopy litter. These samples were then composited for each site, dried, and analyzed for C content in order to calculate red spruce aboveground growth (ABG), which is the annual sum of red spruce biomass increment plus litterfall C (kg C y^{-1}). During this two-year study interval, I also took repeated measurements of net photosynthesis and dark respiration of red spruce foliage and stems to determine whether red spruce growth patterns could be explained by the C balance of aboveground biomass. In contrast with my initial hypotheses, I found that the observed variation red spruce ABG was positively correlated with differences in mean annual temperature (R^2 = 0.92) and stand age (R^2 = 0.58) (Figure 4.2), but was unrelated to both N deposition and N status (Table 4.1). Moreover, we found a weak negative correlation between red spruce ABG and the ratio of foliar dark respiration to net photosynthesis (R^2 = 0.54); whereas, ABG was not associated with differences in wood respiration (Table 4.2). Thus, these results suggest that red spruce growth in these forests is more strongly limited by cooler growth temperatures than by N status or N deposition.

In summary, I did not observe any evidence that N deposition has had cumulative, long-term effects on patterns of N availability, belowground C balance, and red spruce growth in these stands. Instead, I found that the variation in tree species composition had an overwhelming influence on soil N and C cycling, which was likely the result of differences in the quality and decomposability of aboveground litter. Since red spruce litter is of poor quality (i.e. high C:N) and is highly recalcitrant to decay, sites that were dominated by red spruce tended to have lower N availability and lower rates of soil C release. In contrast, sites that had a higher abundance of broadleaf deciduous tree species (e.g. yellow birch and red maple) tended to display characteristics of a more rapid nutrient economy, with higher rates of soil respiration (which partially reflects decomposer activity) and greater N availability. Although total belowground C allocation was positively correlated with N status, aboveground red spruce growth varied primarily as a function of growth temperatures and stand age. Moreover, I found that red spruce growth was negatively associated with the relative balance of C uptake via foliar net photosynthesis and C losses via autotrophic respiration. These result suggests that red spruce growth depended more on favorable climatic conditions than species-related differences in soil fertility.

The results of this research project highlight the importance of tree species composition as a driving force for C and N cycling dynamics in temperate forest ecosystems. In addition, despite being among the more sensitive tree species to the negative effects of N deposition, these red spruce stands appear to be currently unaffected by N deposition, which could be the result of steep declines in pollutant emissions for the central Appalachian Mountains. On the other hand, my research did show that red spruce growth was a) sensitive to climate and b) a "passive observer" to differences in N availability. Given that anthropogenic climate change is projected to cause widespread shifts in tree species composition in the future, it is expected that red spruce will eventually be outcompeted and replaced by the advancement of broadleaf deciduous species migrating to higher elevations. As a consequence, these species shifts could facilitate higher rates of C and N mineralization through increased decomposition of soil organic matter (SOM), resulting in a significant release of soil CO_2 to the atmosphere as well as potential adverse effects from enhanced N availability. Thus, while my results are encouraging in the context of red spruce recovery from pollution, it does provide some preliminary evidence of the potential outcomes that could be expected under future climate change scenarios.

Appendix. Supplementary Tables and Figures.

Table S2.1. Community composition for the seven red spruce stands in the central Appalachian Mountains. Species composition was determined using the point-quarter sampling method during May 2014.

		Relative Importance Value (%)						
Species	Common name	FLR	GKB	MCG	CGL	UMM	SOR	LSB
Acer pensylvanicum	Striped maple	0	0	0	1.5	0	0	0
Acer rubrum	Red maple	19.9	0	2.5	15.9	3.2	6.5	4.8
Acer saccharum	Sugar maple	0	0	0	0	2.7	0	0
Amelanchier canadensis	Shadblow serviceberry	0	0	0	0	0	0	1.5
Betula alleghaniensis	Yellow birch	6.2	0	37.7	12.8	0	18.6	11.7
Betula lenta	Sweet birch	0	0	0	0	2.4	0	1.5
Fagus grandifolia	American beech	0	0	1.5	12.9	2.3	0	0
Ilex montana	Mountain winterberry	0	0	0	0	0	0	2.9
Magnolia fraseri	Fraser magnolia	0	0	1.6	0	0	0	0
Nyssa sylvatica	Black tupelo	0	0	0	0	0	0	10.2
Pinus rigida	Pitch pine	0	0	0	0	0	0	5.2
Picea rubens	Red spruce	71.2	100	20.5	56.9	73.8	27.7	39.8
Prunus serotina	Black cherry	0	0	6.5	0	15.6	12.5	0
Tsuga canadensis	Eastern hemlock	2.6	0	29.7	0	0	34.7	22.4
All Broadleaf Deciduous Species		26.2	0	49.8	43.1	26.2	37.6	32.6
Shannon Diversity Index		0.79	0	1.39	1.23	0.66	1.46	1.7

Table S2.2. Soil descriptions of red spruce study sites. Soil pH determined from 10 g subsamples of homogenized mineral soil (5 cm depth) collected from each site during September 2012. Courtesy: Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for Pocahontas, Randolph and Tucker Cos., WV and for Giles Co., VA. Available online at http://soildatamart.nrcs.usda.gov. Accessed 6 Dec 2012.

