Does enhanced nitrogen input affect the structure and composition of forest vegetation? Results from long-term experiments at the Fernow Experimental Forest

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Abstract

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The effects of nitrogen (N) deposition have been studied in many ecosystems, and one general pattern of response that has emerged from these studies is a decline in species richness. Globally, anthropogenic deposition of N has more than doubled the ambient input of N in terrestrial ecosystems, and the rate at which humans produce reactive N continues to increase annually. As a result of both the current and projected future rates of N input, N deposition is a threat to biodiversity worldwide and is projected to contribute, along with other global change factors, to species extinction. To prevent the loss of biodiversity from N deposition, it is critical to understand the mechanisms by which N deposition causes species loss. In this dissertation I examined some of the direct and indirect mechanisms by which N can influence species richness in the forest herbaceous layer. In *Chapter 1* I outline the effects of N on the herbaceous layer and introduce the long-term fertilization studies at the Fernow Experimental Forest that I used to study these effects. In *Chapter 2*, I tested the rigor of an undocumented method for estimating cover in the forest herbaceous layer. The method was very precise and, when calibrated, potentially accurate for comparison of cover among sites. In *Chapter 3*, I investigated if the interaction between N fertilization and light led to the dominance of *Rubus* spp. in stands of an aggrading forest. Results indicated that added N significantly enhanced the cover of *Rubus* spp. in the forest herbaceous layer only at intermediate and high light levels. In *Chapter 4*, I tested the contribution of two hypothesized mechanisms for species losses (non-random and random species loss) in N-fertilized, N-fertilized and limed, and unfertilized plots. Both mechanisms influenced species richness, with non-random loss becoming the main mechanism over time. I observed that advantages were conferred to some nitrophilic species – particularly *Rubus* spp. – whereas disadvantages were observed in non-nitrophilic species. Additionally, my results indicate that the effect of N on some herbaceous species could be mitigated with the addition of lime. In *Chapter 5*, I examined a potential indirect effect of N fertilization on the herbaceous layer by investigating differences in storm damage experienced by trees growing in N-fertilized, N-fertilized and limed, and unfertilized plots. Trees growing in N-fertilized plots were more susceptible to damage from a wind-storm and the extent of damage depended on the species of tree. There was also evidence that the addition of lime could mitigate the susceptibility of trees to storm damage in N-fertilized plots. As a result of this study, I hypothesized in *Chapter 6* that differential damage to trees among fertilized and unfertilized treatments could result in differential changes in the herbaceous layer, either by increasing the frequency or size of canopy gaps, or by altering the species composition of trees. Overall, this research offers strong support for the idea that both direct and indirect mechanisms will influence species richness in the forest herbaceous layer under the projected increases in global N deposition.

For my Mom, the exemplar of a pioneering spirit

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Chapter 1. Introduction: Nitrogen deposition in temperate broadleaf deciduous forests

1.1 The effects of elevated nitrogen deposition on species composition

Nitrogen and species composition

Nitrogen (N) availability often constrains plant primary productivity worldwide (Vitousek and Howarth 1991), and N additions to terrestrial ecosystems can alter the composition of plant communities in ways that decrease species richness (De Schrijver et al. 2011). Nitrogen additions can change species composition by two primary mechanisms – accelerated mortality across all species (random species loss; RSL) and differential mortality between species, due to advantages (or disadvantages) that are manifest after changes in N availability (non-random species loss - NRSL; Grime 1973; Newman 1973; Goldberg and Miller 1990). These two mechanisms can act simultaneously under enhanced N input to change species composition in favor of few nitrophilic species (Gilliam 2006). So while limited N availability constrains productivity, alleviating N-limitation appears to constrain species richness and, by extension, biological diversity (Suding et al. 2005).

The effects of enhanced N input in terrestrial systems are bipartite. Providing N both alleviates limitation of a critically limiting nutrient and acidifies soil (Driscoll et al. 2001). Nitrogen fertilization and acidification effects can operate concomitantly to alter plant species composition through RSL and NRSL. Differential competition among species emerges because some species may be able to utilize N to outcompete neighbors for light (Hautier et al. 2009), or are better able to endure the effects of soil acidification (Peppler-Lisbach and Kleyer 2009). However, the fertilization and acidification effects of N may, in turn, indirectly affect plant species by altering soil microbial communities (Waldrop et al. 2004) and decreasing litter decomposition rates (Janssens et al. 2010).

Globally, the rate of N input in terrestrial ecosystems has more than doubled due to human activities, including enhanced atmospheric N deposition (Galloway et al. 2004). The trend of ecosystem N input is also steadily increasing as the amount of human-created reactive N increases every year (Galloway et al. 2008). Because of its widespread deposition and its effect on biodiversity, N deposition has been identified as a threat to ecosystems (Sala et al. 2000). As a result, N inputs are likely to be one factor, among many, that may lead to an unprecedented level of species extinction in within the next 50 years (Tilman et al. 2001).

The forest herbaceous layer

The herbaceous layer is the stratum of vascular plants within forests that are one meter tall or less – including both woody and non-woody species. Herbaceous layer plants are often overlooked in forests studies (Gilliam 2007), yet their significance to species richness and diversity cannot be overstated (McCarthy 2003). While contributing to less than 1% of total forest biomass (Muller 2003) and less than 4% of forest net primary productivity (Muller 1978), the herbaceous layer is responsible for more than 80% of the total plant species richness in forests (Gilliam 2007). Disproportionate to its overall biomass and primary productivity, the herbaceous layer also mediates the timing and magnitude of forest nutrient cycling by contributing ca. 12% of total litterfall (Welch et al. 2007) with foliage that contains 30% more N and phosphorus than the foliage of trees (Muller 2003). Additionally, the forest herbaceous layer is home to many species that have been historically used in medicine (Krochmal 1968, Cavender 2006).

The effects of N additions on the forest herbaceous layer have been understudied, relative to other systems (Bobbink et al. 1988, De Schrijver et al. 2011). The dearth of research on this forest stratum may be due to the wide variety of species found there, and the lack of species specific or functional group information about them (Whigham 2004). Despite the lack of information, a general pattern of response to N additions has emerged in which a change in species composition occurs that leads to an overall decline in species richness (Hurd et al. 1998, Small and McCarthy 2005, Gilliam 2006, De Schrijver et al. 2011, Dickson and Gross 2013, Gilliam et al. in press); although, this general response is likely dependent on both the exposure time and the cumulative load of N (Gilliam et al. 2006, Emmett 2007, De Schrijver et al. 2011, Clark et al. 2013). Furthermore, Gilliam (2006) has argued that these N-induced community changes are driven by advantages that are inherent in a few nitrophilic species, and suggested a conceptual framework for the other ecological interactions that can lead to these changes (Figure 1-1).

Forest trees and their interaction with the herbaceous layer

In contrast to plants in the herbaceous layer, trees taller than one meter are responsible for most of the productivity and biomass in forests. The dominant stature of trees, relative to herbaceous layer vegetation, strongly influences species composition the herbaceous layer (Gilliam and Roberts 2014). In addition to controlling the amount of light that reaches the herbaceous layer (Nuefeld and Young 2014), certain tree species have been associated with both the composition and abundance of plants in the herbaceous layer by changing microclimate, increasing competition for nutrients, and by altering litterfall chemistry (Rogers 1981, Crozier and Boerner 1984, Whitney and Foster 1988). However, the herbaceous layer can affect the composition of

overstory tree species by acting as a filter for the recruitment of seedlings (George and Bazzaz 2014).

Much of the research into the effects of N on trees focuses on growth, carbon storage, and physiological effects at relatively high dosages (Magill et al. 2004, Boggs et al. 2005, Thomas et al. 2010). And I am aware of only one experimental study providing evidence for a negative association between N additions and species richness in trees – a small-scale fertilization experiment investigating early succession in a tropical forest (Siddique 2003, Siddique et al. 2010). The paucity of experimental evidence for the effects of N on tree composition may be due to the longevity of trees and the relatively slow rate of species turnover. Moreover, the indirect physiological effects of N on trees, and how those effects may lead to changes in tree composition through disturbance, is also understudied. Additions of N have led to changes in carbon allocation in structural tissues (Chapin 1980, Bloom et al. 1985), root mass (Nadelhoffer 2000, Ostonen et al. 2007, Jourdan et al. 2008, Kobe 2010), and increases in height, and the biomass of both stems and leaves (Miller 1981, Grier et al. 1984). And all of these physiological effects of N may leave trees more susceptible to damage from storms. Given the effects of both light and tree composition on herbaceous layer species composition, increased susceptibility to storms in trees could potentially be a large indirect effect of N on herbaceous layer richness.

1.2 The Fernow Experimental Forest

Location, climate, and vegetation

The Fernow Experimental Forest (FEF) is a 1902 ha research forest located in the Allegheny Mountain physiographic province of north-central West Virginia, near the town of Parsons. The site is thought to be representative of the more than five million ha of mixed mesophytic broadleaf deciduous forests that can be found throughout the Central Appalachian Mountain region. Tree species that are common to these forests are tulip tree (*Liriodendron tulipifera*), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), sweet birch (*Betula lenta*), and American beech (*Fagus grandifolia*). The soils at FEF are predominately fine silt loams derived from non-marine Mississippian sandstone and shale formations with limestone interbeds. Mean annual temperature at FEF is 9.2° C and the mean annual precipitation is 145.8 cm. Land-use history at FEF is well documented, and the site has never been converted to pasture and only experienced grazing in a relatively small area. The only recorded major disturbance at FEF was a site-wide timbering that took place ca. 1900. The lack of agricultural history could have led to a high level of richness at the site, relative to other experimental forests in the Northeastern U. S. (Foster et al. 1998). However, it is likely that the broad-scale timbering left lasting effects on vegetation and nutrient cycling (Goodale and Aber 2001)[1].

[1] The description of FEF was adapted from Kochenderfer (2006).

Long-term study areas

A variety of long-term experimental compartments at FEF were used in this study (Figure 1-2). Four untreated reference areas were used – Watershed 4 (WS4; 39 ha), Watershed 10 (WS10; 15 ha), Watershed 13 (WS13; 14 ha), and the biological control area (BCA; 31 ha). WS4 and WS10 have not been treated since a timber harvest in 1905. WS13 was also cut in 1905, but an additional minor cutting occurred in 1951 (Kochenderfer 2006). The reference watersheds – especially WS4 – serve as a control for the variety of treatments that have been applied on other watersheds at FEF. The BCA is another reference area that has also not been treated since a timber harvest in 1905, however, it is not a complete headwater watershed and differs in the underlying geology from the reference watersheds. In addition to reference areas, two experimental watersheds and a series of experimental plots were examined in this study. Watershed 7 (WS7; 24 ha) was cut in two stages from 1963-1967 and maintained barren with herbicide until 1969. Since then it has been allowed to recover naturally. Watershed 3 (WS3; 34 ha) was last heavily cut between 1969 and 1972 and is currently the site of a whole-watershed fertilization experiment that was initiated in 1989. Since then, 35 kg N ha^{-1} yr^{-1} in the form of ammonium sulfate has been applied to the watershed annually by aircraft.

To supplement findings from WS7 and WS3, I carried out studies in a replicated, plot-scale experiment known as the Long-Term Soil Productivity Experiment (LTSP). The LTSP is a 4 plot × 4 block randomized block design with three treatments plots and one reference plot in each block. Each plot is ~ 0.37 ha and contains a 0.2 ha area in which measurements are made (there is a 7.6-m treated buffer around each plot). The treatments are a whole tree harvest, a whole-tree harvest plus fertilizer (35 kg N ha^{-1} yr^{-1} as ammonium sulfate, hand applied), and a whole-tree

harvest plus fertilizer and lime (22.5 kg Ca as dolomitic lime, hand applied every two years in addition to annual fertilizer applications). The addition of lime to fertilized plots is intended to mitigate the acidification effect of N, so results from these plots can be interpreted as fertilization-only effects of N, and the difference between the limed and un-limed fertilized plots can be interpreted as acidification effects due to the addition of ammonium sulfate. The tree harvest on all treatments included the removal of all aboveground biomass from the site and occurred in 1996. The reference plots have not been cut since ca. 1906 (Adams et al. 2004).

1.3 Objectives of this study

The research undertaken in this dissertation has four main objectives:

1. (*Chapter 2*) – To verify the precision and accuracy of the hand-area method of measuring cover in the forest herbaceous layer at the plant, population, and community scale.

2. (*Chapter 3*) – To determine if the effect of N on *Rubus* spp. cover in the forest herbaceous layer depends on the light level between an N-fertilized and unfertilized watershed and among N-fertilized and unfertilized plots.

3. (*Chapter 4*) – To test the extent to which the decline in species richness in the forest herbaceous layer following N fertilization was due to either random or non-random species loss mechanisms, and to assess the extent of which the decline was due to the fertilizing or acidification effects of N.

4. (*Chapter 5*) – To determine if there is an indirect effect of N on the forest herbaceous layer by measuring tree damage from wind and snow storms in a fertilized and unfertilized watershed, among fertilized, fertilized and limed, and unfertilized plots, and across a native N-availability gradient.

1.4 Tables and Figures

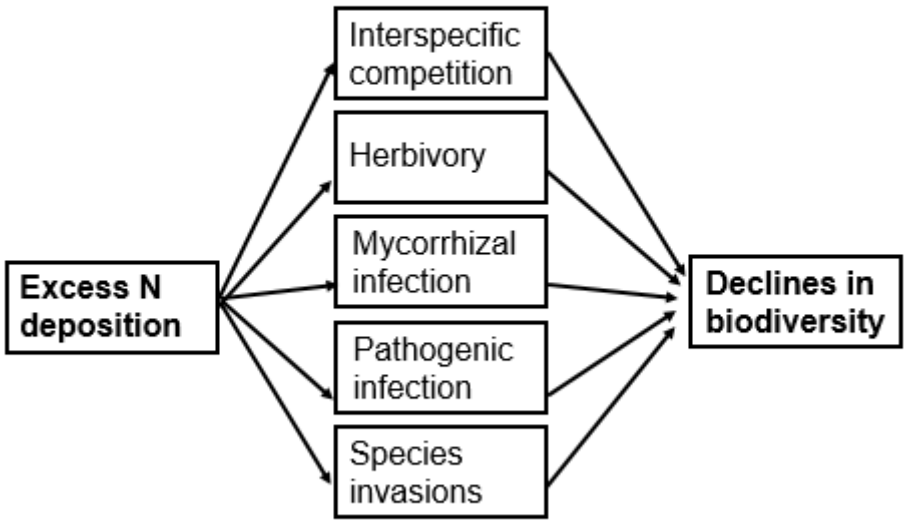

Figure 1-1. A conceptual model of the linkages and feedbacks among biotic factors that lead to declines in forest biodiversity. Adapted from Gilliam (2006).

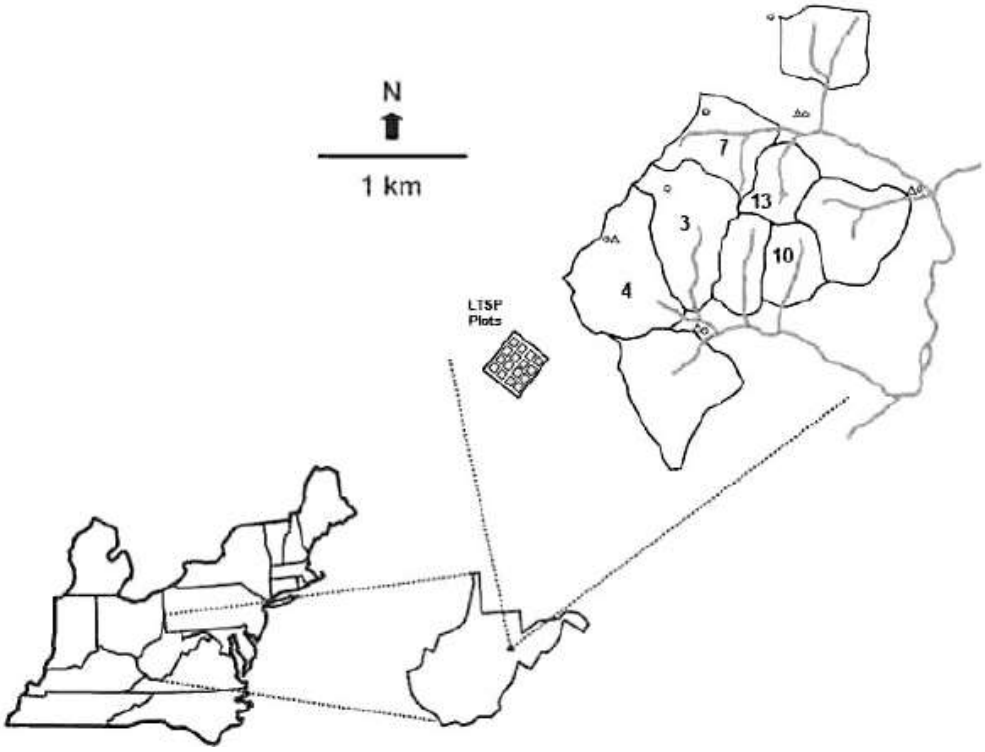

Figure 1-2. Location of the Fernow Experimental Forest and selected experimental compartments within the forest. Used with permission from Fowler (2014).

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Chapter 2. A reference-based approach for estimating leaf area and cover in the forest herbaceous layer

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2.1 Abstract

Cover data are used to assess vegetative response to a variety of ecological factors. Estimating cover in the herbaceous layer of forests presents a problem because the communities are structurally complex and rich in species. The currently employed techniques for estimating cover are less than optimal for measuring such rich understories because they are inaccurate, slow, or impracticable. A reference-based approach to estimating cover is presented that compares the area of foliar surfaces to the area of an observer's hand. While this technique has been used to estimate cover in prior studies, its accuracy has not been tested. I tested this hand-area method at the individual plant, population, and community scales in a deciduous forest herbaceous layer, and in a separate farm experiment. The precision, accuracy, observer bias, and species bias of the method were tested by comparing the hand-estimated leaf area index values with actual leaf area index, measured using a leaf area meter. The hand-area method was very precise when regressed against actual leaf area index at the plant, population, and community scales (R^2 of 0.97, 0.93, and 0.87). Among the deciduous sites, the hand-area method overestimated leaf area index consistently by 39.1% at all scales. There was no observer bias detected at any scale, but plant overestimation bias was detected in one species at the population scale. The hand-area method is a rapid and reliable technique for estimating leaf area index or cover in the forest herbaceous layer and should be useful to field ecologists interested in answering questions at the plant, population, or community level.

2.2 Introduction

Quantitative analysis of the forest herbaceous layer (all vascular plants one meter tall or less) relies on accurate estimates of the cover of plant species. Cover is broadly defined as the percent of ground area covered by individual plants, groups of plant species, or by the entire plant community. However, the term "cover" has many specific and specialized operational definitions (Wilson 2011). Regardless of which type of cover is being measured, cover data are essential in addressing several ecological phenomena including responses to experimental manipulations (Gilliam 2014), successional change (Ladwig and Meiners 2010), ecological restoration (D'Antonio and Meyerson 2002), comparisons of species diversity metrics (Thomas et al. 1999), and tracking the spread of invasive species (Didham et al. 2005).

Cover has been measured using a variety of methods. The more popular methods for estimating cover use visual estimation to assign cover-abundance classes to plants, species, or functional groups, e.g. the methods of Braun-Blanquet (1964), Daubenmire (1959), and Domin and Krajina (see Mueller-Dumbois and Ellenberg 1974). Visual estimations are done by one or more observers that determine the percentage of bare ground covered by individual plants, species, or entire communities. Although visual methods are quick, they rely on subjective classification, which can lead to errors in cover estimates as large as 20% (Sykes et al. 1983; Kennedy and Addison 1987; Hatton et al. 1986; Tonteri 1991). Furthermore, errors in the repeatability of visual estimation methods are due to observer bias that cannot be overcome by observer training (Sykes, Horrill & Mountford 1983; Kercher, Frieswick, & Zedler 2003; but see Leps & Hadincova 1992).

More accurate methods for estimating cover exist, but also have limitations that make them less than ideal for use in the forest herbaceous layer. Allometric relationships between leaf dimensions and leaf area (Wargo 1978) can be more accurate than visual estimations of cover, but this method requires both *a priori* knowledge of the allometry and extensive time measuring one or more dimensions of individual leaves. Line-intercept sampling (Tansley and Chipp 1926; Kent and Coker 1992) can be an accurate technique to measure cover that employs a transect line stretched in a random direction across an area. An observer records, for each species, the length of the line that intercepts that species. The percent cover of a species is then calculated as the distance of the line that was intercepted by that species divided by the total distance of the line and multiplied by 100. Likewise, point-intercept sampling (Drew 1944; Levy and Madden 1933; Goodall 1953) is another technique that can be more accurate than visual estimation. In point-intercept sampling, a gridded frame is placed above the sampling area and a pin is placed vertically from each grid point to the ground. The percent cover of a species is then calculated as the number of pins which intersect the species divided by the total number of pins and multiplied by 100. However, line-intercept sampling is most appropriate for more sparsely vegetated areas like shrublands (Spellman 2011), whereas the point-intercept method is subject to weather-related (e.g., wind and rain) errors in measurement, in addition to being time-consuming when carried out in plant communities with intricate architecture and high species richness (Fenner 1997; Stampfli 1991).

Finally, using ground-based, nadir-facing photography to measure cover is a relatively new method that is at least as accurate as visual methods (Dietz and Steinlein 1996; Macfarlane and Ogden 2012). Photographic methods are done by extending a tripod or frame above the sampling

area and attaching a downward-facing camera. Photographs of the sampling area are taken and the area of plants, species, or the entire community is determined using image processing software. The distinct drawback of the photographic method is that it only measures the uppermost level of vegetation (Dietz and Steinlein 1996; Vanha-Majamaa et al. 2000). While this layer of vegetation – known as "top cover" (Wilson 2011) – can be a useful metric, it is not as robust a measurement for comparing species abundances, determining species richness and diversity, nor measuring vegetation close to the ground. Thus, the photographic method would fail to accurately measure the cover of dense, rich, and structurally complex communities, such as those in the temperate deciduous forest understory.

A simple approach to measure cover is presented, whereby an observer compares the area of their hand to the area of foliar surfaces. This approach has been used successfully to measure herbaceous layer cover in contrasting forest ecosystem types and experimental manipulations. Gilliam and Christensen (1986) used this method to assess effects of varying season and frequency of prescribed burning on the herbaceous layer of a Coastal Plain pine flatwoods. It was used by Gilliam and Turrill (1993) and Gilliam et al. (1995) to quantify effects of forest harvesting on herbaceous layer communities of central Appalachian Mountain deciduous forests, with Gilliam and Turrill (1993) further combining visual estimates along with subsampling of aboveground biomass to allow for extensive non-destructive estimates of herbaceous layer biomass. A more recent focus at this deciduous forest site has been on assessing the effects of experimental additions of nitrogen (Gilliam et al. 2006). However, despite these published applications, this method has yet to be assessed quantitatively with respect to its precision and accuracy.

Accordingly, the objective of this research was to test the precision, accuracy, and potential observer bias of the hand-area method as a rapid and reliable estimate of leaf area and cover in the forest herbaceous layer at three scales: (i) individual plants, (ii) individual populations in small plots, and (iii) the entire plant community in small plots.

2.3 Methods

Study area and species selection

The cover of individual plants, individual populations, and the entire plant community within small plots was estimated, and measured, in the West Virginia University Core Arboretum – a 36.8-ha deciduous forest preserve in the north-central Appalachian Mountain Mountain region of West Virginia, USA (39.6460º N, 79.9801º W). The Core Arboretum supports predominantly mixed mesophytic forest stands of variable age ranging from early successional to old-growth. The dominant tree species include white oak (*Quercus alba*), red oak (*Quercus rubra*), shagbark hickory (*Carya ovata*), pignut hickory (*Carya glabra*), American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), and white ash (*Fraxinus americana*). Similar to other mesophytic forests, the herbaceous layer at the Core Arboretum is diverse, with over 300 non-woody vascular plants present. Vegetation in the herbaceous layer is primarily a mixture of annual, perennial, and biennial forbs, and woody tree seedlings. *Rubus allegheniensis* plants that were being grown at a nearby experimental farm were also included in this study. *R. allegheniensis* plants were grown and measured at the West Virginia University Agronomy Farm (39.6595º N, 79.9028º W), located four miles east of the Core Arboretum. The ability to accurately estimate the cover of *R. allegheniensis* is of particular importance because this species has become increasingly dominant in the herbaceous layer of Appalachian Mountain Mountain forests following enhanced nitrogen inputs (Gilliam 2014; *Chapter 3*).

Experimental design

In order to examine the accuracy and precision of the hand-area method (HA), herbaceous layer cover estimates were compared to measurements made using a leaf area meter (LI-3100, LI-COR, Nebraska, USA). Since cover is typically defined as the proportion of the ground covered by a particular species (i.e. leaves that overlap are not measured separately), measuring each leaf of that species on a leaf area meter would overestimate cover. To work around this potential for overestimation, I measured a particular type of cover using the hand-area method – leaf area index (LAI) which is the leaf area of a plant, population, or community per unit ground area (Wilson 2011). Herbaceous layer LAI was estimated *in situ* using HA at four randomly chosen sites along a transect within the Core Arboretum and at the West Virginia University Agronomy Farm. I defined the herbaceous layer as all vascular plants one meter tall or less (Gilliam and Roberts 2003). Once *in situ* LAI estimates were completed using the hand-area method, the plants were clipped at the base and placed in paper bags for transport to the leaf area meter. The plants were then removed from the bags and the leaves were removed from each plant and passed through the meter to obtain measurements of true LAI. Thus, at the plant, population, and community scale, I had both an estimate of LAI from the hand-area method (LAI_E) and the measurement of actual LAI (LAI_A) from the leaf area meter.

Within each arboretum site, four randomly selected 1-m^2 circular plots were surveyed. Two sites were chosen to estimate the LAI_E of each plant of a randomly selected species in order to test the accuracy of our method at the scale of individual plants. This resulted in the use of 21 plants from four species (*Solidago* spp., *Acer rubra*, *Prunus serotina*, and *Carya glabra*). At the same two sites I also estimated the total LAI_E of each species found in every plot in order to assess the

accuracy of our method at the scale of individual populations. A total of 20 different species were used at the population scale, seven tree species, two woody vine species, and 11 herbaceous species. In the other two sites, I estimated only the total LAI_E of all plants regardless of species in order to assess the accuracy of our method at the scale of the entire plant community found in the small plots. Finally, to strengthen our assessment of this method for estimating the leaf area of individual plants, I used hand-area method to estimate the LAI_E of 42 *R. allegheniensis* plants that were being grown in pots at the West Virginia University Agronomy Farm under a variety of light and fertilizer treatment combinations. At each scale, the plants were harvested and analyzed with a leaf area meter to measure LAI_A.

Hand-area method

The hand-area method (HA) compares the area of a hand with the area of the individual leaves of a plant, a species, or a community. An observer places a hand, palm-down and fingers closed, directly above the foliar surface of a plant. The observer then determines the size of leaf surfaces in relation to their hand (Figure 2-1), either individual leaves or clusters of smaller leaves in increments as small as 0.5 hands. After the total leaf area for each plant was estimated using HA, an observer should have touched all leaves of that plant, comparing their hand area to the leaf area. Likewise when estimating the LAI_E of a population, the observer would have touched all of the leaves of that species and all of the leaves in the entire plot when estimating the LAI_E of a plant community. To improve both the precision and accuracy of the method, two observers made hand-area estimates separately (either at the plant, population, or community scale), then compared their estimates and recorded the average of the two estimates – a process known as active feedback (Wintle et al. 2013). Observers used only their dominant hand for all

measurements and traced the outline of their hands on paper and analyzed them using the leaf area meter to determine the actual area of their hands.

Statistical analysis

To assess both the precision and accuracy of the hand-area method, LAI_E was regressed against LAI_A at the individual plant, population, and community scales. The precision of HA was evaluated using the coefficient of determination (R^2) from regression models, with higher R^2 values indicating a greater precision because LAI_E explained more of the variance in LAI_A.

The accuracy of the hand-area method was assessed by comparing the slopes of regression lines to the 1:1 line using two-tailed, one-sample t-tests. I determined the 1:1 line using the weighted average of the measured hand areas of all observers. The weighted average was used because some pairs of observers measured more plants or plots than others. Slopes significantly lower than the slope of the 1:1 line indicate that HA overestimated LAI_A, and slopes significantly higher than the 1:1 line indicated HA underestimated LAI_A. To test if the accuracies were equal across the plant, population, and community scales, the slopes of regression lines were compared to each other in a pairwise fashion using multiple analysis of covariance tests (ANCOVA; model effects: LAI_E and $LAI_E \times$ scale) without an α-level correction for family-wise error. An α-level correction was not used because it inflates the type-II error rate and increases the likelihood of reporting falsely that HA is equally accurate across all scales (Saville 1990).

In addition to testing the precision and accuracy of our method, I also tested for any species-related and observer biases. I tested for a species-related bias at the individual plant and population scales by comparing the residuals of the LAI_A vs. LAI_E regression line in a one-way analysis of variance (ANOVA; model effect: species). Species with significant negative mean residual distances indicated that HA overestimated LAI_A relative to the regression, and species with significant positive residual distances indicated HA underestimated LAI_A. Species that were only observed once were not included in residual analysis because ANOVA requires a sample size of at least two for each species comparison. A *post hoc* Tukey's honest significant difference (THSD) test was used to compare the mean residual distance among species to determine pairwise differences.

A preliminary test of the effect of leaf morphology on the accuracy of HA was also made at the population scale. The LAI_A was regressed against LAI_E for species with three or more occurrences at the population scale – a total of six species – and the slopes of the lines (i.e. the accuracies) were compared using an ANCOVA. I consider this test to be an initial assessment because I had only 22 observations that could be used to create regression lines (LAI_A vs. LAI_E) for six species. The leaf length-to-width ratio was used as an index of leaf morphology for each species. Leaf length was defined as the length of the axis from leaf petiole to leaf tip, and leaf width was defined as the length of the longest perpendicular axis. I determined the mean ratio for 10 leaves of each species using plants growing in the Core Arboretum or using specimens from the West Virginia University Herbarium. To determine if leaf morphology had an effect on the accuracy of HA, the slopes of the regression lines of LAI_A vs. LAI_E were regressed against the

leaf length-to-width ratios, and that relationship was assessed using R^2 and a one-sample t-test to determine if the slope was different from zero.

To test for observer bias, an ANCOVA (model effects: LAI_E and LAI_E × observer pair) was used to determine if the accuracy of HA depended on the observer pair at each scale. If any significant effects of the LAI_E × observer pair term were found, then they would indicate a bias in HA for at least one observer pair. Two groups of distinct observer pairs were compared at the individual plant scale, three at the community scale, and two at the population scale. Due to the limited degrees of freedom and the complexity of the model, the ANCOVA test at the plot scale could only be applied at seven of the eight plots where all plants were measured together. Furthermore, observer bias could not be tested for leaf area estimates of individual *R. allegheniensis* plants at the West Virginia University Agronomy Farm because the same observer pair measured all of the plants. Two individual plants from the arboretum were identified as outliers using a jackknife distance test based on the multivariate mean of LAI_E and LAI_E, and they were removed from all analyses. All statistical analyses were performed using SAS JMP (SAS Institute 2013), and transformations were applied when appropriate to normalize residuals and meet parametric test assumptions.

2.4 Results

Hand-area precision

Regression of LAI_A vs. LAI_E at the individual plant, population, and plant community scales in the Core Arboretum produced R^2 values of 0.97, 0.93, and 0.87, respectively (Figure 2-2). At the scale of the entire plant community, the leaf area in eight plots was determined by estimating the cover of all plants regardless of species, and the community-scale leaf area of the remaining eight plots was determined by adding the values for the constituent populations. An ANCOVA of LAI_A vs. LAI_E for entire plant communities revealed that the effect of HA on LAI_A did not depend on whether the leaf area of the plants in the plots were estimated together or calculated by adding the estimates obtained for individual populations (one line for both cases in Figure 2-2d). However, the regression of LAI_A vs. LAI_E in the eight plots where the leaf area of the plants was estimated together had an R^2 of 0.80, and in the eight plots where the total leaf area was estimated by adding the values for the constituent populations, the R^2 was 0.95. For individual *R. allegheniensis* plants grown at the agronomy farm, the R^2 was 0.94.

Hand-area accuracy

For individual plants, populations, and entire plant communities, one-sample t-tests confirmed that the slopes of the regression lines of LAI_A vs. LAI_E were all lower than the 1:1 line that was calculated using the weighted mean hand-area of observer pairs (i.e., LAI_E overestimated LAI_A; Figure 2-2). For individual plants in the Core Arboretum, the slope was 39.4% lower than the 1:1 line ($t = 20.438$, $p < 0.0001$). However, for the individual *R. allegheniensis* plants at the agronomy farm, the slope was only 16.5% lower ($t = 3.914$, $p < 0.0001$). At the population scale, the slope of the regression line of LAI_A vs. LAI_E was 41.8% lower than the 1:1 line ($t = 20.981$, p

< 0.0001), and at the community scale, it was 36% lower ($t = 13.188$, $p < 0.0001$). Pairwise ANCOVA tests revealed that the slopes of the regression lines at the plant, population, and community scales in the Core Arboretum were not different from one another, and the mean difference between the 1:1 line and realized slopes was a decrease of 39.1%.

Species-related bias

At the plant scale, an ANOVA determined that there were no differences among species in mean deviation from the regression line of LAI_A vs. LAI_E – and thus no detectable species-related bias. However, at the population scale, there was an effect of species on residual distance ($F = 2.838$, $p = 0.0117$; Figure 2-3), and thus a species-related bias. Specifically, the *post hoc* THSD revealed that the species *Stellaria pubera* had a residual distance that was lower than *Acer rubra* ($p = 0.0029$), *Carya glabra* ($p = 0.0192$), and *Acer saccharum* ($p = 0.0339$). At the population scale, an ANCOVA determined that there was a difference among species in the slopes (i.e. accuracies) of LAI_A vs. LAI_E ($F = 4.262$ $p = 0.0245$; Figure 2-4) and a further regression revealed a negative trend between the species slopes (from the regression of LAI_A vs. LAI_E) and leaf length-to-width ratio ($t = 5.23$, $p = 0.0871$; $R^2 = 0.56$; Figure 2-4 inset).

Observer pair bias

The ANCOVA models testing observer pair bias found no effect of observer pair on the relationship between LAI_A and LAI_E at the scale of the individual plant, population, or entire plant community. Individual hand areas ranged from 115.7-159.9 cm^2, and mean hand areas of observer pairs ranged from 122.3-124.2 cm^2.

2.5 Discussion

The hand-area method of estimating herbaceous layer LAI in a deciduous forest was found to be very precise at the scale of individual plants, plant populations, and entire plant communities. As a result, this method should be very useful for quickly assessing the relative differences in leaf area index and cover that can occur through time, space, or in response to experimental treatments. For studies requiring accurate estimates of leaf area index and cover, this method should also be useful. I found that HA overestimated LAI_A at each scale, but the degree of overestimation was consistently ~39.1% across the scales I examined at the Core Arboretum (Figure 2-2b-d). Thus, at this site, accurate estimates of leaf area can be obtained by simply subtracting 39.1% from each LAI_E value in the dataset – or, equivalently by multiplying each LAI_E value by 0.609. For other investigators, and sites, it is recommended that a simple calibration be performed by harvesting a subset of the plants surveyed and measuring the actual leaf area as was done in this investigation.

Our results also suggest that greater accuracy might be achieved when the reference area (a hand in this case) more closely matches the size and shape of the leaves being measured. For example, at the population scale, the leaf area of *Stellaria pubera* was overestimated relative to estimates obtained for the three tree species in the residual analysis (Figure 2-3), and it was the most over-estimated when compared to five other species in the leaf morphology analysis (Figure 2-4). The leaves of *S. pubera* are typically less than 7.6 cm long and 3.2 cm wide, grow in opposite arrangement around a central stem, and are lanceolate in shape and sessile at the leaf base. By comparison, the leaves of tree and vine seedlings are typically more than twice as long and three times as wide, are more ovate or pinnate, and are more distinct from stems because

they grow from petioles. Thus, the morphological characteristics of the trees and vines more closely resemble those of a hand and should improve the accuracy of the hand-area method. In fact, the average length to width ratio of the observer's hands in this study was 1.79 – equal to the leaf length-to-width ratio of the most accurately estimated plant at the population scale, *Parthenocissus quinquefolia* (Figure 2-4). The idea that leaf morphology affects the accuracy of estimation techniques is also supported by Sykes et al. (1983), who found that observer error using visual estimation techniques was highest among plants with smaller and thinner leaves. Leaf morphology is also the most likely reason why greater accuracy was achieved for *R. allegheniensis* plants (Figure 2-2a). *R. allegheniensis* leaves are typically palmately compound with larger terminal leaflets and smaller lateral leaflets, and the leaflet configuration is very similar to the shape of a hand. Thus, the use of multiple reference areas for different types of leaves might be warranted but the additional effort would be unnecessary if, as in this study, a simple calibration (subtracting 39.1% from each LAI_E observation) results in a robust correction factor.

The effect of leaf morphology on estimation accuracy is not unique to HA. Visual estimation techniques attempt to minimize this error by selecting areas in which to place plots with *a priori* knowledge of species composition (Mueller-Dumbois and Ellenberg 1974). The logic behind this practice is that errors created due to particular leaf morphology will be repeated in subsequent plots. However, practitioners of HA have the potential to disregard the practice of picking plots *a priori*, and quantitatively correct for differences in morphology by using the relationship between the slope of LAI_A vs. LAI_E and a measure of leaf morphology of that species (Figure 2-

4 inset) to estimate a species-specific correction factor – instead of applying the simpler calibration factor, mentioned above, to all species at once.

In addition to the precision and potential accuracy of this method, it is noteworthy that there was no over- or underestimation bias among observer pairs despite differences among the observers in both their experience and hand area. I believe the lack of an observer bias using the hand-area method may be due, in part, to the fact that it employed active feedback which is known to improve measurement accuracy (Wintle et al. 2013). The fact that some observer pairs were trained immediately prior to sampling, while others were experienced practitioners, is an indication that this method is not only robust with respect to its accuracy and precision, but also that it is easy to learn.

The results of this study indicate HA is a precise, accurate (when calibrated), easily learned, and convenient way to assess LAI and cover in the forest herbaceous layer. Therefore, HA should be useful to ecologists who are examining questions relevant to individual plants, plant populations, and entire plant communities in either field or experimental settings.

2.6 Tables and Figures

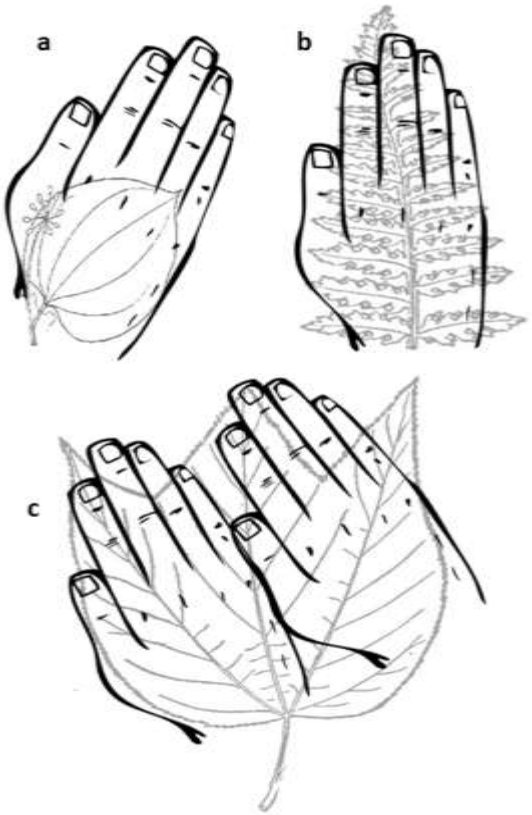

Figure 2-1. Diagram illustrating the hand-area method for measuring the leaf area index of a) *Smilax rotundifolia*, approximately 0.5 hands; b) *Dennstaedtia punctilobula*, approximately one hand; and c) *Acer pensylvanicum*, approximately 2 hands. Plant images from Britton and Brown (1913).

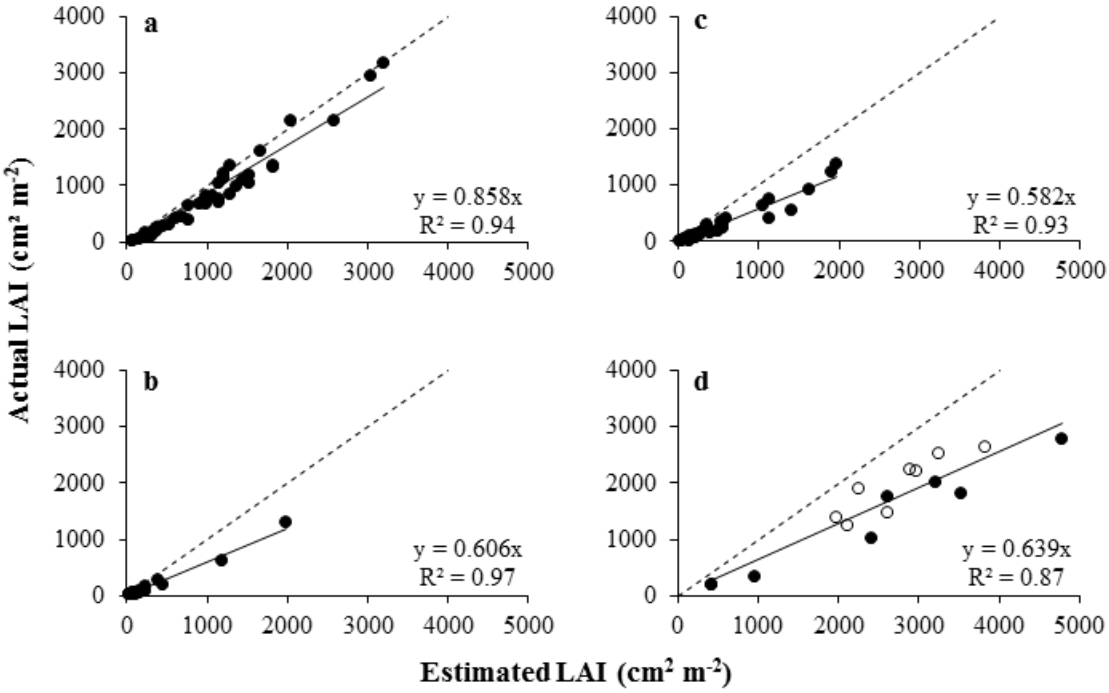

Figure 2-2. Comparisons of estimated leaf area index measured using the hand-area method vs. actual leaf area index measured via leaf area meter for: a) individual plants of *Rubus allegheniensis*; b) individual plants of four different species (*Solidago* spp., *Acer rubra*, *Prunus serotina*, & *Carya glabra*); c) populations of plants (20 different species) within 1-m^2 plots; and d) the entire plant community within 1-m^2 plots. Dashed lines are 1:1 lines, obtained using the weighted average hand area of observer pairs. Open circles in graph d indicate where all plants in 1-m^2 plots were measured together and closed circles are the sum of the populations within 1-m^2 plots. The slope of the 1:1 line equals the weighted mean area (cm^2) of the hands used to make the estimates.

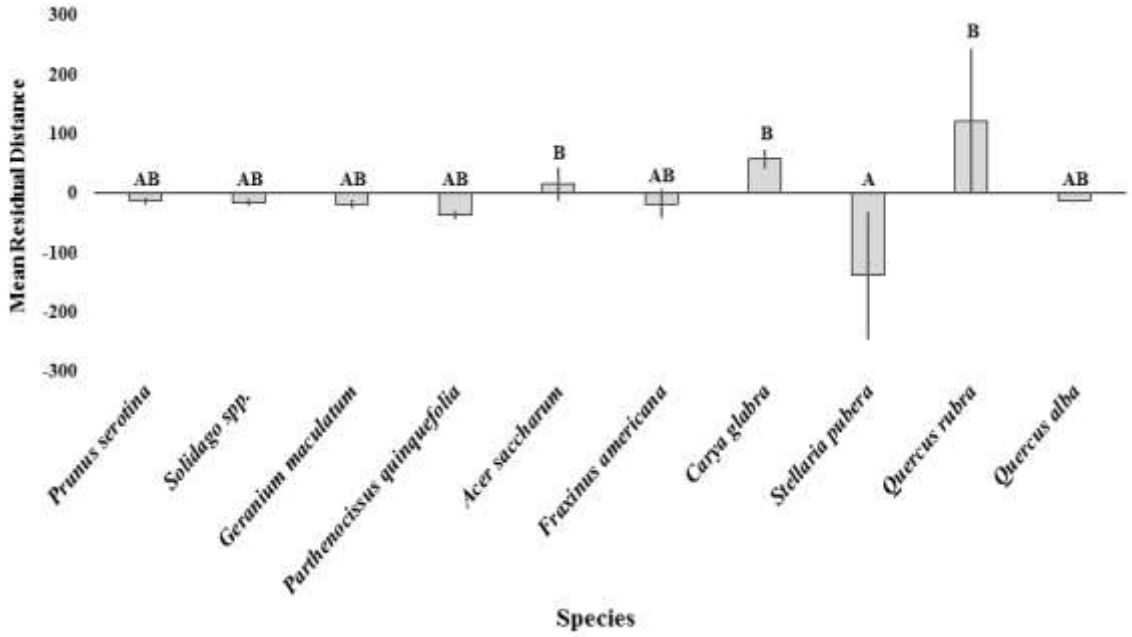

Figure 2-3. The mean residual distance (and SE) by species for regressions of leaf area index (estimated using the hand-area method) vs. leaf area index (measured via meter). Species are presented in ascending order from left to right according to the average leaf area per plant and dissimilar letters indicate significant differences ($p < 0.05$).

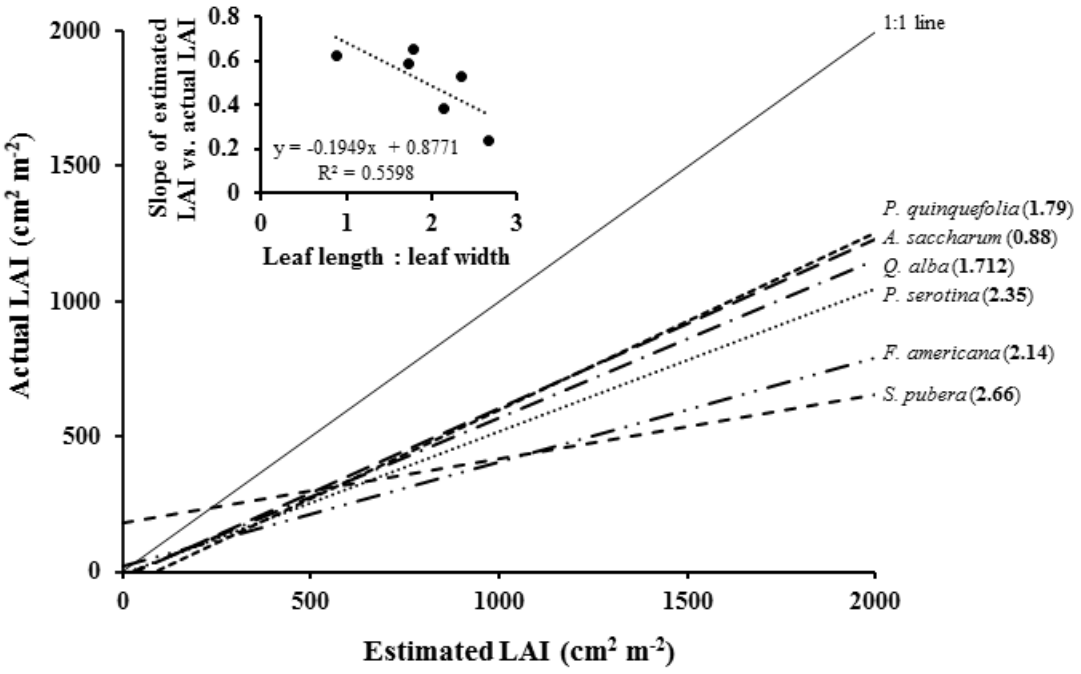

Figure 2-4. Regression lines comparing estimated leaf area index measured using the hand-area method vs. actual leaf area index measured using a leaf area meter for six separate species at the population scale. The numbers in parentheses indicate the leaf length to leaf width ratio. Inset: Leaf length to leaf width ratio vs. the slope of the estimated LAI vs. actual LAI for the same six species at the population scale.

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Chapter 3. Nitrogen fertilization interacts with light to increase *Rubus* spp. cover in a temperate forest

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3.1 Abstract

Nitrogen additions have caused species composition changes in many ecosystems by facilitating the growth of nitrophilic species. After 24 years of nitrogen fertilization in a 40 year-old stand at the Fernow Experimental Forest (FEF) in Central Appalachia, USA, the cover of *Rubus* spp. has increased from 1 to 19% of total herbaceous-layer cover. While *Rubus* spp. are generally associated with high light conditions that are created after a disturbance event, some species are also known to be nitrophilic. I investigated whether the increase in cover in *Rubus* spp. was due to either nitrogen, light, or an interaction between these two factors. To test for the effect of nitrogen and light on *Rubus* spp. cover, I compared the relative cover of *Rubus* spp. among fertilized and un-fertilized watersheds and among fertilized and un-fertilized experimental plots, using estimates of canopy openness as a covariate. *Rubus* spp. plants were also grown *ex situ* in a field experiment using a 2-way factorial design, measuring leaf area, and using two levels of nitrogen and three levels of light. The effect of nitrogen fertilization on relative *Rubus* spp. cover depended on canopy openness in the watersheds ($F = 17.57$, $P = 0.0002$) and experimental plots ($F = 25.04$, $P = 0.0047$). A similar effect for leaf area was also observed among plants grown in the field experiment ($F = 4.12$, $p = 0.0247$). Our results confirm that, although *Rubus* spp. at FEF are nitrophilic, they require sufficient light to increase their cover. Furthermore, the dominance of *Rubus* spp. in the herbaceous layer likely contributes to the observed decline in species diversity.