Study Site	Soil Series	Taxonomic Class	Drainage Class	Description	Soil pH
FLR	Gauley	Frigid Typic Haplorthods	Moderately deep, well drained	Loamy-skeletal, siliceous, superactive	3.24
GKB	Leetonia	Mesic Entic Haplorthods	Deep, well to excessively drained	Sandy-skeletal, siliceous	3.33
MCG	Ernest	Mesic Aquic Fragiudults	Very deep, moderately well to poorly drained	Fine-loamy, mixed, superactive	3.84
CGL	Snowdog	Frigid Typic Fragiudepts	Very deep, moderately well drained	Fine-loamy, siliceous, active	3.75
UMM	Mandy	Frigid Typic Dystrudepts	Moderately deep, well drained	Loamy-skeletal, mixed, active	3.37
SOR	Snowdog	Frigid Typic Fragiudepts	Very deep, moderately well drained	Fine-loamy, siliceous, active	3.32
LSB	Lily (LB Complex)	Mesic Typic Hapludults	Moderately deep, well drained	Fine-loamy, siliceous, semiactive	3.67
	Bailegap (LB Complex)	Mesic Typic Hapludults	Deep, well drained	Fine-loamy, siliceous, semiactive	3.67

Table S2.3. Nutrient supply rates (means and standard error) for each site measured using PRS, Plant Root Simulator™-probes. Data was collected across a 59-day interval during June 2011. Values with an asterisk (*) are significantly greater ($p < 0.05$) than remaining red spruce sites. Italicized values are below method detection limit (MDL) for each measured nutrient.

		PRS™-Probe Supply Rate (μg 10 cm^{-2} 59 days^{-1})						
Nutrient	Statistic	FLR	GKB	MCG	CGL	UMM	SOR	LSB
	N	*4*	*5*	*5*	*5*	*5*	*5*	*5*
Total N	Mean	31	24	100*	92*	30	28	28
	StdErr	5.3	4.1	25.5	12.8	5.2	9.6	7.6
NO_3-N	Mean	21	11	73*	64	21	14	16
	StdErr	5.3	2.4	25.8	13.2	4.1	7.8	8.3
NH_4-N	Mean	10.5	12.8	27.8*	28.5*	9.3	13.7	11.2
	StdErr	0.6	2.5	5.7	8.1	1.4	1.9	0.7
Ca	Mean	266	101	595*	198	102	230	215
	StdErr	70	21	139	43	27	48	55
Mg	Mean	102	43	157*	71	59	130	94
	StdErr	25.1	7.4	30.5	12.5	15.2	28.6	22.3
K	Mean	333	177	146	408*	401*	316	195
	StdErr	71	24	23	59	91	66	22
P	Mean	4.6	1.8	1.5	9.2	6.4	1.9	7.1
	StdErr	1.9	0.6	0.2	3.9	2	0.4	2.1
Fe	Mean	1.5	1.9	5	8.6	9.9*	4.4	6.2
	StdErr	0.1	0.1	1.6	1.7	1.7	1.6	2.7
Mn	Mean	43.3	14.7	62.4*	45.4	11.5	11.4	7.3
	StdErr	7.6	4.4	11.3	14.8	3.6	1.7	0.7
S	Mean	115	179*	144	86	71	57	68
	StdErr	5.7	47.1	22.1	13.1	9.3	7.9	10.7
Pb	Mean	0.9	1.5	3.2*	0.9	0.7	0.8	1.4
	StdErr	0.1	0.2	0.3	0.1	0.1	0.1	0.3
Al	Mean	15	15	36	45*	43*	29	37
	StdErr	1.7	0.5	6.2	9.5	4.3	4.2	6.4
Cd	Mean	*0.04*	*0.06*	0.30*	*0.04*	*0.02*	*0.04*	*0.05*
	StdErr	0.01	0.01	0.05	0.01	0	0.01	0.01

Table S2.4. Geology of red spruce study sites. Associated formations are shown in parentheses. Data derived from geological maps of West Virginia (ca. 1968) and Virginia (ca. 1993), courtesy of the WV Geological and Economic Survey.

Study Site	Period	Group	Type
FLR	Pennsylvanian (Mississippian)	Pottsville (Mauch Chunk)	Sandstone (Shale)
GKB	Pennsylvanian	Pottsville	Sandstone
MCG	Pennsylvanian	Pottsville	Sandstone
CGL	Pennsylvanian	Pottsville	Sandstone
UMM	Devonian	Chemung (Pocono)	Shale (Sandstone)
SOR	Devonian	Chemung	Shale
LSB	Silurian-Devonian	Silurian-Devonian Undivided	Sandstone (Limestone)

Table S2.5. Coefficients of determination (R^2) for bivariate regression models between field-based indices of soil N availability and specific N cycling rates from laboratory incubations.

Index	Fit	Nitrification	Mineralization
PRS^{TM} NH_4^+	Lin	0.75*	0.83**
PRS^{TM} NO_3^-	Lin	0.63*	0.70*
PRS^{TM} Total N	Lin	0.67*	0.75*
Foliar %N	Exp	0.19	0.23
Foliar $\delta^{15}N$	Exp	0.76*	0.85**
Organic $\delta^{15}N$	Exp	0.45	0.67*
Mineral $\delta^{15}N$	Exp	0.52	0.84**
Organic C:N	Exp	0.67*	0.86**
Mineral C:N	Exp	0.71*	0.94***

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Figure S2.1. Linear regressions of mean net N mineralization and net nitrification rates (± std. error bars; *n* = 2) versus total dissolved inorganic N (DIN) supply rates (measured using PRS^{TM} probes across a 59-d burial period) for each study site along a gradient of atmospheric N deposition (*n* = 7). *, $p < 0.05$; **, $p < 0.01$.