3.2 Introduction

Plant community changes in response to nitrogen (N) amendments have been widely observed in grasslands and heathlands (Phoenix et al. 2012; Southon et al. 2013), but less commonly in forest herbs (Gilliam 2006). However, the herbaceous layer (defined as vascular plants < 1-2 m above the ground) comprises, on average, more than 80% of the total plant species richness in forests (Gilliam 2007). Existing studies on forest herbaceous-layer communities in response to increased N deposition have documented a general decline in the cover of many species and an increase in the cover of nitrophilic species (Dirnböck et al. 2014; Suding et al. 2005). Furthermore, a negative relationship between species richness and N availability has been reported in many ecosystems (De Schrijver et al. 2011; Field et al. 2014). Nitrogen additions can change the herbaceous-layer community by increasing the likelihood of mortality in all species and simultaneously select for survival and growth of nitrophilic species (Abrams et al. 1995; Grime 1979; Rajaniemi 2002).

In the Central Appalachian Mountains at the Fernow Experimental Forest (FEF), chronic N fertilization has changed the species composition of the forest herbaceous layer in favor of one particular genus. In a fertilized watershed within the forest, the relative cover of *Rubus* spp. (the percent of total herbaceous-layer cover that is *Rubus* spp.) has significantly increased concomitantly with a substantial decrease in species diversity (Gilliam et al. 2016). Increases in *Rubus* spp. at other sites (hereafter referred to as *Rubus*) are mainly attributed to increases in light (Landhausser et al. 1997) and this genus is often dominant in recently disturbed areas (Hughes & Fahey 1991; Peterson & Pickett 1995; Peterson & Carson 1996). However, many species of *Rubus* are classified as nitrophilic (Hill et al. 1999) and forest disturbances that

enhance light availability to the forest floor typically increase N availability (Vitousek and Melillo 1979). Vegetation surveys at other sites have also documented an increase in *Rubus* cover in response to N additions (Brunet et al. 1998; Falkengrengrerup 1993; Kellner 1993), and N fertilization in large quantities has indirectly increased the amount of light received by the herbaceous layer through tree leaf and branch mortality (Magill et al. 2004). Therefore, increased N availability could both directly and indirectly affect the dominance of *Rubus*, and it seems equally likely that increases in the cover of *Rubus* could be primarily the result of more light, more available N, or an interactive effect between these two factors.

There is experimental evidence that a combination of both N and light are important in *Rubus* germination and growth. Jobidon et al. (1989) observed that the application of mulch in a clearcut balsam fir-spruce (*Abies balsamea*, *Picea mariana*) forest – a practice designed to decrease soil-available N – decreased the cover, frequency, and leaf nitrogen content of *Rubus idaeus*. In a separate study, N fertilization without canopy disturbance in a mature balsam fir-spruce forest stimulated germination of dormant *Rubus idaeus* seeds (Jobidon 1993). However, because of very low light under the closed canopy, the *Rubus idaeus* seedlings that emerged survived less than one year after germination (Jobidon 1993). These results suggest the nitrophilic nature of *Rubus*, and underscore the importance of canopy openness to their survival.

Given previous observations on the effect of both N and light on the growth of *Rubus*, the purpose of this study was to determine if the effect of N on *Rubus* cover depends on the light level in (i) the forest herbaceous layer of both fertilized and unfertilized treatments, and (ii)

among transplanted plants grown in a smaller-scale experiment. These questions were examined at FEF in two long-term fertilization experiments – utilizing the natural variation in canopy openness – and among *Rubus* plants that were transplanted and grown *ex situ* at a farm site in both fertilized and unfertilized soils, and with different levels of artificial shading to experimentally control differences in both N fertilization and light.

3.3 Methods

Study sites & experimental design

The Fernow Experimental Forest (FEF) is a 1902-ha research forest located in the Allegheny Mountain physiographic province of north-central West Virginia, near the town of Parsons (Kochenderfer 2006). Within FEF, two watersheds and a long-term, replicated experiment were chosen to carry out this study. Watershed 3 (WS3; 34 ha) was clearcut between 1969 and 1972 and is currently the site of a whole-watershed fertilization study that was initiated in 1989. Since then, 35 kg N ha-1 yr-1 in the form of ammonium sulfate has been applied to the watershed annually by aircraft (Adams et al. 2006). Watershed 7 (WS7; 24 ha) was clearcut in two stages from 1963-1967 and maintained barren with herbicide until 1969. Since 1969 WS7 has been allowed to recover naturally and serves as the unfertilized reference for WS3 in this study. To control for differences in aspect between watersheds, areas within both WS3 and WS7 were classified based on three aspect strata: 1 – "northeast", 30-90°; 2 – "south", 150-210°; and 3 – "northwest", 270-330°. In each watershed, eighteen 10-m radius plots were randomly chosen from an existing network of study sites in order to establish six plots for each of the three aspect classifications. Within each plot, five 1-m^2 circular sub-plots were randomly selected based on polar coordinates to measure the herbaceous-layer cover and averaged together in the analysis to the plot-level. I defined the herbaceous layer as all vascular plants that were growing one meter above the soil surface or less (Gilliam and Roberts 2014).

The Long-Term Soil Productivity Experiment (LTSP) is a randomized block design that includes four plots of each fertilized and unfertilized treatments (Adams 2004). Each plot is ~ 0.37 ha and contains a 0.2 ha area in which measurements are made (7.6 m treated buffer around each plot).

All aboveground biomass was removed (whole-tree harvesting) in both the unfertilized (WT) in and the fertilized plots (WT+NS) in LTSP in 1996. Since then, WT+NS plots have been treated with 35 kg N ha^{-1} yr^{-1} as ammonium sulfate, applied by hand. In the LTSP, the four replicate plots of each treatment (WT, WT+NS) were used. Within each of these plots, four 1-m radius subplots were randomly located to measure *Rubus* cover. Since the entire LTSP experiment shares the same aspect, no stratification based on aspect was necessary. In both the watershed and LTSP experiments, variation in canopy cover was assumed to be caused by ordinary forest dynamics. I also assumed that differences in soil N availability were not directly affected by canopy openings (e.g. treefalls which increased soil N) – a potential factor-on-factor interaction.

To test the relationship between N fertilization and canopy openness in a controlled setting and to mitigate any potential factor-on-factor interaction, *Rubus* plants were grown ex situ in a two-way factorial experiment with two levels of N-fertilization and three levels of shade. *Rubus* rhizomes were collected on May 27, 2014 from an untreated area adjacent to the LTSP plots and grown in full sunlight at the West Virginia University Agronomy Farm (39.6606º N, 79.9046º W; Sadhu 1989). After the rhizomes were taken from FEF, they were shaken free of soil, trimmed of fine roots, and weighed. The rhizomes were then randomly assigned a treatment and planted in 12.7 cm wide × 18.4 cm tall circular, plastic pots. The potting soil was a 2:1 mixture of PRO-MIX BX (sphagnum moss, perlite, and vermiculite) and Turface MVP (clay soil conditioner). To prevent the pots from drying quickly, the entire pot was buried so that the top of the pot was level with the top of the soil in the farm field. The pots were planted randomly in a 4×15 grid with a 1.5 m space between each pot to prevent shading between the plants. Sixty plants were initially planted – ten receiving each treatment – but some rhizomes never sprouted

canes and others died while sprouting at the beginning of the experiment. The final number of replicates for each treatment was five for low-shade/low-N, nine for low-shade/high-N, nine for medium-shade/low-N, six for medium-shade/high-N, six for high-shade/low-N, and seven for high-shade/high-N (Figure 3-1). The rhizomes were planted on May 29 and the plants were harvested on July 30, 2014.

The shade levels in the field experiment were achieved by placing wire cages above the pots and covering all sides of the cages with shade cloth. The shade cloth levels were selected based on the nominal percentage of direct light that they block and were used to simulate the broad range of light levels from canopy openings that are found in both WS3 and WS7. The low light level used 90% shade cloth, medium light level used 60% shade cloth, and the high light level used 30% shade cloth. The actual light levels achieved by these treatments were measured using HOBO pendant light sensors, model UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). Sensors were placed randomly in two pots of each shade treatment and one sensor was placed in full light to measure ambient levels. The light intensity was measured in Lux over 25 days by each of the sensors and the mean intensity recorded for each light treatment level was compared to the mean intensity measured for ambient light. These measurements revealed that the low light level received 5% of ambient light, the medium light level received 11.4% of ambient light, and the high light level was received 50% of ambient light. The two N-fertilization levels in the factorial experiment were designed to match the soil N availability in both the unfertilized WS7 (low N) and the fertilized WS3 (high N). Nitrogen was applied to the plants using a nutrient solution modified from Johnson et al. (1957; Table 1) and the low N level was half of the concentration of 200 μM N found in the soil water of a reference area at FEF

(Edwards et al. 2006). The nutrient solution in the high N level was the same except that it included an additional 35 kg N ha^{-1} as ammonium sulfate over the duration of the experiment – the same amount of fertilizer that WS3 receives annually. The nutrient solution was delivered to the plants in ten separate 500-ml applications over the course of the experiment. Therefore, in each application, the low N level plants received 100 µM of N, and the high N level plants received 1,244 µM of N (Figure 3-1).

Forest experiment measurements

Plant cover was measured in each subplot by comparing the area of the plant with the area of an observer's hand. Observers estimated the cover of herbs by placing a hand, palm-down and fingers closed, directly above the foliar surface of a plant. The observer then determined the size of the leaf in relation to their hand. The units of measure were "hands" and observers worked in pairs to estimate cover separately, then averaged their estimates together to improve precision through active feedback (Wintle et al. 2013). This method proved to be very precise when hand-measured leaves were regressed against the same leaves measured using a leaf area meter (average $R^2 = 0.94$ for individual *Rubus* plants; Walter et al. 2015; See *Chapter 2*). Two categories of plant cover were measured in each subplot – the cover of *Rubus*, and the total cover of all herbaceous-layer plants. The vast majority of *Rubus* plants at FEF are *Rubus allegheniensis*, but *Rubus idaeus* has also been observed. Since *Rubus* species hybridize and can be difficult to identify in the field, *Rubus* cover was measured on plants identified at the genus level. The relative *Rubus* cover was calculated as the fraction of all herb cover in a plot that was *Rubus*. To determine the effect of canopy light on *Rubus* cover in both the watersheds and LTSP plots, I relied on the strong association between the amount of photosynthetically active radiation

that reaches the forest floor and canopy openness (Becker et al. 1989; McCarthy and Robison 2003; Rich 1990). A spherical densiometer was used to measure the canopy openness inside each of the 1-m^2 subplots. One densiometer reading was taken in each of the cardinal directions in the subplot and averaged to estimate the mean canopy openness. The relative *Rubus* cover and canopy openness were measured in the watersheds and LTSP WT and WT+NS treatments in June 2012.

Field experiment measurements

To test for differences in plant cover, the leaf area of each *Rubus* plant was measured using a leaf area meter (LI-3100, LI-COR, Nebraska, USA). The height and dry biomass of each plant was also measured. To determine if N fertilization led to an increase in leaf chlorophyll and/or leaf N, the relative leaf chlorophyll content was estimated using a SPAD meter (SPAD-502, Spectrum Technologies, Aurora, IL, USA). SPAD was measured and on the terminal leaflet of five leaves on each plant and averaged to obtain a mean SPAD value for each plant. SPAD measurements are unit-less, and provided a non-destructive, relative measure for leaf chlorophyll and nitrogen content. To measure leaf N concentrations directly, the leaves of each plant were dried, ground, and analyzed for their N content using a Carlo Erba NCS elemental analyzer, model NA 1500. Total leaf N for each plant was then calculated by multiplying the concentration of leaf N (% N by mass) by the total leaf mass per plant.

Statistical analysis

To test if the effect of N fertilization on *Rubus* cover depended on canopy openness in the forest measurements, a two-way analysis of covariance (ANCOVA) was performed for the two watersheds (WS3 and WS7) and both LTSP treatments (WT and WT+NS). The order of the relationship (linear vs. polynomial) between canopy openness and relative *Rubus* cover in the ANCOVA models was determined by choosing models with the lowest corrected Akaike information criterion statistic. One-way analysis of variance tests (ANOVA) were used to compare the mean relative *Rubus* cover and mean canopy openness between the watersheds and LTSP treatments. To test if the effect of N fertilization on *Rubus* leaf area depended on the light level in the ex situ field experiment, a two-way ANCOVA was used to test for differences in leaf area, SPAD, percent leaf N, and total leaf N. Initial rhizome mass was used as a covariate in the ANCOVA models to correct for any contributions to growth from larger rhizomes. Student's t-tests were used for post-hoc pairwise comparisons of means because family-wise error correction in multiple comparison tests inflates the probability of Type II errors (Saville 1990) and because of the relatively small number of comparisons. All statistical analyses were performed using SAS JMP (SAS Institute 2013). Transformations to normalize residuals and independent samples ANOVA tests were applied when appropriate.

3.4 Results

Forest experiments

The effect of N fertilization on relative *Rubus* cover in the watersheds depended on canopy openness ($F = 17.57$, $P = 0.0002$). Specifically, the mean relative *Rubus* cover in WS3 was 84.2% higher than in WS7 at the highest level of canopy openness, but equal at the lowest level of canopy openness (Figure 3-2a). The best fit ANCOVA model included watershed (WS), canopy openness (C), WS × C, C^2, and WS × C^2 effects. The effect of canopy openness on relative *Rubus* cover in the LTSP treatments (WT compared to WT+NS) was also dependent on fertilization ($F = 25.04$, $P = 0.0047$). At the highest canopy openness, the relative *Rubus* cover was 85.7% higher in WT+NS when compared to the WT, but equal at the lowest level of canopy openness (Figure 3-2b). The best fit ANCOVA model for LTSP included the effects of treatment (T), C, and T × C. Overall, the mean relative *Rubus* cover was higher in both WS3 ($t = 5.71$, $P < 0.0001$) and the LTSP WT+NS treatment ($t = 2.03$, $P = 0.0444$) when compared to their unfertilized counterparts, WS 7 and LTSP WT. However, the average canopy openness did not differ between the fertilized and unfertilized watersheds nor between the LTSP treatments.

Field experiment

The effect of N fertilization on *Rubus* leaf area per plant depended on the light level ($F = 4.12$, $p = 0.0247$). The initial rhizome mass also had a significant positive effect on leaf area ($F = 5.46$, $p = 0.0253$). Post hoc comparisons using t-tests determined that leaf area at the high-N/high-light treatment was significantly greater than the leaf area of the plants grown at low-N/high-light ($t = 2.13$, $p = 0.0401$). Specifically, the mean leaf area at the high-N/high-light treatment was

130.2% greater than in the low-N/high-light treatment (Figure 3-3). Additionally, the t-tests revealed that mean leaf area at the high-N/high-light treatment was 83.3% greater than at the high-N/low light treatment ($t = 2.04$, $p = 0.0489$). The final ANCOVA model included light (L), nitrogen (N), initial root mass, and L × N as effects.

The effect of N fertilization on *Rubus* SPAD and percent leaf N did not depend on the light level and N fertilization alone did not have an effect. However, light did have a positive effect on both SPAD ($F = 4.85$, $p = 0.0138$) and percent leaf N ($F = 10.19$, $p = 0.0003$; Figure 3-4). Post hoc t-tests determined that SPAD was 16.9% higher at the high-light level when compared to low-light ($p = 0.0231$) and percent leaf N was 35.9% higher at the high-light level when compared to low-light ($t = 5.12$, $p < 0.0001$; Figure 3-4). Percent leaf N was also found to be higher at the high-light level when compared to medium-light ($t = 3.00$, $p = 0.0047$) and higher at medium-light when compared to low-light ($t = 2.25$, $p = 0.0302$). The effect of N fertilization on *Rubus* total leaf N did not depend on the light level and there were no additive effects of light or N-fertilization on the total leaf N. Yet, there was a significant positive effect of initial root mass on total leaf N ($F = 14.13$, $p = 0.0010$). Light (L), nitrogen (N), initial root mass, and L × N were included as effects in the final ANCOVA models for SPAD, percent leaf N, and total leaf N.

3.5 Discussion

In this study, I investigated the effect of N and light on the cover of *Rubus* in forest and field experiments. Chronic N fertilization in the forest experiment led to a striking increase in the relative *Rubus* cover in the fertilized watershed and LTSP plots. Species of the *Rubus* genus are typically found in abundance after forest canopy disturbances, when light levels are high (Hughes and Fahey 1991; Peterson and Carson 1996; Peterson and Pickett 1995; Phillippe et al. 2010). However, in the absence of forest disturbances or differences in canopy openness between fertilized and unfertilized treatments, the relative *Rubus* cover was considerably higher in the fertilized treatments (Figure 3-2). Yet, light was indeed an important factor, as the effect of N on the cover of *Rubus* depended on canopy openness. Therefore, the increase in the relative *Rubus* cover in the fertilized treatments was only realized because of the increase in cover in areas with higher canopy openness. The differential effect of N and light was also observed among *Rubus* plants grown the field experiment. At the highest light level in the field experiment, *Rubus* leaf area was substantially higher in the plants grown at high-N when compared to those grown at low-N (Figure 3-3). These results demonstrate that *Rubus* plants growing in fertilized areas were able to utilize the increased light from larger canopy openings to increase cover.

Interactions between light and nutrients have been documented in other herbaceous-layer plants (Baeten et al. 2010; Eickmeier and Schussler 1993; Rodriguez-Garcia and Bravo 2013) and in coniferous forest systems (Hedwall et al. 2010; Hedwall et al. 2013; Strengbom and Nordin 2012; Thomas et al. 1999), but less so in temperate deciduous forests (Gilliam 2007). Whereas light is thought to be the most limiting resource in the forest herbaceous layer (Coomes and Grubb 2000; Neufeld and Young 2014), the effect of light has been observed to be dependent on

the level of soil N (Walters and Reich 1997). However, light was the major factor affecting *Rubus* leaf N content in our field experiment (Figure 3-4). *Rubus* plants grown in the field experiment had higher leaf N concentrations in higher light regardless of their level of N fertilization. The lack of a differential effect between N and light on leaf N concentration is consistent with previous leaf research that has determined that light is the major factor controlling leaf N (Evans 1989). At the lowest level of light, fertilized plants appeared to have a lower leaf area (although not statistically significant) and the same concentration of chlorophyll (and foliar N) compared to unfertilized plants – suggesting that fertilized plants had a lower efficiency (chlorophyll-to-leaf area ratio) of light capture. At greater levels of light, this apparent difference in the efficiency of light capture was exaggerated since the leaf area of fertilized plants became greater than the leaf area of unfertilized plants while the concentration of chlorophyll did not differ from unfertilized plants. Although resource-use efficiencies can vary in response to resource availability, the exact mechanisms for the patterns I observed as light increased was not determined.

The substantial increase in the relative cover of *Rubus* under N-fertilization suggests that *Rubus* species at FEF are indeed nitrophilic (Craine 2009; Dirnböck et al. 2014). Nitrophilic plants also often have thorns and a planophilic leaf angle distribution (Craine 2009), both notable traits of *Rubus* (Balandier et al. 2013). Under N fertilization, nitrophilic plants can cause shifts in herbaceous-layer species composition through increased competition for resources (Clark et al. 2007; Cleland et al. 2008; Suding et al. 2005). Thus, plants that respond to N-fertilization by increasing cover can potentially out-compete neighboring plants for light (Newman 1973; Wilson and Tilman 1991). Specifically in *Rubus*, increases in cover at other sites have led to

decreases in tree seedling growth and survival by creating deep-shade (Balandier et al. 2013). Furthermore, the ability of *Rubus* to propagate vegetatively allows it to reproduce and spread quickly (Eilts et al. 2011) – which is likely the major factor causing the decline in diversity observed after 25 years of experimental N fertilization in WS3 at FEF (Gilliam et al. 2016).

Whereas changes in interspecific competition help to explain the dominance of *Rubus* following N fertilization, other N-mediated processes could be shifting simultaneously in the forest herbaceous layer. Higher soil N can result in increased plant N uptake which, in turn, increases the quality of plant tissue for foraging herbivores (Throop and Lerdau 2004). Increased N availability can also lead to increases in pathogenic infections (Mitchell et al. 2003; Strengbom et al. 2002), increased susceptibility to species invasion (Cassidy et al. 2004), and composition shifts in soil microbial communities (Brandrud and Timmermann 1998; Compton et al. 2004). However, the *Rubus* plants grown in the field experiment experienced neither competition, species invasion, nor obvious signs of herbivory or pathogens, and leaf area was considerably higher at the high-N treatment when compared to the low-N treatment at the highest level of light. Therefore, a shift in herbaceous-layer composition toward nitrophilic species in N-fertilized treatments at FEF is likely due primarily to a decline in the heterogeneity of soil nutrients under N fertilization (Beatty 2014; Eilts et al. 2011; Small and McCarthy 2003), and not due to other secondary N-mediated processes, consistent with the predictions of the N homogeneity hypothesis (Gilliam et al. 2016).

Overall, our results underscore the effect of both N and light on *Rubus* in the forest herbaceous layer. These effects were observed over a large span of temporal and spatial scales – from a 1-year pot experiment, a 16-year early successional plot experiment, and a 23-year aggrading forest watershed experiment. At each level, the response of *Rubus* under N-fertilization at FEF follows the pattern suggested by the soil nutrient homogeneity hypothesis, whereby a more homogeneous soil nutrient environment enhances the advantages of nitrophilic species and species richness can be reduced (Gilliam 2006). If our results are indicative of herbaceous layers in other temperate forest regions, then there is still potential for large losses of biodiversity under continued N deposition – at least, in part, driven by an increased dominance of nitrophilic species like *Rubus*.

3.6 Tables and Figures

Table 3-1. The concentrations of chemical constituents used in the nutrient solution applied to *Rubus* plants grown in the field experiment, modified from Johnson et al. (1957).

Constituent	Concentration (µM)
KNO_3	50
$Ca(NO_3)_2 \bullet 4H_20$	25
NH_4NO_3	50
KH_2PO_4	6.25
$MgSO_4 \bullet 7H_20$	12.5
KCl	20
H_3BO_3	25
$MnSO_4 \bullet H_20$	2
$ZnSO_4 \bullet 7H_20$	2
$CuSO_4 \bullet 5H20$	0.5
Na_2MoO_4	0.5
$CoCl_2 \bullet 6H_20$	0.5
$C_{10}H_{12}N_2NaFe0_8$	20

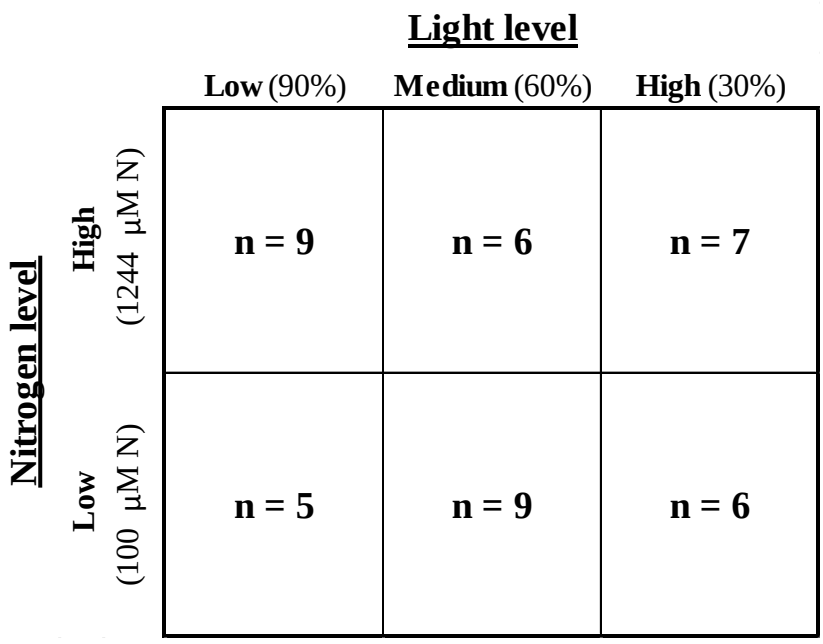

Figure 3-1. Experimental design, treatment groups, and sample sizes used in the two-way field experiment. The values within light treatment indicate the percentage of ambient light purportedly blocked by the shade cloth, and the values within nitrogen treatment indicate the amount of nitrogen delivered at each of the 10 fertilizer applications.

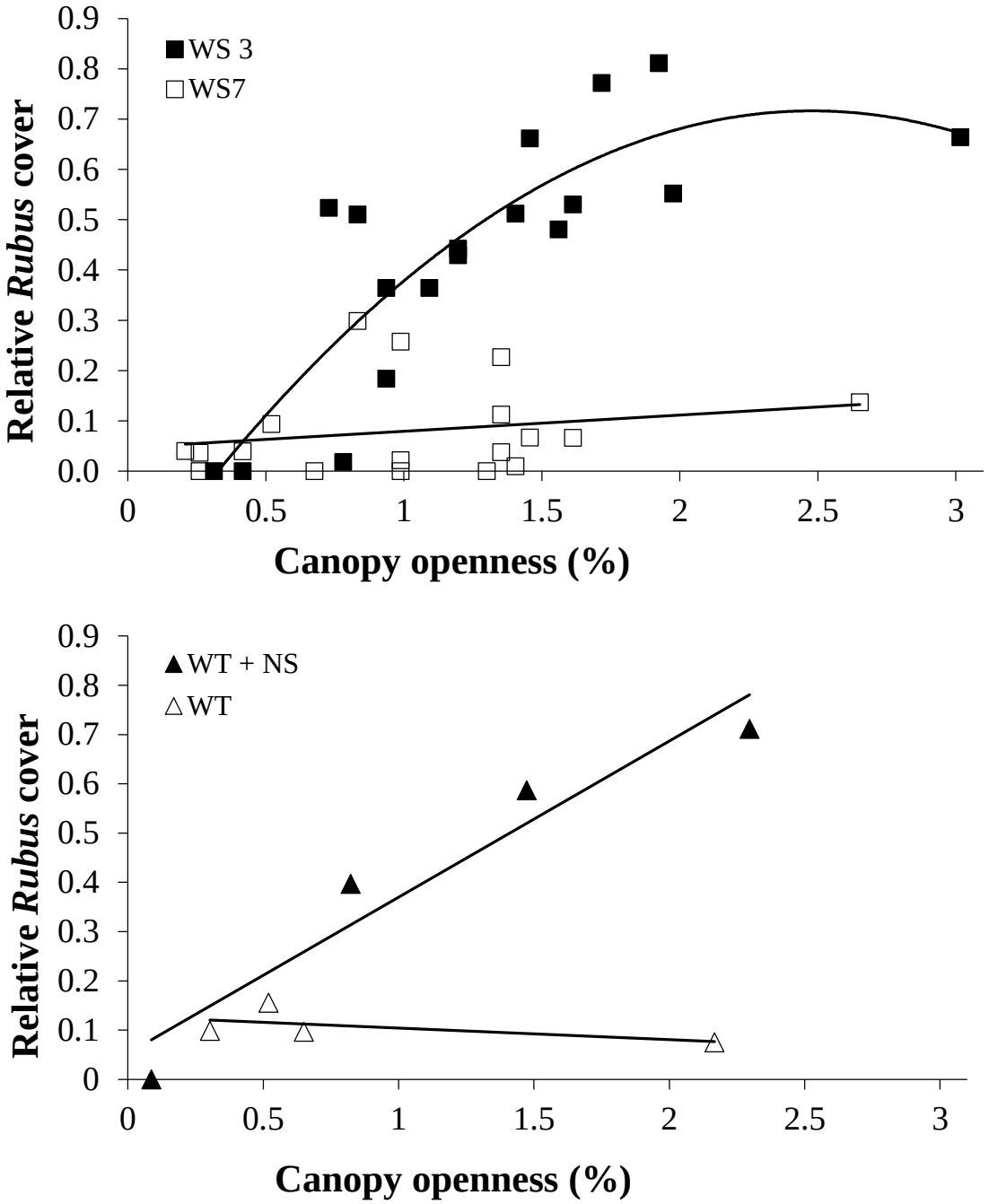

Figure 3-2. The relative *Rubus* cover (the proportion of total herbaceous layer cover in a plot that is *Rubus*) in a fertilized (WS3) vs. unfertilized (WS7) watershed (A) and in fertilized (WT+NS) and unfertilized (WT) treatments in LTSP (B) vs. canopy openness as measured by a densiometer.

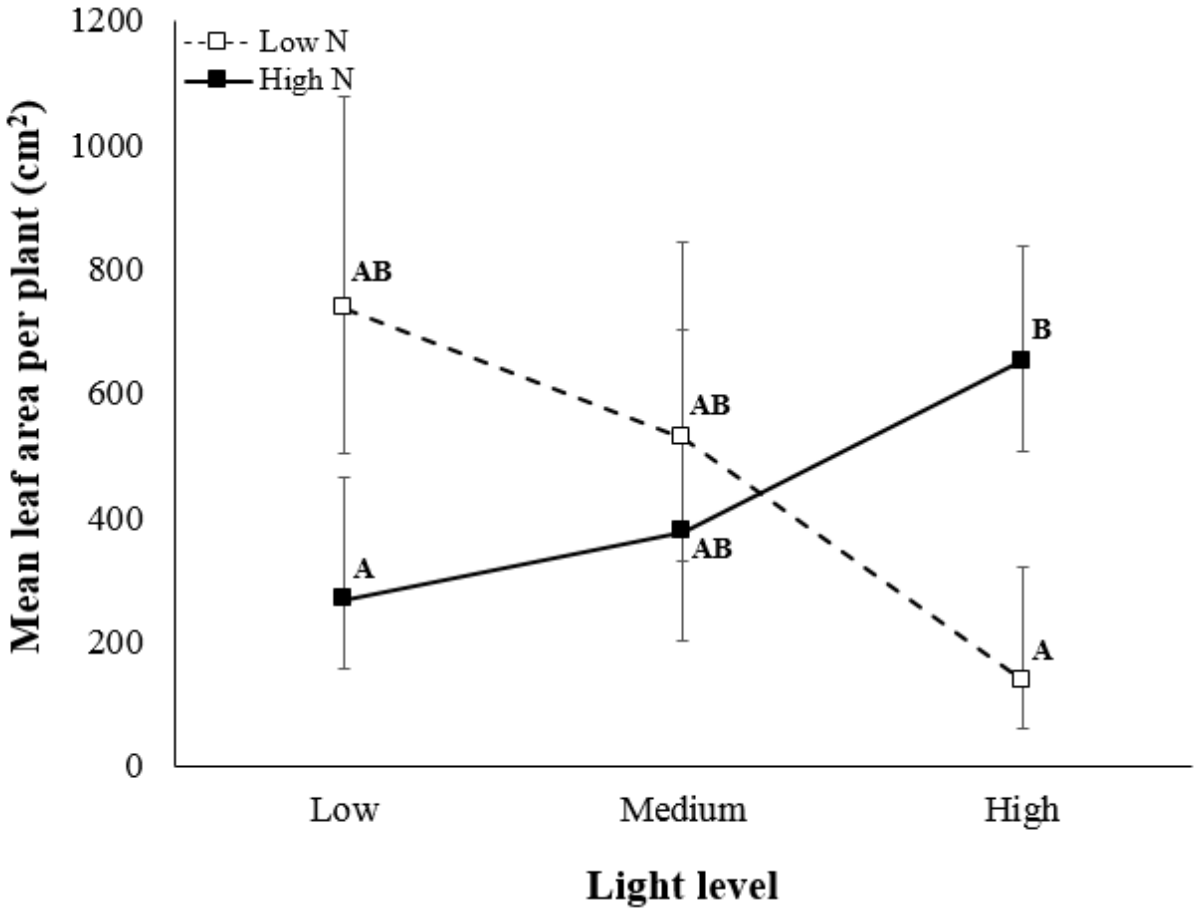

Figure 3-3. Mean leaf area per *Rubus* plant grown at two nitrogen levels and three light levels, achieved by using three densities of shade cloth designed to block a percentage of ambient light – high light used 30%, medium used 60%, and low used 90%. The means were back-transformed after analysis from log-transformed data and the error bars represent 95% confidence limits. Differing letters indicate significant differences ($p < 0.05$) between means using Student's t-test.

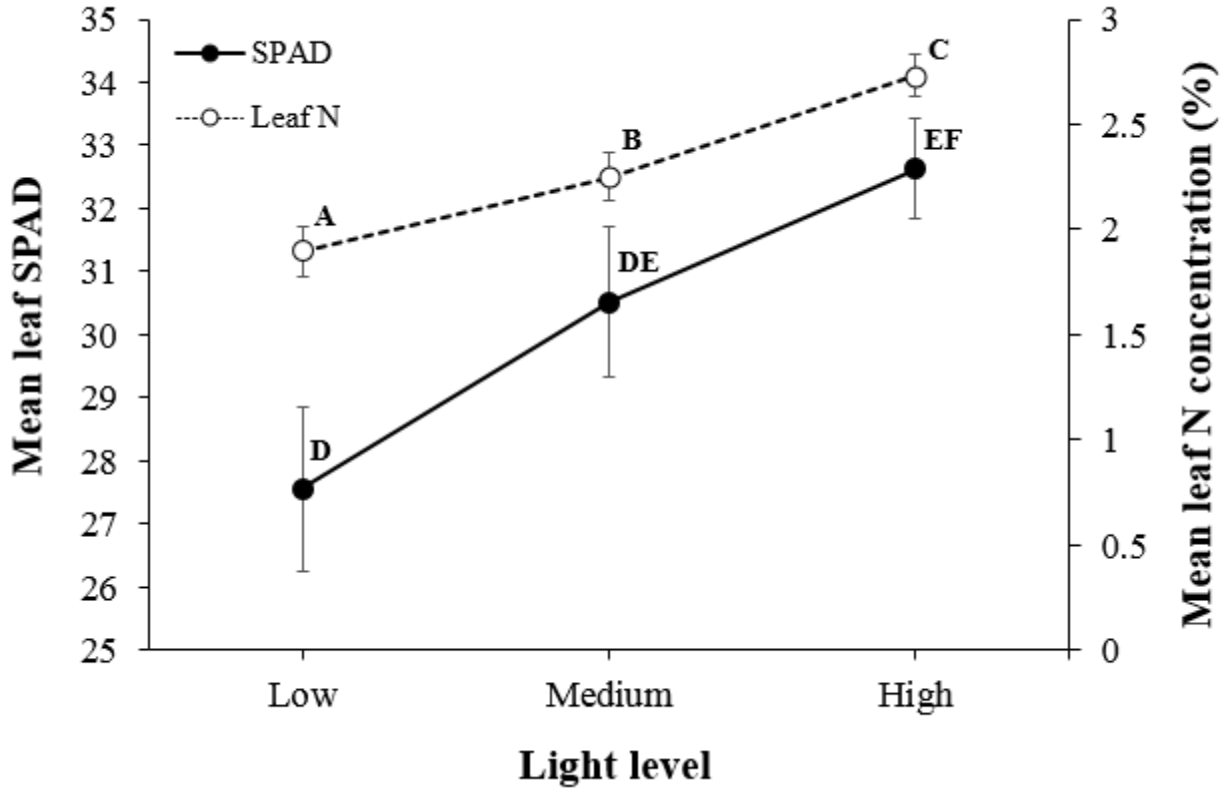

Figure 3-4. Mean *Rubus* leaf SPAD and percent leaf nitrogen in plants grown across three light levels achieved by using three densities of shade cloth designed to block a percentage of ambient light – high light used 30%, medium used 60%, and low used 90%.. Differing letters indicate significant differences ($p < 0.05$) at each light level using Student's t-test and error bars represent one standard error.

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Chapter 4. Nitrogen-induced species loss in the herbaceous layer of a broadleaf deciduous forest: A comparison of random and non-random mechanisms.

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4.1 Abstract

Nitrogen (N) additions have decreased species richness (*S*) in broadleaf deciduous forest herbaceous layers, yet the functional mechanisms for these declines have not been explicitly evaluated. I tested two hypothesized mechanisms in the forest herbaceous layer of a long-term, plot-scale fertilization experiment in the central Appalachian Mountains, USA – the random species loss (RSL) and non-random species loss (NRSL) hypotheses. Using a random thinning algorithm, I simulated changes in the density of each species under RSL and compared the simulated densities to the observed densities among N-fertilized (+N), N-fertilized and limed (+N+L), and reference (REF) plots in regenerating forest stands. I found a decline in richness among +N and +N+L treatments when compared to REF, over all of the years surveyed, and determined that many species had either an advantage or disadvantage under N additions because they occurred at densities different than those expected due to random thinning. Furthermore, I observed different responses for some species to the +N and +N+L treatments, providing evidence that species can respond to either the fertilization or acidification effects of added N. Species identified as being nitrophilic responded to N additions by achieving a greater density than what would be expected due to random thinning. However, despite changes due to random thinning in many species, the reduction in richness observed in N-fertilized treatments was a function of both RSL and NRSL. Thus, my results indicate that declines in *S* in the forest herbaceous layer under N fertilization are due to both random and non-random species loss, and that changes in composition can be influenced by the inherent advantages of a small number of nitrophilic species.

4.2 Introduction

A negative relationship between nitrogen (N) inputs and plant species richness has been reported in many ecosystems (De Schrijver et al. 2011). This relationship has been widely observed in grasslands (Dupre et al. 2010; Stevens et al. 2004), heathlands (Phoenix et al. 2012; Southon et al. 2013) and, to a lesser extent, the forest herbaceous layer (Gilliam 2006; Hurteau and North 2008). However, fewer studies have investigated the mechanisms responsible for N-mediated declines in the species richness of plant communities. Since excessive N additions can threaten the biodiversity of terrestrial ecosystems (Sala et al. 2000) and contribute to species extinction (Tilman et al. 2001), understanding how N lowers species richness is critical for developing strategies to preserve biodiversity (Suding et al. 2005).

Globally, N availability constrains primary productivity (Vitousek and Howarth 1991), and N additions usually increase plant productivity by alleviating N limitation (LeBauer and Treseder 2008). The relationship between productivity and species richness is often unimodal, where richness is highest at an intermediate level of productivity (i.e. the "hump-backed model"; Grime 1973; but see Adler et al. 2011). There are two primary mechanistic hypotheses which explain why species are lost under N fertilization at the highest levels of productivity. The non-random species loss hypothesis (NRSL) is an explanation of species loss where species that are superior in nutrient acquisition, growth rate, pathogen resistance, and other properties will displace species with inferior levels of those traits (Newman 1973; Tilman 1984; Wilson and Tilman 1993). With increased soil fertility, the superior species indirectly suppresses the growth of the subordinate species and different mortality rates between the two emerge. In contrast, the random species loss hypothesis (RSL) contends that mortality is equal among all species, and

that the change in species composition under increased fertility is an effect of enhanced density-dependent mortality, where uncommon species are lost by chance (Goldberg and Miller 1990; Oksanen 1996; Stevens and Carson 1999). Neither NRSL nor RSL are necessarily mutually exclusive and the degree to which either mechanism alters community composition varies across systems (Suding et al. 2005), scales (Gross et al. 2000), and sites (Clark et al. 2007; Gough et al. 2000).

Beyond increasing productivity, N additions also have the potential to acidify soil which, in turn, can decrease the concentration of base cations in the soil and increase the solubility of toxic metals (Vitousek et al. 1997; Driscoll et al. 2001). Thus, nitrophilic species – species that are associated with environments that have high N availability – may be able to either: 1) utilize excess N to out-compete or gain other advantages over non-nitrophilic species (Hautier et al. 2009); 2) withstand the secondary effects of soil acidification (Peppler-Lisbach and Kleyer 2009; Schuster and Diekmann 2003); or 3) do both simultaneously. However, distinguishing a species or community response between the fertilization and acidification effects of N additions is difficult because multiple, interacting soil factors may be changed with N additions that can confound expected plant responses (Schaffers and Sykora 2000), and alter the degree to which NRSL and RSL mechanisms affect species richness.

Most research testing NRSL and RSL mechanisms on species richness has been done in grassland and old-field communities (Thomas et al. 1999) – systems dominated by herbaceous plants with relatively low species richness at broad scales. While these studies have helped spur

changes in plant community theory (Fraser et al. 2014), their results may not be generally applicable to forested systems. In contrast to grasslands and old-fields, the herbaceous layer of broadleaf deciduous forests are species rich communities of mostly perennial herbs, canes, graminoids, woody shrubs, and tree seedlings. Additionally, competition within this community for light, water, and nutrients occurs both within the herbaceous layer and between herbaceous layer plants and overstory trees (Gilliam and Roberts 2014; Neufeld and Young 2014). Community changes in the herbaceous layer of forests are of critical importance for managers interested in protecting biodiversity, because this forest stratum is responsible for more than 80% of plant species richness in broadleaf deciduous forests (Gilliam 2007). Yet, to our knowledge, no tests of NRSL vs. RSL hypotheses have explicitly been done in a broadleaf deciduous forest herbaceous layer.

Accordingly, the objectives of this research were to: (1) determine the extent of N-mediated changes in plant density and species richness, diversity, and evenness; (2) explicitly test the extent to which the NRSL or RSL mechanisms could be responsible for the changes in those community metrics; (3) separate the effects of N fertilization from those of soil acidification; and (4) understand the effect of nitrophilic species on community composition under experimental N fertilization in a broadleaf deciduous forest herbaceous layer. To meet these objectives, I analyzed long-term data collected from a plot-scale fertilization experiment located in the central Appalachian Mountains, USA.

4.3 Methods

Study area and sampling

This research was carried out at the Fernow Experimental Forest (FEF) in West Virginia, USA, in the long-term soil productivity experiment (LTSP; 39.0563, -79.6979). The FEF is a 1902-ha research area that primarily contains a mixed mesophytic forest (Kochenderfer 2006). The LTSP is a four plot × four block randomized design (four plots per block) that includes three experimental treatments and one uncut area in each block (Figure 4-1). For the purpose of this research, the uncut area was not examined. The three experimental treatments were all harvested (removal of all aboveground biomass) in the winter of 1996 (Adams et al. 2004). Since 1996, four plots have been fertilized at a rate of 35 kg N ha^{-1} yr^{-1} with ammonium sulfate, applied by hand (+N). Another four plots have been fertilized with ammonium sulfate at the same rate and limed at a rate of 22.5 kg Ca ha^{-1} yr^{-1} with dolomitic lime (+N+L). And the remaining four plots have been allowed to regrow naturally with no experimental additions and are used as the reference in this experiment (REF). Each plot is 0.4 ha and contains a 0.2 ha area in which measurements are made (a 7.6 m treated buffer surrounding each plot). Herbaceous layer sampling was done by randomly selecting five 1-m circular subplots from a gridwork of 16 reference points within each plot (Figure 4-1). The herbaceous layer was defined as all vascular plants that were one meter tall or less (Gilliam 2007), and individual plants were defined as one stem coming from the ground. Within each subplot, each plant was identified and counted. The subplots within each plot were summed to determine the density of each species per five m^2. Sampling was done between the months of June and July in 1996 (prior to treatment), and during the same months in 1997, 2001, 2006, and 2011.

Community metrics

Total plant density and species richness, diversity, and evenness were calculated in each plot for each sampling year. Total plant density (*D*) was calculated as the sum of all individuals per 5-m^2, and species richness (*S*) was defined as the total number of species per 5-m^2. Species diversity (*H'*) was calculated using the Shannon-Weaver index, and evenness was calculated using Pielou's evenness metric (*J*; Hill 1973). To examine differences in *D, S, H', and J*, a three-way analysis of variance (ANOVA; with model effects being block, treatment, year, and treatment × year) was used, testing each metric for the effect of both treatment and year, and the differential effect of the two factors. Since there was only one replicate of each treatment in each block, model effects testing interaction with block could not be included in the model. Tukey's HSD test (THSD) was used to determine pairwise differences in means among years, among treatments, and among both factors simultaneously. Since the 1996 sampling period occurred prior to treatment, the three-way ANOVA and THSD tested only the post-treatment sampling years of 1997, 2001, 2006, and 2011. To test for pre-treatment differences in *D*, *S*, *H'*, *and J* among the treatments, a two-way ANOVA (with model effects being block and treatment) and THSD were used to analyze the 1996 sampling year. A block × treatment term could not be included in the model because there was only one treatment in each block. Assumptions of normality and homoscedasticity were tested in ANOVA residuals and no data transformations were necessary. The community metrics were all calculated using R package vegan (Oksanen et al. 2015), and ANOVAs were performed using SAS JMP (SAS Institute 2015).

NRSL vs. RSL

To test the NRSL vs. RSL hypotheses, a simulated random thinning replicated what would occur if RSL was the only mechanism determining composition in the herbaceous layer. More specifically, an algorithm was used to randomly thin all plants from each plot from their density in 1997, to their density in a later sampling year (2001, 2006, or 2011). The thinning simulation was then repeated to obtain a bootstrap distribution of the RSL density of each species (D_S). Then, D_S in each plot was compared to the observed species density within the plot (D_O). The difference between D_O and D_S is a distribution of differences and was denoted as δ. If the mean δ was positive, then there was evidence that the species has an advantage. Likewise, a negative δ indicated a disadvantage for the species. Differences in mean δ should be expected in the herbaceous layer of an early successional forest, as many factors could influence NRSL among species. To gain a more detailed understanding how N affects NRSL, differences between the mean δ among treatments were compared for each species. The simulation approach I used was modified from Stevens and Carson (1999), and consisted of the following steps:

1) Four of the plots from each treatment were selected randomly, with replacement.
2) Within each plot, individual plants were randomly selected from the total community of plants within the plot in 1997, without replacement. The number of randomly selected plants was determined by the D_O of that same plot in 2001. The plants that were randomly selected represented the remaining community after random thinning (RSL).
3) The mean density of each remaining species was calculated for each plot.
4) The simulation was repeated 15,000 times to create a distribution for each treatment of the mean density values for each species (D_S).

5) Since differences between D_O and D_S are expected during succession, the differences among the treatments between D_O and D_S were calculated:

$$\delta_{t1} = \left[D_{O_{t1}} - D_{S_{t1_i}}\right] \quad \textit{resulting in 15,000 values of } \delta_{t1}$$

$$\delta_{t2} = \left[D_{O_{t2}} - D_{S_{t2_i}}\right] \quad \textit{resulting in 15,000 values of } \delta_{t2}$$

And then the mean differences were compared using a probability test:

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{t1_i}}{n} < \frac{\sum_{i=1}^{n} \delta_{t2_i}}{n} \quad \textit{then} \quad \text{p} = \frac{\sum_{i=1}^{n} [\delta_{t1_i} < \delta_{t2_i}]}{n}$$

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{t1_i}}{n} > \frac{\sum_{i=1}^{n} \delta_{t2_i}}{n} \quad \textit{then} \quad \text{p} = \frac{\sum_{i=1}^{n} [\delta_{t1_i} > \delta_{t2_i}]}{n}$$

$$\text{If, } \frac{\sum_{i=1}^{n} \delta_{t1_i}}{n} = \frac{\sum_{i=1}^{n} \delta_{t2_i}}{n} \quad \textit{then} \quad \text{p} = 1$$

where t_1 and t_2 are two treatments for comparison and i denotes iteration number. The p-value is the probability that the mean δ is different (larger or smaller) between two treatments – a p-value of one indicates no difference between means.

6) The entire process was repeated for 2006 and 2011, using the initial density in 1997 for each year.

7) To control for the potential of type II errors in multiple comparisons, I used a sequential Bonferroni test on the p-values from step five across all species, treatments, and years (Holm 1979, Rice 1988).

The algorithm is predicated on the observation that N additions decrease the density of individuals at the community level. As such, this procedure estimates the maximum loss of species from RSL, and the minimum loss of species from NRSL. Since some population dynamics cannot be included in this approach, the loss of individuals represents the net loss between two sample periods, since new stems could have been added to the populations. The

thinning simulation was performed using R (R-Core-Team 2015) and R package vegan (Oksanen et al. 2015).

To determine the effect of NRSL on *S*, I modified the species equilibrium theory equation (MacArthur and Wilson, 1967) to partition species losses within a treatment into losses from RSL, and losses from NRSL:

$$\Delta S_{t_0-t_x} = G_{t_0-t_x} - (L_{RSL_{t_0-t_x}} + L_{NRSL_{t_0-t_x}})$$

where $\Delta S_{t_0-t_x}$ is the change in *S* from 1997 to time *x* (2001, 2006, or 2011), $G_{t_0-t_x}$ is the input of new species from 1997 to time *x*, $L_{RSL_{t_0-t_x}}$ is the difference between observed *S* in 1997 and the expected *S* under RSL at time *x* (loss of species due to RSL), and $L_{NRSL_{t_0-t_x}}$ is the number of species lost due to NRSL from 1997 to time *x*. The mean *S* calculated by the random thinning simulation is used as the estimate of $L_{RSL_{t_0-t_x}}$. Using this estimate, the equation can be modified to solve for the number of species lost due to NRSL:

$$L_{NRSL_{t_0-t_x}} = G_{t_0-t_x} - \mathrm{E}(L_{RSL_{t_0-t_x}}) + \Delta S_{t_0-t_x}$$

where $\mathrm{E}(L_{RSL_{t_0-t_x}})$ is the expected loss of species due to RSL, estimated from the random thinning simulation. This equations makes two assumptions: (1) that the error in detecting a species is equal to the error in not detecting a species in a plot, and (2) that populations are undergoing RSL simultaneously to NRSL. Since $\mathrm{E}(L_{RSL_{t_0-t_x}})$ is calculated from a bootstrap sampling procedure that used the plots as the unit of analysis, no statistical comparisons could be made to test differences in the % contribution of NRSL and RSL among treatments. However, to test for differences between RSL and NRSL in each treatment, means of observed species richness (S_{t1}) and means of species richness that did not include species additions since 1997

(S_{cohort}) were compared to the expected species richness under RSL (S_{RSL}). The difference between S_{cohort} and S_{RSL} is the difference in S due to NRSL. To compare means, 99.7% confidence intervals of S_{RSL} were calculated to correct the for family-wise error rate at $\alpha = 0.05$. Means of S_{t1} and S_{cohort} that fell outside of the confidence intervals were considered to be significantly different.

Nitrophilic species

In order to examine the presence and performance of nitrophilic species, information on the association between plants and N availability must be used. Since a database of nitrophily does not exist for the United States, I used published information to assign a nitrophily status to each species I found in the LTSP plots (Table A-1). Where possible, I used species-specific experimental or observational results from the eastern North American broadleaf deciduous forest region. If regional results were not available, species-specific results from other regions were used. In many cases, I used a nitrophilic classification scheme for European plants – the Ellenberg index (Hill et al. 1999). The Ellenberg index assigns species to a number from one to nine based on their association with soil N availability (nine being the highest level of nitrophily). Some of the species in the LTSP plots were listed in the Ellenberg index and, in those cases, I used the published Ellenberg value. Some species were not in the Ellenberg index, but their congeners were. In those cases, the median Ellenberg nitrophily score of all congeneric species was assigned to an LTSP species. Since I frequently relied on the Ellenberg index, species whose nitrophily status was determined from studies other than Hill et al. (1999) were also assigned an Ellenberg nitrophily score based on their long-term response to differing levels of N.

To test the effect of N addition on nitrophilic species, the nitrophily index values were treated as a binary nominal variable. Species with index values greater than five were categorized as nitrophilic, and species with index values equal to, or less than five were categorized as non-nitrophilic. This step of assigning nitrophily into two categories was undertaken to help overcome the lack of species-specific nitrophily information (i.e. using congeners in nitrophily status assignment) and the subjective classification of non-Ellenberg listed species that were found in the published studies. A three-way analysis of covariance (ANCOVA) was used to compare differences in mean δ between nitrophilic and non-nitrophilic species, among the treatments, and among the years 2001, 2006, and 2011. Since a full three-way ANCOVA model included seven effects, and because of the potential of false (i.e. non-significant) heterogeneity in slopes may diminish detection of treatment effects (Engqvist, 2005), corrected Akaike's Information Criterion (AICc) statistics were used to determine the best final model in the absence of any significant effects or interactions. A THSD of the final model was also used to test for differences between nitrophilic and non-nitrophilic species among treatments and years. I was unable to determine the nitrophily status for three species: *Zanthoxylum americanum*; *Podophyllum peltatum*; and *Streptopus lanceolatus*, and these species were excluded from the ANCOVA and THSD tests. A transformation to normalize residuals was not successful, therefore I performed the ANCOVA without transformation and rely on the robustness of ANOVA procedures to deviations from normality – particularly because the data were balanced (Quinn and Keough 2002).

Plant life-forms

In addition to examining the response of different functional groups, I also examined if different plant life-forms (graminoids, non-woody herbs, shrubs, trees and vines) were favored or placed at a disadvantage by the experimental treatments. This was accomplished by comparing the δ values from the random thinning simulation (difference between simulated and observed density for each species) among five plant life-form groups in a two-way ANOVA. Three separate ANOVAs were used to determine if the effect of treatment (REF, +N, or +N+L) on mean δ depended on the plant life-form in any one of the three simulation years (2001, 2006, and 2011). THSD tests were used to determine pairwise differences in mean δ.

4.4 Results

Community metrics

Prior to the beginning of treatment, there were no differences in density (*D)*, richness (*S)*, diversity (*H*'), or evenness (*J)*. For the years following the beginning of experimental treatments, the effect of the various treatments on *D* did not depend on year. However, the experimental treatments did have an effect on *D* ($F = 6.74$, $p = 0.0035$), as did year ($F = 39.92$, $p < 0.0001$). Across all years, *D* was 37.8% lower in the +N treatment than in the REF treatment ($t = -3.64$, $p = 0.0026$). Across all treatments, *D* decreased 39.4% between 1997 and 2001 ($t = 8.03$, $p < 0.0001$), 74.4% between 1997 and 2006 ($t = 9.93$, $p < 0.0001$), and 55.4% between 1997 and 2011 ($t = 8.38$, $p <0.0001$; Figure 4-2a). There were also multiple pairwise differences in *D* among both years and treatments (Table A-2).

There was no differential rate of change of *S* among treatments. However, treatment alone had an effect on *S* ($F = 9.04$, $p = 0.0007$), as did year ($F=3.12$, $p = 0.0389$). Across all years, *S* was 23.5% lower in +N ($t = -4.03$, $p = 0.0009$) and lower by 18.0% in +N+L when compared to REF ($t = -3.18$, $p = 0.0088$; Figure 4-2b). When averaged across all treatments, *S* declined between 1997 and 2011 ($t = 2.91$, $p = 0.0310$). Among both treatments and years, when compared to REF in 1997, *S* in the +N treatment was 38.7% lower in 2001 ($t = -3.53$, 0.0478), 42.7% lower in 2011 ($t = -3.83$, $p = 0.0228$), and 38.7% lower in the +N+L treatment in 2001 ($t = -3.53$, $p = 0.0478$; Table A-2). Additionally, there was evidence of a trend toward lower *S* when REF in 1997 was compared to the +N treatment in 2006 ($t = -3.43$, $p = 0.0606$), and +N+L in 2011 ($t = -3.33$, $p = 0.0762$).

There was no differential effect of treatment and year on *H*'. Treatment also did not have an effect on *H*', however, year did (F = 5.17, p = 0.0049). Across all treatments, there were decreases in *H*' for the years 1997-2011 (16.0% decline; t = 3.52, p = 0.0066), 2001-2011 (13.2% decline; t = 2.87, p = 0.0342), and 2006-2011 (14.3% decline; t = 3.12, p = 0.0187; Figure 4-2c). Pairwise comparisons among both years and treatments determined a 31.8% decline in H' in REF between 1997 and 2011 (t = 3.99, 0.0153), and a trend toward a 27.0% decline in REF between 2001 and 2011 (t = 3.30, p = 0.0821; Table A-2).

With respect to *J*, the effect of treatment did not depend on the year. Treatment did have an effect on *J* (F = 5.4661, p = 0.0089), as did year (F = 4.93, p = 0.0061). Across all years, the only difference in *J* among treatments was between REF and +N. Specifically, *J* in REF was 0.658, and *J* in +N was 0.719, a difference of 8.9% (F = 3.29, p = 0.0065; Figure 4-2d). Across treatments, there was an 8.9% decrease in *J* from 1997 to 2011 (t = 2.74, p = 0.0457), a 10.6% decrease from 2001 to 2011 (t = 3.31, p = 0.0117), and a 10.4% decrease from 2006 to 2011 (t = 3.25, p = 0.0136). There were multiple pairwise differences in *J* among both years and treatments (see Table A-2), and a trend toward a difference between REF in 2011 and +N in 2011 (t = 3.35, p = 0.073).

NRSL vs. RSL

For the species examined, 54.1% of the net density changes found were the result of NRSL, rather than a consequence of RSL. Furthermore, for the seven species with the highest δ values (δ >10 for one or more years), the treatment differences in δ tended to be enhanced through time, with the exception of *Smilax rotundifolia* where δ values were consistently greater for the +N

plots compared to those for the REF plots (Figure 4-3). Overall, there were differences in δ among treatments for 45 of the 83 species in one or more sampling years (Tables A-3 through A-5). The most frequently detected treatment effect for species with high δ values was between the REF and +N plots. For four species (*Rubus spp, Polygonum spp*., *Rosa carolina,* & *Smilax rotundifolia*), the plants in the +N plots had a greater advantage (typically in the later years) compared to those growing in the REF plots (i.e. they had a significantly higher δ). For one species (*Acer rubrum*) the reverse was true, with the plants in the +N plots thinning randomly whereas those in the growing in the REF plots had an advantage by achieving much higher densities than expected by random thinning (Figure 4-3).

With respect to the contributions of NRSL and RSL on species losses, I found that NRSL was an important mechanism affecting *S*. The contributions to species loss from NRSL ranged from 28.6-72.5% across all treatments and years (Table 1), and the average was 50.8%. Across all years, the average contribution to species losses from NRSL was 60.1% in REF, 43.8% in +N+L, and 48.6 in +N. In 2001 and 2011, the percent contribution to species loss from NRSL was highest in REF and lowest in +N+L. However, in 2006 this pattern changed and NRSL was most dominant in +N+L and least dominant in +N (Table 1). When the expected richness under RSL (S_{RSL}) was compared to observed richness without species additions (S_{cohort}), NRSL was the dominant mechanism responsible for reduced *S* in all years, in REF. When compared among S_{cohort} in the +N+L treatment, *S* was reduced by NRSL in 2006 and 2011, but by RSL in 2001. In the +N treatment, NRSL was the dominant force acting on *S* only in 2011, and RSL was responsible for a greater level of reductions in *S* in both 2001 and 2006.

Nitrophilic species

In REF plots, both nitrophilic and non-nitrophilic species were thinning randomly in 2001. However, a large difference emerged by 2011, when nitrophilic species were much less advantaged than non-nitrophilic species (Figure 4-4a). The addition of N, with or without lime, suppressed any temporal transitions in the average advantage of nitrophilic and non-nitrophilic species, and both were found to be thinning randomly throughout the study period (Figure 4-4b & 4-4c).

The effect of nitrophily status on the mean δ depended on the year ($F = 3.00$, $p = 0.0502$). When averaged across treatment, there were no significant differences in mean δ among years or nitrophily status. However, the largest non-significant difference in mean δ occurred between non-nitrophilic species in 2011 (1.23 individuals/5 m^2) and nitrophilic species in 2011 (-1.80 individuals/5 m^2; $t = 2.31$, $p = 0.1902$; Figure 4-4d). When averaged across both years and treatments, there was also a trend in the effect that nitrophily status had on mean δ ($F = 2.76$, $p = 0.0971$), with nitrophilic species exhibiting less of an advantage (mean δ = -0.73 individuals/5 m^2) than non-nitrophilic species (mean δ = 0.51 individuals/5 m^2; $t = 1.66$, $p = 0.0971$). There was no main effect of year or treatment on mean δ. The model was best fit by using the effects of year, nitrophily status, treatments, and year × nitrophily status.

Plant life-forms

With regard to the different life-forms that were either advantaged or disadvantaged, the effect of treatment on δ (difference between simulated and observed density) did not depend on life form. Moreover, there was no effect of treatment on mean δ, when averaged across plant life-forms.

However, there was an effect of plant-life form on mean δ in 2001 ($F = 6.89$, $p < 0.001$) and 2006 ($F = 2.6867$, $p = 0.0319$; Figure 4-7). Shrubs had a density advantage over herbs ($t = 5.10$, $p < 0.0001$), trees ($t = 4.70$, $p < 0.0001$), and vines ($t = 3.69$, $p = 0.0025$) in 2001, and herbs had a trend toward a density advantage over vines in 2006 ($t = 2.63$, $p = 0.0680$).

4.5 Discussion

My results show that N additions to the herbaceous layer in a broadleaf deciduous forest reduced both *D* and *S*, and both NRSL and RSL mechanisms appeared to make significant contributions to those reductions. Tests of the bootstrap simulation found that 54.1% the species present in the sampling years from 2001-2011 were either more or less dense than predicted under random thinning in at least one sampling year. Application of the species richness equation indicates that 48.6% of species loss in the +N treatments, and 43.8% of the species lost in the +N+L treatments were due to NRSL. Therefore, both mechanisms (NRSL and RSL) contributed to the decrease in density that was observed between REF and +N treatments, and the decrease in richness between REF and both +N and +N+L treatments (Figure 4-2).NRSL was the dominant factor affecting species losses in the REF treatment, and the effect of NRSL generally increased through time in the +N and +N+L treatment (Figure 4-5). Across all lines of evidence in this study, there was a general increase in the relative importance of NRSL over time in fertilized treatments (+N+L and +N), and a steady contribution from NRSL over the years in REF treatments.

Studies of the effects of N in grassland and old-field systems have discovered evidence for species loss due to both NRSL (Hautier et al. 2009) and RSL (Stevens and Carson 1999), and evidence for both occurring simultaneously (Suding et al. 2005). Research testing the two mechanisms in the understory a fertilized needle-leaf evergreen forest determined that RSL was the mechanism responsible for declines in *S* (Thomas et al. 1999). Discrepancies in results among these studies, and ours, are likely due to a variety of varying environmental factors that may favor certain species, and the collection of plant functional types that are present in the herbaceous community at each site (Suding et al. 2005). The sites examined in previous studies

vary widely with respect to ecosystem type, N-fertilization amounts, land-use history, and cumulative N load – all factors could affect NRSL and RSL mechanisms. Additionally, differential advantages among species under N additions can also indirectly occur because of shifts in soil microbial communities (Johnson et al. 2003), increases in plant litter accretion (Foster and Gross 1998, Lamb 2008) and changes in herbivory, pathogenic infections and earthworm activity (Gilliam 2006).

One, or many, of the previously documented N-induced, indirect environmental changes could explain the advantages and disadvantages I observed among species in the forest herbaceous layer at the Fernow Experimental Forest (Rajaniemi 2003). However, the dominant resource influencing the response of a species under N additions is likely to be light (Hautier et al. 2009, DeMalach et al. 2016), as competition among species shifts from belowground nutrient acquisition to aboveground light acquisition (Newman 1973, Tilman 1987). Therefore, the response of nitrophilic species, like *Rubus* spp. (Figure 4-3), under N additions is probably the result of increased competition for light, and not the result of other indirect effects. Results from my field experiment on *Rubus* support this idea (*Chapter 3*). In that study, I found that, at high light levels, N fertilization caused a substantial increase in the leaf area of *Rubus allegheniensis* in the absence of changes in herbivory, earthworm activity, or obvious pathogenic infections.

Previous studies of forest herbaceous layers typically find N-induced reductions in *H*' (Strengbom and Nordin 2008, Hedwall et al. 2011, Gilliam et al. *in press*). In contrast, I found that species diversity and evenness did not decrease after 15 years of N fertilization. Instead, both *H*' and *J* were lower in 2011 in REF treatments when compared to both +N and +N+L

treatments, suggesting that in this experiment N fertilization maintained higher *H*' early in succession (Figure 4-1). Since *H*' is a function of both *S* and *J*, the decrease in *H*' observed in REF after 15 years was driven mainly by a decrease in *J*, since *S* was not significantly different among the treatments in 2011. However, Gilliam et al. (*in press*) did not observe a decrease in *H*' in the forest herbaceous layer of a nearby location until ca. 25 years of fertilization at 35 kg N ha^{-1} yr^{-1}, suggesting that a similar decrease in *H*' in response to N fertilization in the LTSP may not be realized until later in succession.

Gilliam (2006) used the nitrogen homogeneity hypothesis to argue that the dominance of nitrophilic species should cause both *J* and *S* to decrease after chronic N additions, leading to a decrease in *H*'. However, our results show that both *J* and *H*' were higher in the N-fertilized treatments after 15 years of application, with no significant differences in *S* among treatments (no differences among treatments in *S* in 2011). One explanation for why, after 15 years, there were lower values of *H*' in REF plots, rather than in the fertilized plots, may be the increase in heterogeneity of – and greater competition for – light due to significant damage to the forest canopy in the LTSP plots that occurred in December 2009 during a localized wind storm (*Chapter 5*). Since competition for light is a major factor influencing diversity under N-additions (Hautier et al. 2009), it seems plausible that a greater, storm-induced heterogeneity in light at the forest floor stimulated enhanced competition for light across all LTSP treatments. Simultaneously, the species composition in +N and +N+L treatments may have already been dominated by nitrophilic species (e.g. *Rubus* spp.; Figure 4-6) that were all competing for light, leading to a higher eveness, whereas fewer nitrophilic species in REF plots could have led to lower levels of species eveness. There is also evidence that tree damage was greater in N-

fertilized plots (*Chapter 5*), which may have led to differences among treatments in post-storm effects on the herbaceous layer.

Unlike most studies of N effects on plant communities, my results allow me to separate the acidification and fertilization effects of N additions on plant species. If the effect on δ of N additions was only attributable to the fertilization effect, I would expect the same response in mean δ in each species, between +N and +N+L treatments. Although a variety of response patterns were observed when these two treatments were compared (Tables A-3 through A-5), the addition of lime usually diminished the magnitude of the advantage, or disadvantage, shown by a given species due to N fertilization (Table 4-1; Figure 4-3). Additionally, responses to lime additions were not observed among plant life-forms (Figure 4-7). Although no clear pattern emerged by comparing the response of different plant life-forms, the addition of lime appeared to hasten the role of NRSL. Specifically, I found that the species richness in +N+L plots was affected by NRSL in 2006, but was not affected by NRSL in +N plots until 2011.

The use of an index of nitrophily status as a functional trait appears to be meaningful since species classified as nitrophilic responded to N additions in ways that were likely to contribute to species losses through NRSL. In general, non-nitrophilic species had a greater advantage (a higher mean δ) in REF plots than nitrophilic species (Figure 4-4a), and their advantage relative to non-nitrophilic species increased through time. However, this pattern was diminished in both the +N and +N+L treatments where the changes in density of the two functional groups were consistent with the effect of random thinning alone (Figures 4-4b and 4-4c). These results contrasted with the analysis of treatments among plant life-forms (Figure 4-7). Using traits that

characterized life form were not as powerful as nitrophily status at predicting advantageous or disadvantageous outcomes under fertilization.

After 15 years of experimental treatments, *Rubus* spp. and *Polygonum* spp. were the two nitrophilic species that showed the greatest positive response to N additions (Figures 4-3b and 4-3c), consistent with the observations that *Rubus* spp. has increased dramatically in a nearby, fertilized watershed at FEF (WS3) since 1989 (Gilliam et al. *in press*), and has increased in response to N additions in boreal forests (Strengbom and Nordin 2008, Hedwall et al. 2011). By 2011, the percentage of the herbaceous layer density consisting of *Rubus* spp. averaged 14.1% in the fertilized treatments (+N+L and +N), and only 2.6% in REF (Figure 4-6). Further evidence from FEF (including the LTSP plots) indicates that *Rubus* spp. utilize excess N to increase cover in areas of high light (*Chapter 3*). Similarly, research in a European deciduous forest found that *Polyganum* spp. is an indicator of high N-availability (Bernhardt-Romermann et al. 2010). In contrast to nitrophilic species, the two species that appeared to suffer the most from the effects of N additions were *Viola* spp. and *Acer rubrum*. Since both species were primarily affected by N additions without lime (+N), this suggests that the effect of acidification had a greater influence than the effect of added N (Figures 4-3a and 4-3d). Furthermore, both species have declined after 25 years of N fertilization in a watershed-scale experiment (WS 3) at the FEF (Gilliam et al. *in press*).

Overall, this research demonstrates a substantial role for the mechanisms of non-random species loss under N additions, particularly as time – and the cumulative load of N – increased. More specifically, the N-induced reduction of *S* in fertilized treatments was hastened by an increase in

NRSL through time. As previously suggested by Gilliam (2006), changes in the herbaceous layer in our study sites due to N additions appear to be influenced by the advantage gained by a few nitrophilic species. Changes in herbaceous layer composition also occurred by non-nitrophilic species being put at a disadvantage by the N additions, chiefly in response to the acidification effects of the added N. Thus, in forests receiving high levels of atmospheric N deposition, the overall loss of plant biodiversity (concentrated in the herbaceous layer) may be mitigated to some extent by the addition of lime. Our research also demonstrates that understanding the response of the herbaceous layer in a broadleaf deciduous forest to N additions requires long-term experiments that monitor these complex communities through time.

4.6 Tables and Figures

Table 4-1. Variables used in the calculation of species loss due to non-random species loss (NRSL) and random species loss RSL, and the estimated percent contribution of NRSL to species losses in reference (REF), fertilized and limed (+N+L), and fertilized plots (+N).

	2001			2006			2011		
Metric	REF	+N+L	+N	REF	+N+L	+N	REF	+N+L	+N
S_{1997}	27.0	21.0	21.0	27.0	21.0	21.0	27.0	21.0	21.0
S_{t1}	23.8	18.3	18.3	24.3	21.5	18.5	20.3	18.8	17.5
$\Delta S_{1997\text{-}t1}$	3.3	2.8	2.8	2.8	-0.5	2.5	6.8	2.3	3.5
Number of spp. additions	6.3	5.5	5.5	7.8	9.0	7.5	5.5	7.0	6.8
Expected spp. loss due to RSL	2.6	5.4	3.9	6.3	4.3	7.1	3.9	4.8	3.7
Expected *S* due to RSL	24.4	15.6	17.1	20.7	16.7	13.9	23.1	16.2	17.3
Spp. loss due to NRSL	6.9	2.8	4.4	4.2	4.2	2.9	8.3	4.4	6.6
% spp. loss due to NRSL	72.5	34.2	53.0	39.7	49.5	28.6	68.0	47.7	64.2

Notes: *S* denotes species richness and t_1 = year indicated in column

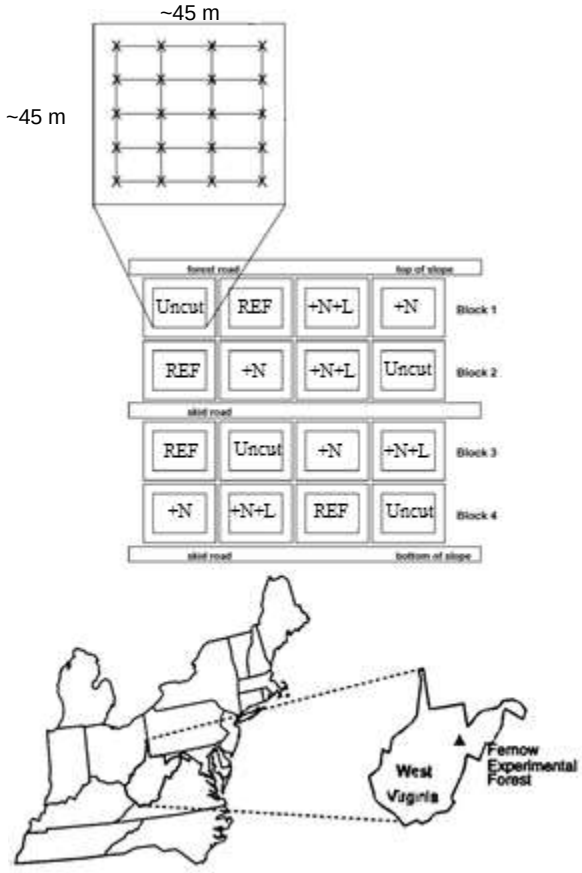

Figure 4-1. Location and layout of the LTSP plots detailing the grid within each plot, from which five subplots were randomly chosen.

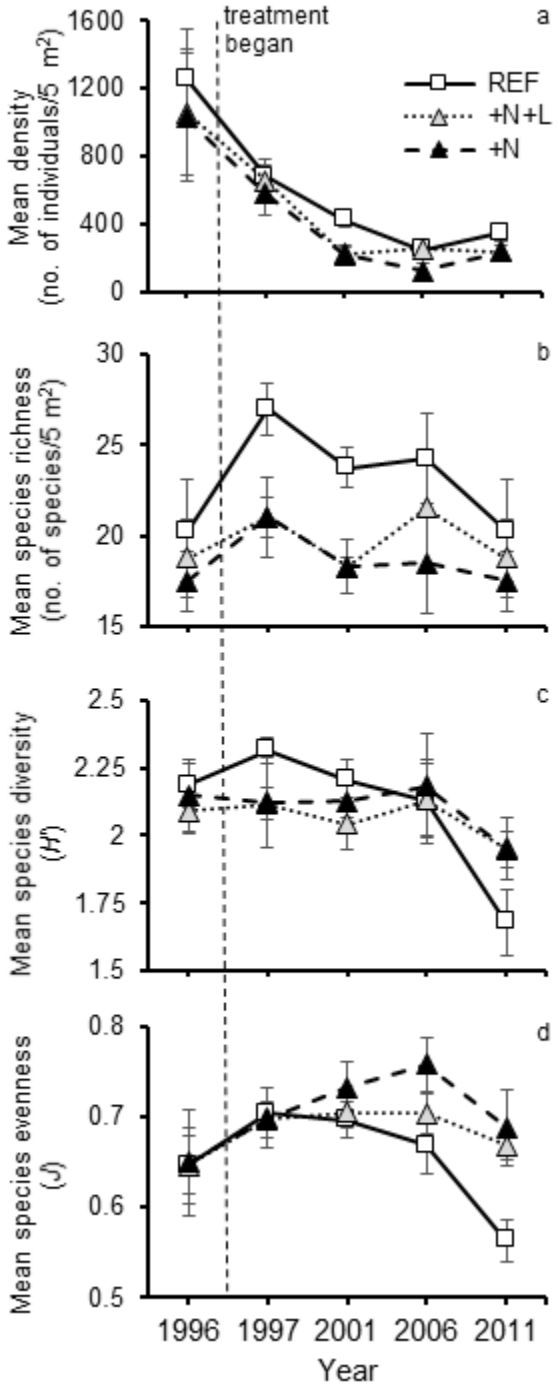

Figure 4-2. Species metrics in the reference (REF), N-fertilized (+N), and N-fertilized and limed treatments (+N+L). Error bars represent one standard error.

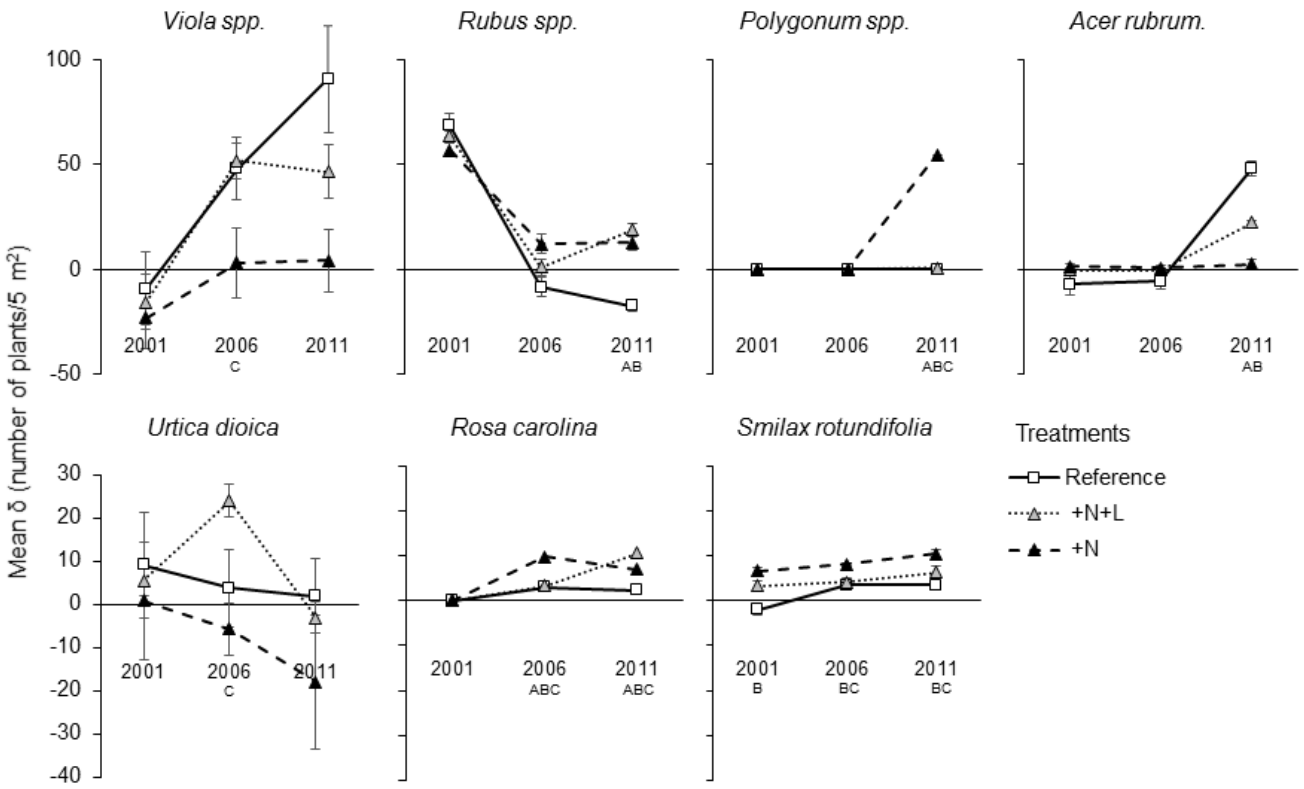

Figure 4-3. Mean difference in simulated vs. observed density (δ) for the seven species with δ values greater than ten in at least one sampling year among reference (REF), N-fertilized (+N), and N-fertilized and limed treatments (+N+L). Error bars represent one standard error and significant sequential Bonferroni p values testing pairwise comparisons are indicated as (A) REF vs. +N+L, (B), REF vs. +N, and (C) +N vs. +N+L.

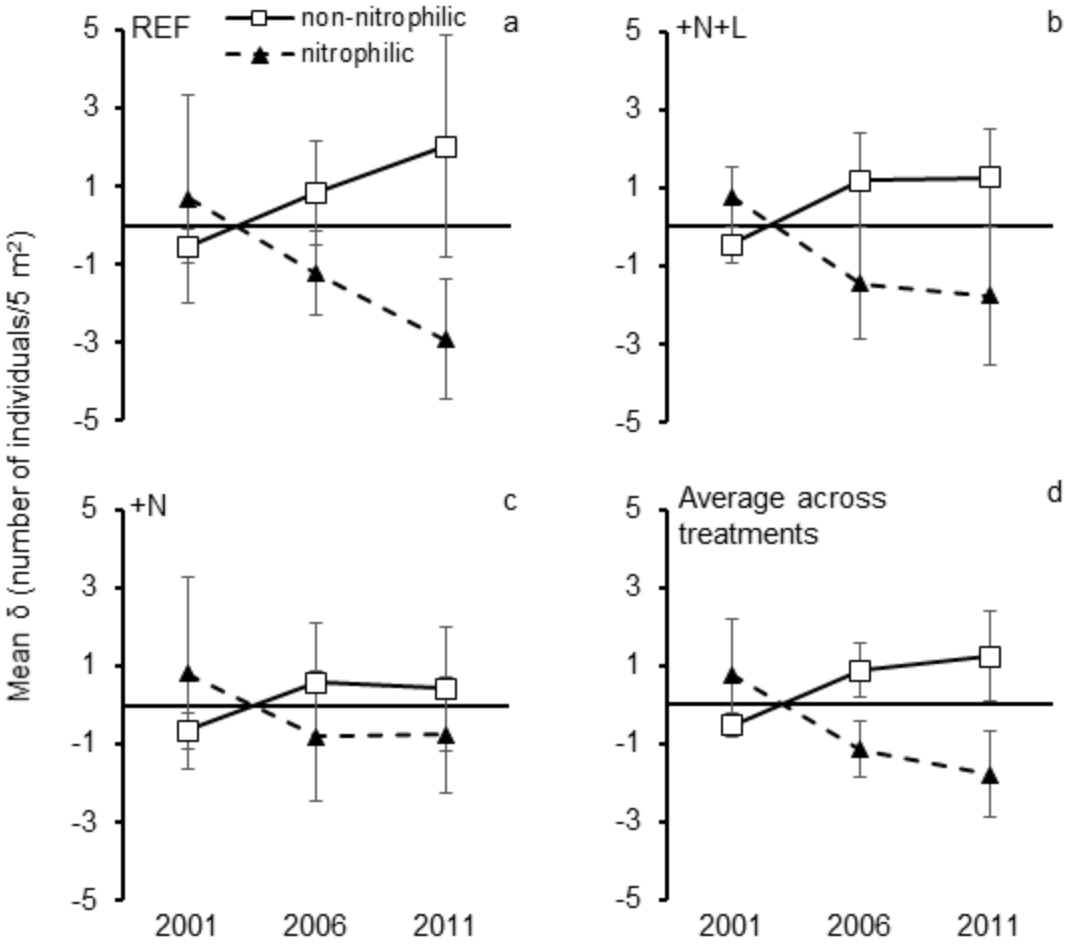

Figure 4-4. Mean difference in simulated vs. observed density (δ) between nitrophilic (closed triangles) and non-nitrophilic (open squares) species among reference (REF), N-fertilized (+N), N-fertilized and limed treatments (+N+L), and averaged across all treatments. Error bars represent one standard error.

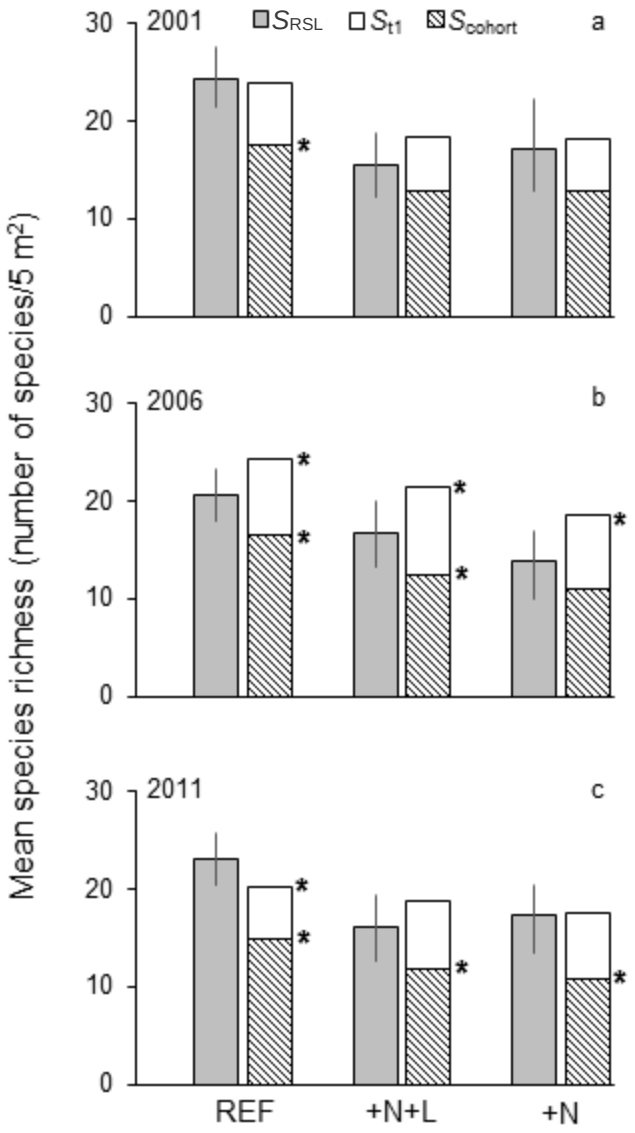

Figure 4-5. Mean species richness among N-fertilized (+N), and N-fertilized and limed treatments (+N+L) expected under RSL (S_{RSL}), observed during that sampling year (S_{t1}), and observed minus species additions since 1997 (S_{cohort}). Error bars represent a 99.7% confidence interval to correct for family-wise error rate at $\alpha = 0.05$ and asterisks denote significant differences between means.

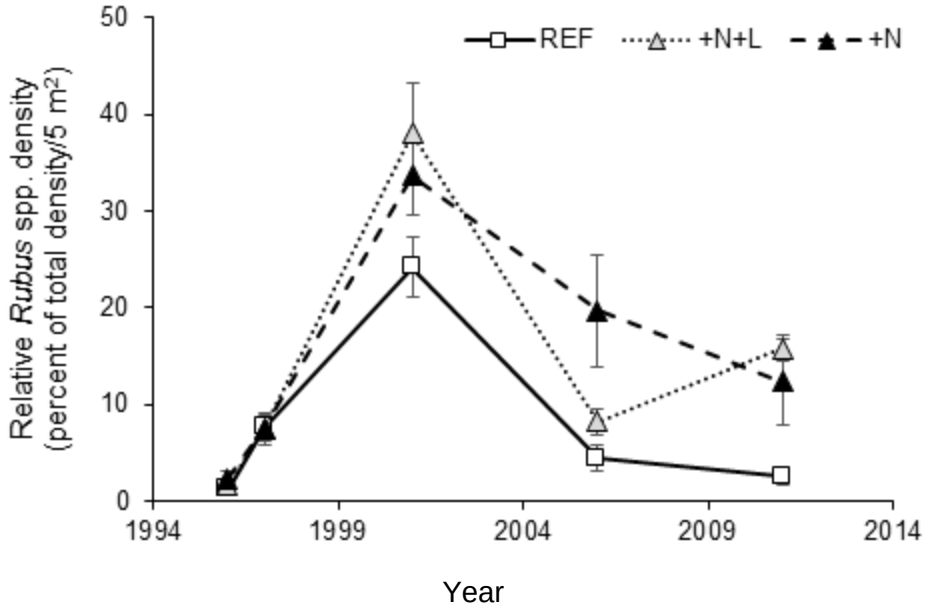

Figure 4-6. Mean relative density of *Rubus* spp. among reference (REF), N-fertilized (+N), N-fertilized and limed treatments (+N+L). Error bars represent one standard error.

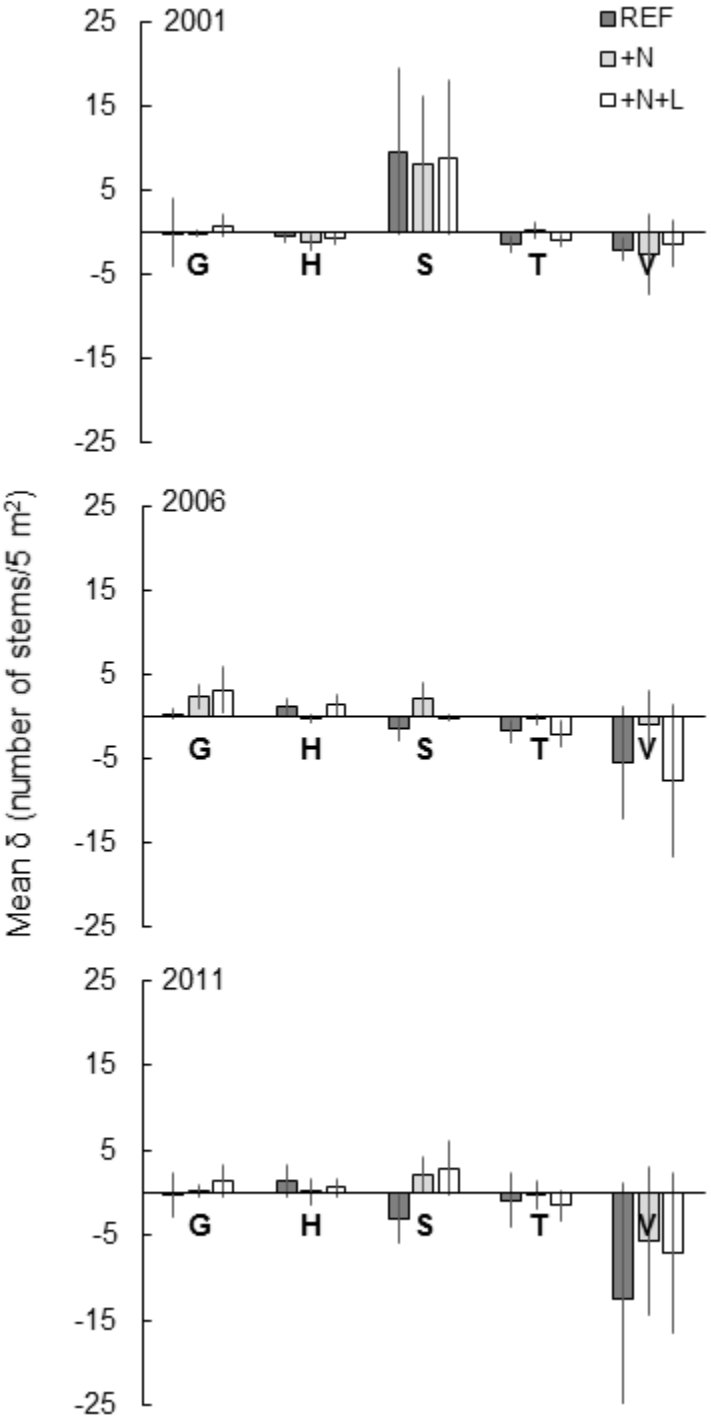

Figure 4-7. Mean δ of plant life-form groups – graminoid (G), herb (H), shrub (S), tree (T), and vine (V) – in unfertilized (REF), fertilized (+N), and fertilized and limed (+N+L) treatments.

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Chapter 5. Does soil nitrogen availability affect storm damage in stands of broadleaf deciduous forest in the central Appalachian Mountains?

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5.1 Abstract

Storms are the greatest natural disturbance in the forests of eastern North America. Damage from storms can, among other things, devalue timber, change species composition and recruitment, and alter canopy structure. Increased nitrogen (N) availability could make trees more susceptible to storm damage (especially wind damage) by changing root architecture, wood fiber arrangement, altering leaf morphology, and by decreasing canopy density. Greater storm damage to trees growing in N-rich environments can also indirectly impact the herbaceous layer by creating larger canopy openings that increase the amount of light reaching the forest floor. To understand how N availability affects susceptibility to storm damage in temperate broadleaf deciduous forests, I took advantage of a unique opportunity to survey the damage caused by two severe storms that occurred in pre-existing fertilized and unfertilized areas at the Fernow Experimental Forest, and across a native N-availability gradient. I found that both the percentage of basal area (BA) and the percentage of stems damaged by a localized winter wind storm were higher in N-fertilized plots than in unfertilized plots in early successional stands. In contrast, both the percentage of BA and the percentage of stems damaged by a late-fall severe snow storm – a consequence of Superstorm Sandy – were lower in a fertilized watershed, when compared to an unfertilized watershed. However, the lower damage from the snow storm in the fertilized watershed was likely the result of a greater abundance of less susceptible species in the fertilized watershed. No overall difference in damage was detected across a native N-availability gradient following the severe snow storm, but the differences in the percentage of BA and stems damaged among the species may have depended on the level of N availability. Our results suggest that the influence of long-term changes in N availability on storm damage in a temperate forest depends on the nature of the storm and the tree species, with the effects of severe winds being different

than the effects of heavy snowfall. Understanding the complexities of the relationship between storm damage and N availability is of particular importance in the forests of eastern North America because this region has a history of chronic N deposition, and the probability of large magnitude, Atlantic origin storms – particularly storms that cause high wind speeds – is likely to increase as the climate changes.

5.2 Introduction

Storm damage is the most significant natural disturbance in the forests of eastern North America (Fischer et al. 2013), and can disrupt forest ecosystems in a variety of ways and at a diversity of spatial scales. The effect of storm damage on individual trees is often positively associated with tree diameter at breast height (DBH; Zimmerman 1994, Platt et al. 2000, Van Bloem et al. 2006) suggesting that larger trees are the most susceptible. However, smaller understory trees may also be damaged (bent or broken) when overstory trees fall. The damage to overstory and understory vegetation can alter the forest environment in several ways that may, in turn, alter species composition and diversity. For example, at the stand level, tree damage can increase maximum canopy gap sizes by more than 30% (Xi et al. 2008) and increase canopy gap light levels by more than 45% (Sherman et al. 2001), which can have dramatic effects on the herbaceous layer vegetation. And at the forest scale, storm-created canopy disturbance has caused lasting changes in tree recruitment (Baldwin et al. 2001; Batista and Platt 2003; Pascarella 1997), diversity (Uriarte et al. 2004), and species composition (Merrens and Peart 1992).

How storms interact with nutrient additions to alter forest disturbance is less understood, but is a topic of increasing importance since inputs of N to the terrestrial biosphere have grown (Galloway et al. 2004), and since the frequency of intense storms is expected to increase in a warmer world. Increased soil nutrients can lead to several changes that should leave forests more susceptible to damage by intense storms (Deangelis et al. 1989). Trees growing in areas with high nutrient availability typically allocate less carbon to wood structural fibers (Bloom et al. 1985; Chapin 1980; Pitre et al. 2007), yet are often taller, and have greater stem and leaf biomass (Grier et al. 1984; Miller 1981). Increased nutrient availability can also reduce the specific length

(root length : root dry mass) and total mass of roots, and alter three-dimensional root architecture (Ostonen et al. 2007, Valverde-Barrantes 2007, Jourdan et al. 2008, Kobe 2010, Domenicano et al., 2011; but see Nadelhoffer 2000). And tree stands in areas of very high N availability may experience branch and leaf mortality (Magill et al. 2004) that could decrease the canopy density and lower the extent that a forest canopy dampens wind speeds. Thus, it appears that increased N availability could produce trees that may be more susceptible to wind damage because they are taller, have fewer roots, and a greater mass of leaves. A greater mass of leaves may also increase damage by a heavy snowfall if the storm were to occur before leaf senescence was complete. At the stand and forest level, increased soil nutrients can change tree seedling composition in ways that alter adult tree composition in a forest (Lu et al. 2010; Siddique et al. 2010). Thus, if tree species differ in their susceptibility to storm damage, then soil fertility could indirectly influence storm damage by altering tree species composition. In either case, through changes in tree composition or increases in damage, the effects of N on trees could also have an indirect effect on the composition of the herbaceous layer.

Evidence of the effect of increased nutrient availability on the susceptibility of forest stands to storm disturbance is sparse and largely limited to tropical regions because large storms are more likely to strike equatorial latitudes. In studies across nutrient gradients in tropical forests, areas with higher soil nutrients were damaged more by hurricanes and cyclones than nutrient-scarce areas (Beard et al. 2005; Gleason et al. 2008). In a fertilization study, Herbert et al. (1999) measured hurricane damage in a phosphorus-limited tropical forest that included short-term nitrogen and phosphorus amended plots. They discovered that a hurricane caused more stem damage and leaf area loss in the fertilized plots when compared to the unfertilized plots. The

increase in susceptibility was attributed to an enhanced leaf area in the fertilized trees that increased the wind-drag (Herbert et al. 1999). However, tropical forests on old soils are often thought to be more limited by phosphorus than N (Vitousek 1984; Elser et al. 2007), so structural changes in tropical trees due to increased N availability may not be as severe as those in temperate forests. Additionally, studies in tropical forests investigate only one major aspect of storms – heavy winds – and, as a consequence of their location, cannot examine the effects of heavy snowfall.

Understanding the impact of soil nutrition on forest susceptibility to storms in eastern North America is important because forests in this region have experienced chronic N deposition that began in the late 1800's (Galloway et al. 2004), and because the intensity of Atlantic-origin storms is increasing with climate change (Bender et al. 2010; Emanuel 2005; Grinsted et al. 2012; Grinsted et al. 2013). The occurrence of two severe storms (one windstorm and one heavy snowfall) on existing long-term fertilization experiments, and across a native N-availability gradient, at the Fernow Experimental Forest created a unique opportunity for us to assess whether temperate forests with greater N availability were more susceptible to damage by severe storms, and for us to understand if this effect depended on whether the primary destructive force of the storm was from wind or snow.

5.3 Methods

Study site

The Fernow Experimental Forest (FEF) is a 1902-ha research forest located in the Allegheny Mountain physiographic province of north-central West Virginia (Kochenderfer 2006). Five watersheds, a stand of mature trees (the Biological Control Area), and a long-term, replicated experiment within FEF were used to carry out this study (Table 1; Figure 1-1). Watershed 4 (WS4; 39 ha), Watershed 10 (WS10; 15 ha), Watershed 13 (WS13; 14 ha) and the Biological Control Area (BCA; 31 ha) were last cut ca. 1900, and serve as reference areas for an assortment of experiments at the FEF. Watershed 3 (WS3; 34 ha) was last cut between 1969 and 1972 and is currently used as a whole-watershed acidification experiment. Since 1989, 35 kg N ha^{-1} yr^{-1} as ammonium sulfate has been applied to the watershed by aircraft. Watershed 7 (WS7; 24 ha) was cut in two phases between 1963 and 1967 and was maintained barren with herbicide until 1969. Since the stands in WS7 and WS3 are similar ages, WS7 served as an unfertilized reference for WS3 in this study.

The Long-Term Soil Productivity experiment (LTSP) is a randomized block design (4 plot × 4 block) with three treatment plots and one reference plot in each block. Each plot is 0.4 ha and contains a 0.2-ha area in which measurements are made (7.6-m treated buffer around each plot). The three LTSP treatments used in this study were the whole-tree harvest (REF), whole-tree harvest plus fertilizer (+N; 35 kg N ha^{-1} yr^{-1} as ammonium sulfate, hand applied), and whole-tree harvest plus fertilizer and lime (+N+L; 35 kg N ha^{-1} yr^{-1} as ammonium sulfate and 22.5 kg Ca ha^{-1} yr^{-1} as dolomite, hand applied). All aboveground biomass was harvested and removed in all three LTSP treatments in 1996 (Adams et al. 2004). The REF treatment served as an unfertilized

reference for the +N and +N+L treatment in this study. The addition of the +N+L treatment allows me to test whether the effects of ammonium sulfate application were due to direct effects of N fertilization, or due instead to secondary effects of N additions which are ameliorated by the addition of dolomitic lime; effects such as soil acidification, an increase in toxic aluminum levels, and/or the depletion of soil base cations.

Experimental design

To test the susceptibility of trees at various levels of soil N availability, I took advantage of severe storm damage from a strong winter wind event in 2009, and damage from heavy snows caused by Superstorm Sandy in 2012. The wind event occurred in late December of 2009 along a portion of Fork Mountain where the LTSP plots are located. Damage from the storm was highly localized, suggesting that it was the result of a microburst. Superstorm Sandy occurred three years after the localized wind event and caused widespread damage to trees throughout FEF. Prior to being downgraded to a Superstorm, Sandy was the largest diameter Atlantic hurricane ever recorded. Even though the center of the storm made landfall ca. 550 km east and 160 km north of the FEF, its effects were observed across a large portion of the eastern United States. By the time it reached the FEF on October 30th, 2012 it was downgraded to a super storm and there was only a mild increase in wind speed. The majority of damage to trees at the FEF (many still retaining a significant number of leaves) was caused by snow, with an accumulation estimated to have been as high as 1-m during the first 24 hours (C. Cassidy, on-site forest technician). To test the effect of N availability on storm damage to trees by severe wind (the 2009 storm), I compared damage across treatments in the LTSP experiment. To test the effect of N availability on storm damage to trees caused by a severe snow storm (the 2012 storm), I measured the

damage found in both WS3 (fertilized) and WS7 (unfertilized), and across LTSP treatments (compared using the difference in damage between storms). To determine if any patterns resulting from experimental fertilization could occur under less extreme differences in N availability, I also examined tree damage from snow across a native N-availability gradient in the uncut reference areas of the FEF - WS4, WS10, WS13, and the BCA.

Damage estimates and N availability measurements

Damage to trees was measured in all areas and used to form a binary classification - damaged or not damaged. Trees that had any crown damage, were bent, snapped, leaning, or tipped over were all classified as damaged. In the uncut reference areas (WS4, WS10, WS13, and BCA) 25-m radius permanent growth plots were surveyed. The permanent growth plots were established between 1990 and 1996 and are used to track all trees > 2.54 cm diameter at breast height (DBH). A total of 27 growth plots were surveyed, five in the BCA, WS10, and WS13, and 12 in WS4. In both the fertilized (WS3) and unfertilized (WS7) areas, eighteen 10-m radius plots were selected randomly from a network of existing study sites and then surveyed for damage. The plots in WS3 and WS7 were equally divided among three aspect strata: 1 – "northeast", 30-90°; 2 – "south", 150-210°; and 3 – "northwest", 270-330°. Damage was measured among the three LTSP treatments using six randomly located 130-m^2, square sub-plots in each of the four replicated treatment plots. The damage measured in each sub-plot was then summed for each plot. Within each plot I studied, every tree >2.54 cm DBH was identified by species, its DBH measured, and it was categorized as either damaged or not damaged. This information was then used to calculate the percentage of stems and basal area (BA) damaged in each plot. Damage

from the 2009 storm was surveyed in June 2011, and damage from the 2012 storm was surveyed in June 2013.

To characterize N availability across the uncut reference areas, potential net N mineralization rates were measured using laboratory incubation. Within each plot, eight soil cores (2.2-cm diameter) of the mineral soil were taken to a depth of 5 cm and composited to create a single sample per plot. The soils were then sieved through a 5.6-mm mesh and approximately 10 g (wet weight) of the soil was placed individual plastic cups – one pre-incubation cup and one post-incubation cup per plot. The soils were allowed to acclimate in the dark at room temperature (21-24 ºC) for five days. Nitrate and ammonium were extracted from the soils in the pre-incubation cups by shaking the soil in 100 mL of 1 M KCl for 15 minutes. The extractant was filtered through a 0.45-µm filter and frozen until analyzed. Soils in the post-incubation cups were incubated 30 days before being extracted and the extracts frozen. Extracts were analyzed using Lachat QuickChem 8500 Series 2 Auto-analyzer, method 12-107-04-1-B for nitrate, and method 12-107-06-2-A for ammonium with 1 M KCl as a carrier. Net N mineralization rates were calculated by dividing the change in inorganic N (nitrate + ammonium) per gram of soil that occurred during the incubation by the number of incubation days.

Statistical analysis

To test the effect of a native N-availability gradient – across plots in uncut reference areas – on both the percentage of basal area (BA) and percentage of stems damaged in the 2012 storm, these values were regressed against the measured net N mineralization rates. Slopes from these

two regressions were tested using t-tests. Since differential damage may occur among species, a two-way analysis of covariance (ANCOVA) that included species as a factor was used. The ANCOVA included model effects of N mineralization (NMIN), species (S), and NMIN × S, and the covariate in the model was NMIN. Only species occurring in four or more plots were used in the analysis. To test the effect of N fertilization between fertilized (WS3) and unfertilized (WS7) watersheds, I used t-tests that compared both the percentage of stems and BA damaged in the 2012 storm. Similar to the N-gradient analysis, species-specific damage response to storms, may exist. To account for differences in species composition among WS3 and WS7, I employed a two-way analysis of variance (ANOVA). The ANOVA included model effects of watershed (WS), S, and WS × S, and only species occurring in four or more plots were used in the analysis. An ANOVA was also used to test treatment effects (+N, +N+L, and REF) on the percentage of stems and BA damaged in the LTSP experiment by both the 2009 and 2012 storm, separately for each storm. A t-test for differences in species composition was not used to test damage effects among LTSP treatments, because the LTSP is dominated by one species – *Prunus pensylvanica* (Fowler 2014). The ANOVA model included the effects of block, treatment (T), S, and T × S, and only species occurring in six or more plots were used in the analysis. Tukey's HSD analysis was used to test for pairwise differences between treatments and species for WS 3 and WS7, and among LTSP treatments. Overall, 24 species were used in the analyses (Table 2), and all analyses were performed using SAS JMP. Transformations to ensure normality and homoscedasticity in residuals were applied when appropriate.

5.4 Results

Wind storm in December 2009

Across the entire LTSP, the 2009 wind storm damaged 26.2% of stems, and 31.9% of BA on average. The effect of species on the mean percentage of BA damaged by the 2009 wind storm did not depend on LTSP treatment. However, there were main effects of both species ($F = 15.32$, $p < 0.0001$) and treatment ($F = 3.88$, $p = 0.0268$). With respect to the differential damage among tree species, both *Betula lenta* ($t = 3.35$, $p = 0.0297$) and *Prunus pensylvanica* ($t = -3.16$, $p = 0.0498$) were damaged to a much greater extent (> 3x) than *Acer rubrum* (Figure 5-1c). With respect to differential damage among the experimental treatments, the mean percentage of BA damaged increased from REF, to +N+L, to +N (Figure 5-1a), with the mean BA damage being 18.7% lower in REF than in +N ($t = 2.71$, $p = 0.0244$). Similar to the results for BA damage, the effect of species on the mean percentage of stems damaged did not depend on treatment. However, there was no main effect of species, but there were significant differences in the effect of the experimental treatments ($F = 3.46$, $p = 0.0386$). Specifically, the mean percentage stems damaged increased from REF, to +N+L, to +N (Figure 5-1b), with the mean stem damage being 18.9% lower in REF than in +N ($t = -2.63$, $p = 0.0298$).

Heavy snow-fall in October 2012

By 2012, the snow storm, in addition to the damage from the wind storm of 2009, damaged 55.3% of the stems, and 69.9% of the BA on average in the LTSP. There were no differential or main effects of either species or treatment on the mean percent of BA or stems damaged by heavy snow fall in the LTSP. However, a trend was apparent in the effect of species on mean

percent stem damage (F = 2.16, p = 0.0527) such that proportion of stem damage in *Quercus rubra* may have been greater than that experienced by both *Prunus serotina* (t = 2.94, p = 0.0840) and *Sassafras albidum* (t = 2.88, p = 0.0981).

Across fertilized (WS3) and unfertilized (WS7) watersheds, 49.6% of BA was damaged, and 58.4% of stems were damaged by the snow storm of 2012. Comparing damage between the two watersheds, there was no difference in the percentage of stems that were damaged, but the percentage of BA area damaged was lower in WS3 (t = 2.23, one-tail p = 0.0163). WS3 experienced damage to 44.8% of its BA as a result of the heavy snow fall in contrast to 54.6% of the BA being damaged in WS7. When species composition was included in the analysis, the effect of species on the mean percentage of BA damaged depended on the watershed (F = 2.06, p = 0.0178; Figure 5-2a). In addition, when the mean percentage of BA damaged was averaged across watersheds, there was a main effect of species (F = 2.30, p = 0.0071), but no main effect of watershed was found when values were averaged across species. When the watersheds were compared with respect to the percentage of stems damaged, the effect of species depended on the watershed in a manner that was very similar to that observed for the percentage of BA damaged (F = 2.16, p = 0.0123; Figure 5-2b). There was also a main effect of species on the percentage of stems damaged when values were averaged across watersheds (F = 2.19, p = 0.0109), but no effect of watershed when values were averaged across species.

Across the native N-availability gradient within the older, long-term reference areas, 38.5% of BA was damaged and 48.7% of stems were damaged by the snow storm of 2012. There was no

difference in either percent BA or stem damage among the N-availability levels. However, when species composition was included in the analysis, an trend indicated that the effect of species on the percent BA damaged by Superstorm Sandy may have depended on the level of net N mineralization ($F = 1.15$, $p = 0.0801$). Species differed in the percentage of BA damaged ($F = 3.22$, $p < 0.0001$; Figure 5-3a), but there was no main effect of net N mineralization. Among the tree species present, the percentage of BA damaged was lower for *L. tulipifera* than *Acer pensylvanicum* ($t = 4.55$, $p = 0.0016$), *R. psuedoacacia* ($t = 4.30$, $p = 0.0044$), *Acer saccharum* ($t = 4.18$, $p = 0.0069$), *Oxydendrum arboretum* ($t = 3.85$, $p = 0.0225$), and *Fagus grandifolia* ($t = 3.84$, $p = 0.0235$). The percentage of BA damaged was also lower for *Q. rubra* than *A. pensylvanica* ($t = 3.69$, $p = 0.0385$), and there was a trend towards lower damage for: (1) *Q. rubra* compared to *R. psuedoacacia* ($t = 3.57$, $p = 0.0570$); (2) *Quercus prinus* compared to both *A. pensylvanicum* ($t = 3.56$, $p = 0.0586$) and *R. psuedoacacia* ($t – 3.54$, $p = 0.0630$); and (3) *Fraxinus americanus* compared to both *A. pensylvanicum* ($t = 3.52$, $p = 0.0661$) and *R. psuedoacacia* ($t = 3.52$, $p = 0.0666$).

Analysis of the percentage of stems damaged across the N-availability gradient resulted in a trend that indicated that the effect of species depended on the level of net N mineralization ($F = 1.61$, $p = 0.0504$). Individual slopes of the relationship between N mineralization and damage for each species were not different from zero. There was a main effect of species on the percentage of BA damaged ($F = 3.45$, $p < 0.0001$; Figure 5-3b), but no main effect of net N mineralization. The percentage of stems damaged was lower in *L. tulipifera* than *A. pensylvanicum* ($t = 4.06$, $p = 0.0096$), *R. psuedoacacia* ($t = 3.99$, $p = 0.0141$), and *A. saccharum* ($t = 3.88$, $p = 0.0205$). The percentage of damaged stems was also lower for *Q. prinus* compared to *R. psuedoacacia* (t =

3.62, p = 0.0488). There was a trend toward lower percent stem damage when *Q. prinus* was compared to *A. pensylvanicum* (t = 3.59, p = 0.0533), when *L. tulipifera* was compared to *O. arboretum* (t = 3.58, p = 0.0551), and when *Q. rubra* was compared to both *A. pensylvanicum* (t = 3.46, p = 0.0792) and *R. psuedoacacia* (t = 3.44, p = 0.0792).

5.5 Discussion

By assessing the impact of two severe storms that occurred in pre-existing fertilized and unfertilized areas at the Fernow Experimental Forest, and across a native N-availability gradient, I found evidence that differences in N availability can alter the percentage of both BA and the number of stems that were damaged. However, the influence of long-term changes in N availability on storm damage in our study sites depended on both the nature of the storm, and the species of tree.

For a localized but severe wind storm, the results from the LTSP experiment indicate that strong winds damaged trees growing in N-fertilized stands (+N) more than those growing in unfertilized stands (REF). However, for the heavy snowfall associated with Superstorm Sandy, there was less overall damage in the N-fertilized watershed (WS3), when compared to the unfertilized watershed (WS7). And the lower percentage of damaged trees in WS3 seems to result, at least in part, from a differential effect of N fertilization on the damage experienced by various species. Furthermore, while some species tended to have a higher percentage of damage in WS3 (e.g. *F. americanus* and *R. psuedoacacia*), major differences in the abundance of different tree species may have had the strongest influence over the amount of total damage within each watershed. For example, there was a much greater abundance of canopy trees in the fertilized watershed (51% BA in WS3 vs. 20% BA in WS7) of a species that appears to have been less damaged by heavy snowfall (*P. serotina*). Although a conservative Tukey's HSD test did not detect differential damage in *P. serotina* between watersheds, the apparently lower level of damage to this species in WS3 (Figure 5-2), when combined with the much greater abundance of *P.*

serotina in WS3, may have contributed to the lower overall damage experienced in the fertilized watershed.

Not surprisingly, previous studies have found that storm damage affects individual species differently (Everham and Brokaw 1996; Foster 1988; Rebertus et al. 1997). However, even for a given species, the nature of the damage may depend on the type of storm causing the damage. For example, one study found that *P. serotina* was more susceptible than other species to damage by ice storms (Bruederle and Stearns 1985), whereas our study suggests that, if anything, this species was less damaged than some species by strong winds and heavy snowfall. The reasons for inter- and intra-species differences in susceptibility to storm damage are undoubtedly numerous and complex. However, for *P. serotina* at the FEF I speculate that its relatively thin canopy and early leaf drop, relative to other broadleaf deciduous species like *Q. rubra* (C. A. Walter, *unpublished*), prevented a significant retention of snow in the canopy during an late autumn snow event. A thin canopy, if associated with a lower density of canopy branches, could also explain why it had one of the lowest mean percentages of BA damage among species affected by the 2009 wind storm in LTSP plots (Figure 5-1).

The potential for significant indirect effects of storms on forest communities have been noted in previous studies. For example, storm damage can have drastic and lasting effects on tree composition, favoring the recruitment and regeneration of *Betula nigra* and *Acer rubrum* (Favjan et al. 2003). Disturbances that change tree composition can also lead to changes in the composition of the forest herbaceous layer (Whitney and Foster 1988). And, disturbance events

that that lead to large canopy openings have the potential to change the light levels in the herbaceous layer, ultimately leading to increased competition for this limiting resource among plants growing near the forest floor (Rajaniemi 2003).

Although strong winds and heavy snowfalls in late autumn are currently exceptional events, it seems likely that strong winds will occur more frequently as Earth's climate continues to change (Bender et al. 2010; Emanuel 2005; Grinsted et al. 2013). Thus, if our results are generally applicable, then forests that have experienced high levels of N input in the past (such as forests in the eastern US), or forests that are currently experiencing increases in N deposition (such as forests in eastern China), may be more susceptible to damage by high winds in the future – which has potential effects for both tree and herbaceous layer composition. This research also underscores the importance of long-term ecological research sites that allow for opportunistic, and realistic, assessments of factors that may influence the consequences of unique weather-related events in the future.

5.6 Tables and Figures

Table 5-1. Study sites within the Fernow Experimental Forests that were used in the analysis of storm damage.

Area	Treatment	Approximate stand age (years)	Number of plots
LTSP	Fertilization experiment	19	12
WS7	Cut and unfertilized	45	18
WS3	Cut and fertilized	45	18
BCA	Uncut reference	115	5
WS4	Uncut reference	115	12
WS10	Uncut reference	115	5
WS13	Uncut reference	115	5

Table 5-2. Species used in analysis of storm damage across the Fernow Experimental Forest.

Species	Code
Acer pensylvanicum	ACPE
Acer rubrum	ACRU
Acer saccharum	ACSA
Amelanchier arborea	AMAR
Aralia spinosa	ARSP
Betula lenta	BELE
Carya cordiformis	CACO
Fagus grandifolia	FAGR
Fraxinus americanus	FRAM
Liriodendron tulipifera	LITU
Magnolia accuminata	MAAC
Magnolia fraseri	MAFR
Nyssa sylvatica	NYSY
Ostrya virginiana	OSVI
Oxydendrum arboreum	OXAR
Prunus pensylvanica	PRPE
Prunus serotina	PRSE
Quercus alba	QUAL
Quercus prinus	QUPR
Quercus rubra	QURU
Robinia pseudoacacia	ROPS
Sassafras albidum	SAAL
Tilia americana	TIAM
Ulmus rubra	ULRU

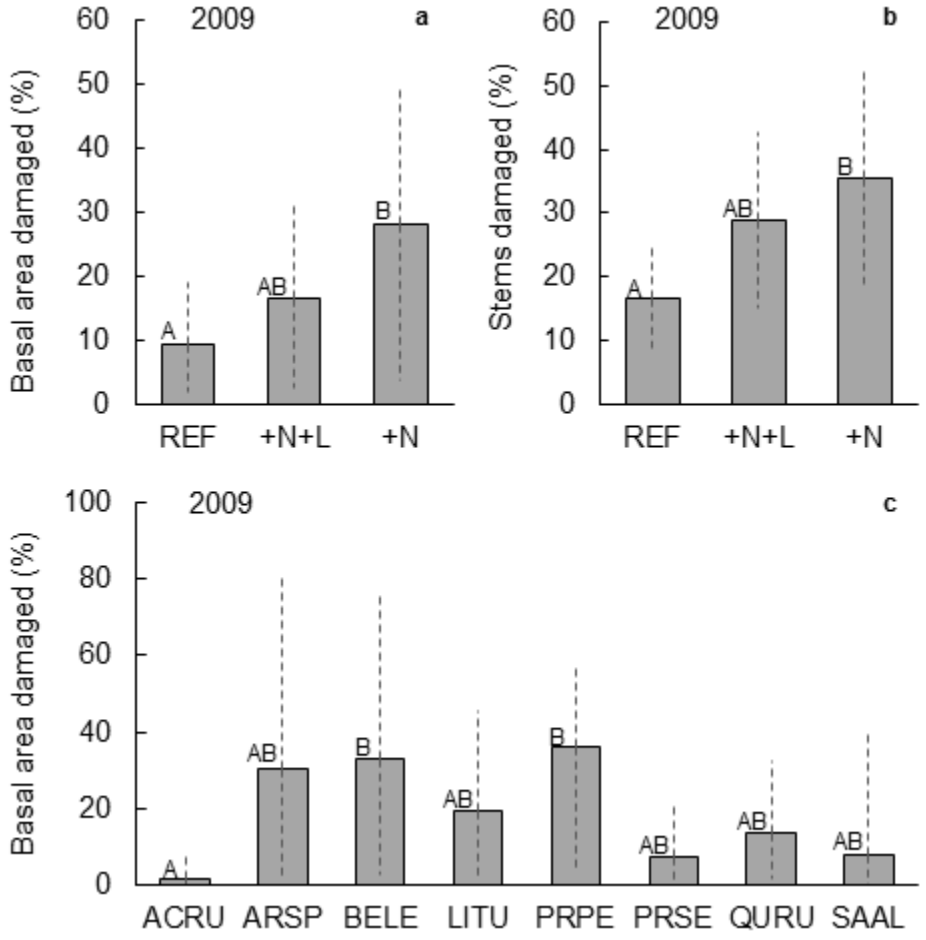

Figure 5-1. Mean percentage of basal area and stems damaged across treatments and species in unfertilized (REF), fertilized (+N), and fertilized and limed (+N+L) treatments in the LTSP from a wind storm in 2009. Error bars are 95% confidence intervals, since mean percent basal area damage and 2012 percent stem damage were back-transformed from square root transformations. Differing letters indicate mean differences from a Tukey's HSD test at $p < 0.05$ and species codes are located in Table 5-2.

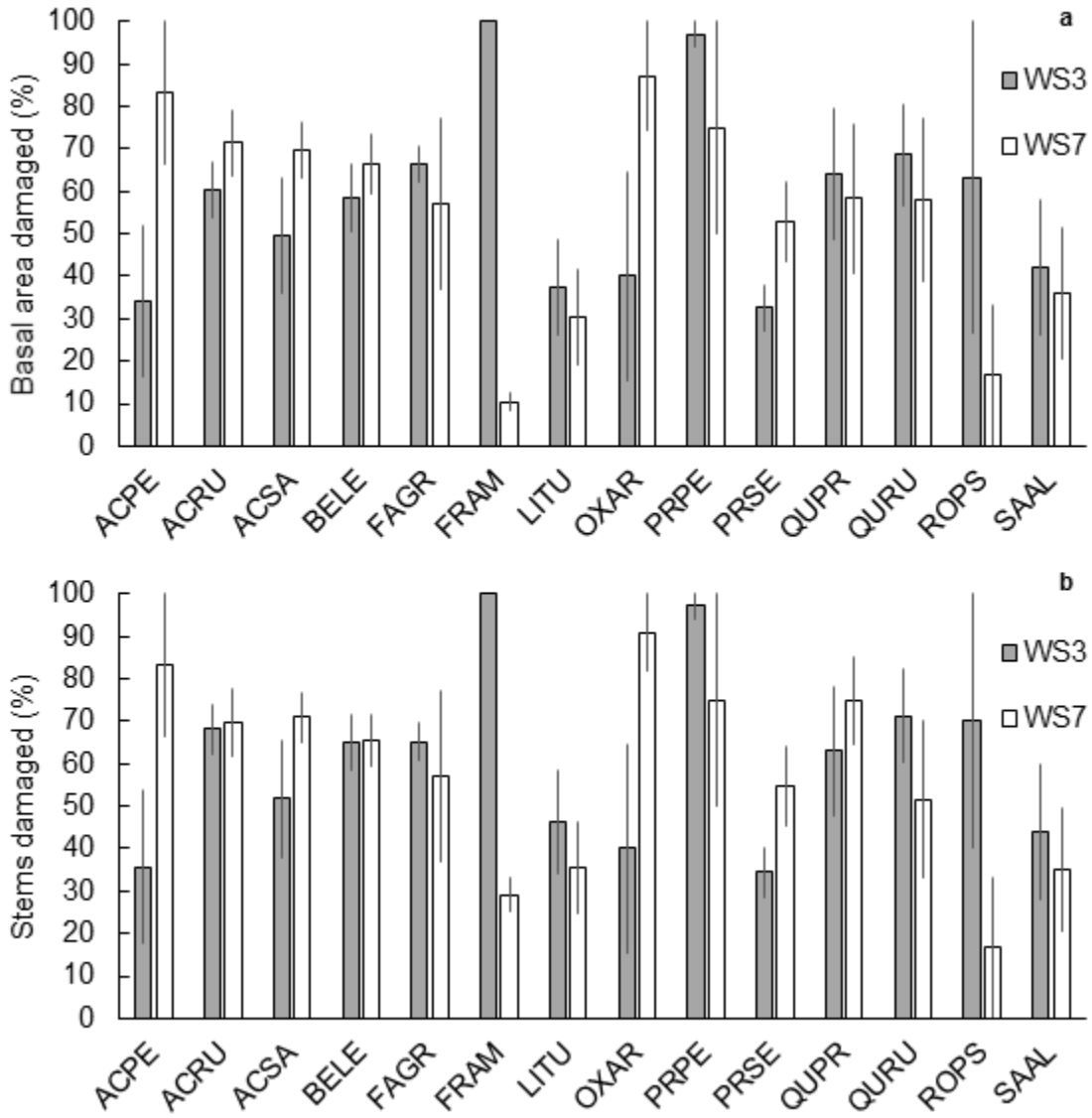

Figure 5-2. Mean percent basal area and stems damaged by species in unfertilized (WS7) and fertilized (WS3) watersheds. Error bars represent one standard error. Species codes are located in Table 5-2.

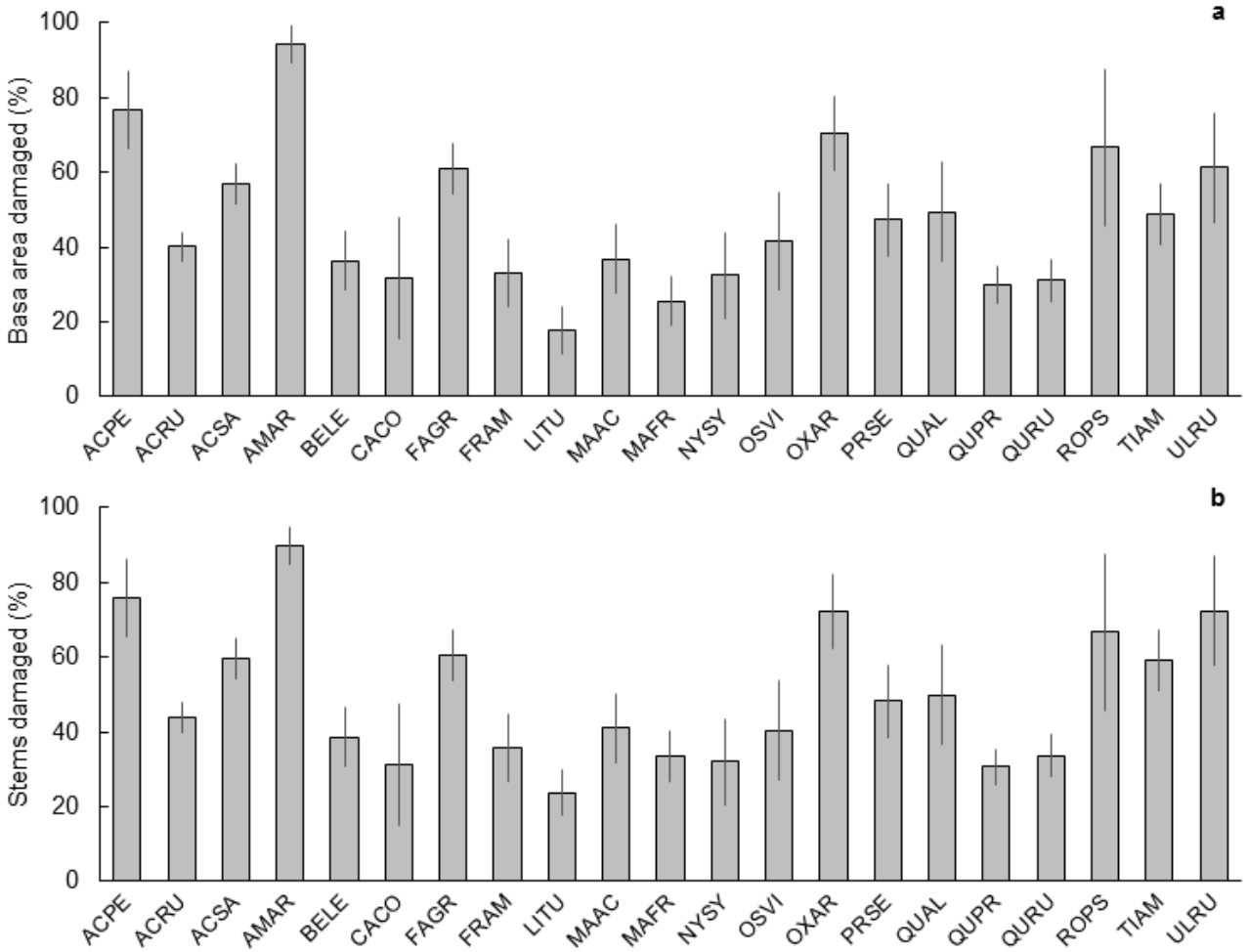

Figure 5-3. Mean percent of stems and basal area damaged by species in the 2012 snow storm across uncut reference areas. Error bars represent one standard error and Tukey's HSD ($p < 0.05$) test revealed % BA damaged of LITU was lower than ACPE, ROPS, ACSA, OXAR, and FAGR. The % BA damaged was also lower for QURU than ACPE. The % stems damaged was lower in LITU than ACPE, ROPS, and ACSA. The % stems damaged was also lower for QUPR ROPS. Species codes are located in Table 5-2.

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Chapter 6. Conclusion: Advancing our understanding of the role of nitrogen addition in shaping the forest herbaceous layer

Summary of results

I used a variety of long-term fertilization experiments and reference compartments throughout the Fernow Experimental Forest (FEF) to: 1) verify the precision and accuracy of the hand-area method of measuring cover in the forest herbaceous layer at the plant, population, and community scale; 2) determine if the effect of nitrogen (N) on *Rubus* spp. cover in the forest herbaceous layer depends on the light level between an N-fertilized and unfertilized watershed, and among N-fertilized and unfertilized plots; 3) test the extent to which the decline in richness in the forest herbaceous layer following N fertilization was due to either random species loss (RSL) or non-random species loss (NRSL), and to assess the extent to which the decline was due to the fertilizing or acidification effects of N; and 4) determine if N additions might have an indirect effect on the herbaceous layer by increasing tree damage from severe wind and snow storms.

The hand-area method was found to be a very precise and potentially very accurate (when calibrated) approach for estimating forest herbaceous layer leaf area or cover. The method was precise because the relationship between estimated leaf area index (LAI) and actual LAI was consistent at the plant, population, and community scale. Further, there was no bias introduced from observers, which is a clear advantage of this approach over other visual estimation methods. The method tended to overestimate actual LAI by 39.1%, consistently across scales. Thus, to improve accuracy, the 39.1% overestimation could be subtracted from each estimated LAI value. Such a correction factor could be calculated by individual practitioners of the method to obtain accurate LAI estimates for comparisons with other sites. Conversely, the method could

be used without a correction factor to compare relative differences in cover or leaf area within a site.

With respect to the effects of N and light on *Rubus* spp. cover, I found a significant interaction between the two factors. At the highest light levels, the relative *Rubus* spp. cover was ca. 85% higher in N-fertilized fertilized treatments than unfertilized treatments. Yet, when light levels were low, there was no difference in relative *Rubus* spp. cover between fertilized and unfertilized treatments. These results confirm that *Rubus* spp. at FEF are nitrophilic because they were able to utilize excess N to compete better for light. Since similar increases in relative *Rubus* spp. cover were observed over a large timespan – experiments ranging from one growing season to 23 years – I infer that the dominance of *Rubus* spp. in response to N happens quickly, and is long lasting. Therefore, changes in species composition in the herbaceous layer in response to N deposition that are driven by nitrophilic species can occur immediately, and persist with continued input.

Testing the mechanisms that drive changes in species richness under N fertilization, I discovered that species richness declined in N-fertilized plots, when compared to unfertilized plots. The mechanisms of random species loss (RSL) and non-random species loss (NRSL) in the forest herbaceous layer both operated to decrease species richness in the forest herbaceous layer, with NRSL dominating in the final sampling year of the experiment. Across all sampling years, 48.6% of species losses in N-fertilized plots could be attributed to NRSL, and 60.1 % of the species losses in unfertilized plots could be attributed to NRSL. These mechanisms were driven, in part, by the advantage conferred to a few nitrophilic species – particularly *Rubus* spp. After 15

years of fertilization, ~ 15% of total herbaceous layer density was occupied by *Rubus* spp. in fertilized plots. By comparison, *Rubus* spp. only made up ~ 3% of the total density in unfertilized plots.

Finally, testing the effect of N on storm damage in forest trees, I found that 18.7% more basal area, and 18.9% more stems were damaged by a severe wind storm in fertilized plots, when compared to unfertilized plots. In contrast, I discovered that the percent of basal area damaged was lower in a fertilized watershed than an unfertilized watershed in response to Superstorm Sandy, a severe snow storm that occurred in late fall. The damage by the snow storm was driven by differences in the composition of tree species between the watersheds, since there was evidence that the effect of N fertilization on damage depended on the species of tree. A similar differential pattern of N availability and species was observed when damaged trees were surveyed across a native N-availability gradient.

Implications for the existing conceptual framework

The results of this dissertation suggest that three modifications should be considered to the conceptual framework presented by Gilliam (2006; Figure1-1) for understanding the effects of N on the species composition of the forest herbaceous layer: (1) the inclusion of the effects of random species loss (RSL); (2) an emphasis on the magnitude of both the interspecific competition portion of non-random species loss (NRSL) and RSL, relative to more distal and secondary effects of N; and (3) the inclusion of the indirect effect of N on herbaceous layer communities, by increasing the susceptibility of forest trees to wind damage.

Results from *Chapter 4* highlighted the effect of both NRSL and RSL in decreasing species richness in the forest herbaceous layer. Previous studies in forests also have found evidence of reductions in richness due to RSL (Thomas et al. 1999), while studies in herb-dominated systems have found evidence that both NRSL and RSL mechanisms decrease richness (Stevens and Carson 1999, Hautier et al. 2009). In light of my results, and the results from previous work, it is clear that both NRSL and RSL may have a significant role in shaping the herbaceous layer composition of a forest under N additions, and that the magnitude of these effects is large relative to more indirect effects of N (Figure 6-1).

Results from *Chapter 3* also helped to determine the magnitude of the direct effects of N additions on NRSL. In one study contained in that chapter, *Rubus allegheniensis* plants were taken from the FEF and transplanted in a field experiment to determine if the effect of N on the leaf area of *R. allegheniensis* depended on the level of light. I found that leaf area was 130.2% greater in the N-fertilized plants at high light, when compared to unfertilized plants at high light. Growing these plants in a field experiment over one growing season allowed me to control for some of the indirect N effects proposed in the Gilliam (2006) conceptual framework – particularly those of herbivory, species invasions, and exotic earthworm activity. Additionally, we can reasonably infer from the experimental design that other indirect effects of N were minimal, if not, completely absent since there were no obvious signs of pathogenic infection, and the potting soil used came from a homogenous mixture, presumably meaning that the potential for mycorrhizal inoculation was equal for each plant. Controlling for these factors in this experiment, I conclude that the large increase in leaf area in fertilized *R. allegheniensis* plants under high light levels is a direct effect of N. Accordingly, I suggest that the conceptual

framework indicate a greater magnitude of direct N effects on the contribution of interspecific competition to NRSL, and the magnitude of those effects be carried through to affect species richness (Figure 6-1).

Results from *Chapter 5* present a potentially novel indirect effect of N that could lead to changes in species composition in the forest herbaceous layer. In that chapter I discovered that a wind storm caused more tree damage in fertilized plots than those that weren't fertilized, and that the percentage of damaged trees was different among species. These results pose two potential scenarios, each with implications for species composition in the herbaceous layer: 1) canopy gaps created by wind-storm damage are greater in both quantity and size under N additions, which causes more light to reach the herbaceous layer; and 2) differential damage among species could lead to changes in tree composition that, in turn, affect herbaceous layer composition later in succession. In *Chapter 3* I demonstrated that the effect of N on *Rubus* spp. was only realized when there was sufficient light. Under the first scenario (more and larger canopy gaps), storm damage that led to more light in an N-fertilized area could dramatically enhance the cover of *Rubus* spp., increase the role of non-random species loss (NRSL), and decrease species richness. Thus, competition for light in this scenario emerges as the major factor affecting composition in N-amended areas (Hautier et al. 2009). Under the second scenario (differential species damage), storm damage that led to a shift in tree species could affect the herbaceous layer composition by increasing competition, and/or changing litter chemistry (Crozier and Boerner 1984, Whitney and Foster 1988). Thus, I suggest that storm damage be added an intermediary step between N deposition and both NRSL and RSL mechanisms. Although the current relative magnitude of this effect is low, it is likely to increase as the frequency of large-magnitude storms increases in a

warmer world (Emanuel 2005, Bender et al. 2010, Grinsted et al. 2012, 2013). Additionally, Since the level of tree damage from storms is species specific, and the herbaceous layer can influence the composition of trees by altering tree recruitment (Balandier et al. 2013; George and Bazzaz 2014), I also suggest that there is a potential for feedback from herbaceous layer to the level of damage from storms (dashed arrows in Figure 6-1).

Implications for biodiversity

The eastern broadleaf deciduous forest region of North America is ranked as globally outstanding, in terms of biological distinctiveness – a metric that accounts for the diversity of species, ecosystems, and ecological processes (Ricketts et al. 1999). And it is the richness of the herbaceous layer that drives this globally distinctive diversity ranking (Gilliam 2007). Therefore, if biodiversity is to be preserved in these forests, it is of critical importance to understand the underlying mechanisms that lead to declines in richness in the herbaceous layer, under N additions. This research reaffirms the direct effects that N can have on the forest herbaceous layer and explores in more depth some mechanistic functions for changes in species composition in response to N. Additionally, the results bring to light a potentially novel indirect effect of N on the composition of the herbaceous layer – increasing tree susceptibility to storm damage – that is likely to become more significant as the frequency of large-magnitude storms increases under climate change. Thus, if these results are indicative of broadleaf deciduous forests everywhere (e.g. Chinese forests, which are currently receiving historically high rates of N deposition), then forest biodiversity is threatened from N deposition, and the loss of forest species is likely to be exacerbated by climate change.

6.1 Tables and figures

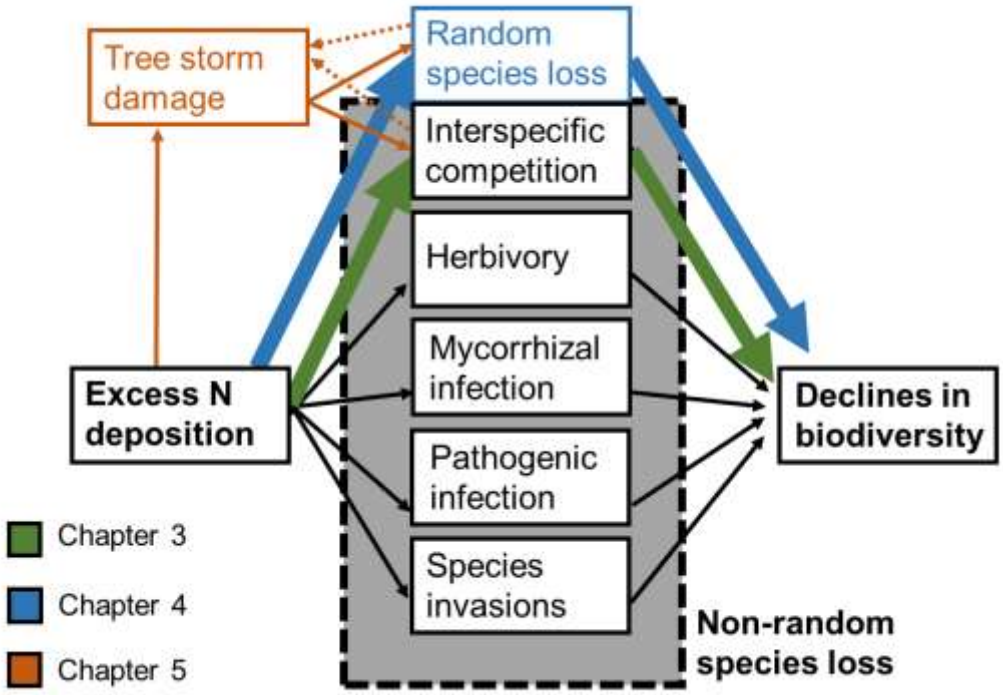

Figure 6-1. A conceptual model of the linkages and feedbacks among biotic factors that lead to declines in forest biodiversity under N deposition, modified from Gilliam (2006). Colored boxes and arrows indicate modifications suggested by the results of this dissertation, the magnitude of effects is indicated by the size of the arrow, and dashed arrows indicate a potential feedback whereby herbaceous layer community can affect recruitment of overstory tree species.

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Appendix A. Supplementary Tables

Table A-1. Nitrophily status of plants in the Long-Term Productivity Experiment. Index values were assigned or based on prior observations and experiments, the species-specific index value in the Ellenberg index (Hill et al. 1999), or the median index value from the congeners from the Ellenberg index when species-specific values were unavailable. Index values greater than five were categorized as nitrophilic.

		Nitrophily		
Taxon	**Form**	**Index**	**Status**	**Source**
Acer pensylvanicum	tree	6	nitrophilic	Hill et al. 1999
Acer rubrum	tree	3	non-nitrophilic	Peterjohn et al. 2015
Acer saccharum	tree	8	nitrophilic	Peterjohn et al. 2015
Actaea pachypoda	herb	6	nitrophilic	Hill et al. 1999
Actaea racemosa	herb	6	nitrophilic	Hill et al. 1999
Ageratina altissima	herb	7	nitrophilic	Wang and Feng 2005
Amaranthus spp.	herb	7	nitrophilic	Hill et al. 1999
Amelanchier arborea	shrub	1	non-nitrophilic	Peterjohn et al. 2015
Aralia nudicaulis	herb	6	nitrophilic	Allen 2004
Arisaema triphyllum	herb	3	non-nitrophilic	Fraterrigo et al. 2009
Aristolochia spp.	vine	5	non-nitrophilic	Ulrey 2002
Aster spp.	herb	6	nitrophilic	Hill et al. 1999
Athryum filix-femina	herb	7	nitrophilic	Brunet at al. 1998
Betula alleghaniensis	tree	4	non-nitrophilic	Hill et al. 1999
Betula lenta	tree	4	non-nitrophilic	Peterjohn et al. 2015
Boehmeria cylindrica	herb	4	non-nitrophilic	Welch et al. 2007
Cardamine angustata	herb	6	nitrophilic	Hill et al. 1999
Carex spp.	graminoid	3	non-nitrophilic	Hill et al. 1999
Carya cordiformis	tree	4	non-nitrophilic	Smalley 1990
Caulophyllum thalictroides	herb	6	nitrophilic	Spies and Barnes 1985
Chamerion angustifolium	herb	5	non-nitrophilic	Hill et al. 1999
Circaea lutetiana	herb	6	nitrophilic	Hill et al. 1999
Clematis virginiana	vine	5	non-nitrophilic	Hill et al. 1999
Collinsonia canadensis	herb	5	non-nitrophilic	Rees 2003
Convallaria majuscula	herb	5	non-nitrophilic	Hill et al. 1999
Cornus alternifolia	tree	6	nitrophilic	Hill et al. 1999
Dennstaedtia punctilobula	herb	3	non-nitrophilic	Brach 1993
Dichanthelium clandestinum	grass	2	non-nitrophilic	Rentch et al. 2005
Dioscorea villosa	herb	4	non-nitrophilic	Ulrey 2002
Dryopteris carthusiana	herb	4	non-nitrophilic	Hill et al. 1999
Fagus grandifolia	tree	5	non-nitrophilic	Hill et al. 1999
Fraxinus americana	tree	4	non-nitrophilic	Welch et al. 2007
Galium spp.	herb	3.5	non-nitrophilic	Hill et al. 1999
Geranium maculatum	herb	6	nitrophilic	Hill et al. 1999
Goodyera pubescens	herb	2	non-nitrophilic	Hill et al. 1999

Table A-1 continued

Taxon	Form	Nitrophily Index	Nitrophily Status	Source
Graminoid	graminoid	6	nitrophilic	Hill et al. 1999
Hexastylis virginica	herb	7	nitrophilic	Bernhardt-Romermann et al. 2007
Impatiens pallida	herb	6.5	nitrophilic	Hill et al. 1999
Lindera Benzoin	shrub	5	non-nitrophilic	Welch et al. 2007
Liriodendron tulipifera	tree	6	nitrophilic	Peterjohn et al. 2015
Lycopodium spp.	herb	3	non-nitrophilic	Aerts and Bobbink 1999
Magnolia acuminata	tree	2	non-nitrophilic	Peterjohn et al. 2015
Magnolia fraseri	tree	4	non-nitrophilic	Johnson et al. 2010
Medeola virginiana	herb	4	non-nitrophilic	Kenlan et al. 2009
Monarda clinopodia	herb	3	non-nitrophilic	usda
Monotropa uniflora	herb	2	non-nitrophilic	Hill et al. 1999
Nyssa sylvatica	tree	3	non-nitrophilic	Peterjohn et al. 2015
Osmorhiza clatonia	herb	3	non-nitrophilic	Welch et al. 2007
Ostrya virginiana	tree	2	non-nitrophilic	Talhelm et al. 2013
Oxalis stricta	herb	4.5	non-nitrophilic	Hill et al. 1999
Oxydendron arboreum	tree	3	non-nitrophilic	Fabio 2006
Parthenocissus quinquefolia	herb	4	non-nitrophilic	Chapman et al. 2015
Phytolacca americana	herb	6	nitrophilic	Cahill and Casper 1999
Podophyllum peltatum	herb	unknown	unknown	NA
Polygantum biflorum	herb	5	non-nitrophilic	Hill et al. 1999
Polygonum spp.	herb	5.5	nitrophilic	Fraterrigo et al. 2009
Polystichum acrostichoides	fern	6	nitrophilic	Welch et al. 2007
Potentilla simplex	herb	2	non-nitrophilic	Hill et al. 1999
Prenanthes altissima	herb	7	nitrophilic	Fraterrigo et al. 2009
Prosartes maculata	herb	3	non-nitrophilic	Kaye et al. 2008
prunus pensylvanica	tree	6	nitrophilic	Hill et al. 1999
Prunus serotina	tree	6	nitrophilic	Hill et al. 1999
Pycanthemum virginianum	herb	6	nitrophilic	McPhee 2013
Quercus alba	tree	4	non-nitrophilic	Hill et al. 1999
Quercus prinus	tree	4	non-nitrophilic	Hill et al. 1999
Quercus rubra	tree	5	non-nitrophilic	Peterjohn et al. 2015
Robinia pseudoacacia	tree	6	nitrophilic	Hill et al. 1999
Rosa carolina	herb	4	non-nitrophilic	Hill et al. 1999
Rubus spp.	shrub	8	nitrophilic	Walter et al. 2015
Rumex spp.	herb	7	nitrophilic	Hill et al. 1999
Sambucus canadensis	shrub	7	nitrophilic	Hill et al. 1999
Sassafras albidum	tree	2	non-nitrophilic	Hutchinson et al. 1999
Smilax ecirrhata	herb	4	non-nitrophilic	Martin 2013
Smilax rotundifolia	vine	4	non-nitrophilic	Martin 2013

Table A-1 Continued

Taxon	Form	Nitrophily Index	Nitrophily Status	Source
Streptopus lanceolatus	herb	unknown	unknown	NA
Thelypteris noveboracensis	herb	5	non-nitrophilic	Hill et al. 1999
Tilia americana	tree	6	nitrophilic	Hill et al. 1999
Tussilago farfara	herb	6	nitrophilic	Hill et al. 1999
Urtica dioica	herb	8	nitrophilic	Hill et al. 1999
Viburnum acerifolium	shrub	5.5	nitrophilic	Hill et al. 1999
Viola spp.	herb	2	non-nitrophilic	Hill et al. 1999
Vitis spp.	vine	3	non-nitrophilic	Strenbom et al. 2003
Zanthoxylum americanum	tree	unknown	unknown	NA

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Table A-2. Results of Tukey's HSD tests of pairwise comparisons in plant density (*D*), species richness (*R*), diversity (*H*'), and evenness (*J*) among years and treatments.

Contrast			Density		Richness		Diversity		Evenness	
			Diff.	t-ratio	Diff.	t-ratio	Diff.	t-ratio	Diff.	t-ratio
+N (1997)	vs.	+N (2001)	355.5	4.78 **	2.8	1.11	-0.004	-0.03	-0.035	-0.94
+N (1997)	vs.	+N (2006)	450.3	6.05 ****	2.5	1.01	-0.061	-0.39	-0.060	-1.61
+N (1997)	vs.	+N (2011)	339.3	4.56 **	3.5	1.41	0.172	1.08	0.009	0.25
+N (1997)	vs.	REF (1997)	-102.3	-1.37	-6.0	-2.42	-0.193	-1.21	-0.005	-0.15
+N (1997)	vs.	REF (2001)	148.5	1.99	-2.8	-1.11	-0.082	-0.52	0.001	0.04
+N (1997)	vs.	REF (2006)	331.3	4.45 **	-3.3	-1.31	-0.005	-0.03	0.029	0.78
+N (1997)	vs.	REF (2011)	225.3	3.03	0.8	0.30	0.443	2.78	0.135	3.60 *
+N (2001)	vs.	+N (2006)	94.8	1.27	-0.3	-0.10	-0.057	-0.36	-0.025	-0.67
+N (2001)	vs.	+N (2011)	-16.3	-0.22	0.8	0.30	0.176	1.1	0.045	1.20
+N (2001)	vs.	REF (1997)	-457.8	-6.15 ****	-8.8	-3.53 *	-0.189	-1.18	0.030	0.80
+N (2001)	vs.	REF (2001)	-207.0	-2.78	-5.5	-2.22	-0.078	-0.49	0.037	0.98
+N (2001)	vs.	REF (2006)	-24.3	-0.33	-6.0	-2.42	0.000	0	0.065	1.73
+N (2001)	vs.	REF (2011)	-130.3	-1.75	-2.0	-0.81	0.447	2.81	0.170	4.55 **
+N (2006)	vs.	+N (2011)	-111.0	-1.49	1.0	0.40	0.233	1.46	0.070	1.87
+N (2006)	vs.	REF (1997)	-552.5	-7.42 ****	-8.5	-3.43 º	-0.131	-0.83	0.055	1.47
+N (2006)	vs.	REF (2001)	-301.8	-4.05 *	-5.3	-2.12	-0.021	-0.13	0.062	1.65
+N (2006)	vs.	REF (2006)	-119.0	-1.60	-5.8	-2.32	0.057	0.36	0.090	2.40
+N (2006)	vs.	REF (2011)	-225.0	-3.02	-1.8	-0.71	0.504	3.16	0.195	5.22 ***
+N (2011)	vs.	REF (1997)	-441.5	-5.93 ****	-9.5	-3.83 *	-0.365	-2.29	-0.015	-0.40
+N (2011)	vs.	REF (2001)	-190.8	-2.56	-6.3	-2.52	-0.254	-1.6	-0.008	-0.21
+N (2011)	vs.	REF (2006)	-8.0	-0.11	-6.8	-2.72	-0.176	-1.11	0.020	0.53
+N (2011)	vs.	REF (2011)	-114.0	-1.53	-2.8	-1.11	0.271	1.7	0.125	3.35 º
+N+L (1997)	vs.	+N (1997)	76.8	1.03	0.0	0.00	-0.005	-0.03	-0.001	-0.03
+N+L (1997)	vs.	+N (2001)	432.3	5.81 ****	2.8	1.11	-0.009	-0.06	-0.036	-0.97
+N+L (1997)	vs.	+N (2006)	527.0	7.08 ****	2.5	1.01	-0.066	-0.42	-0.061	-1.64
+N+L (1997)	vs.	+N (2011)	416.0	5.59 ***	3.5	1.41	0.167	1.05	0.009	0.23
+N+L (1997)	vs.	+N+L (2001)	429.3	5.77 ****	2.8	1.11	0.074	0.47	-0.008	-0.21
+N+L (1997)	vs.	+N+L (2006)	396.8	5.33 ***	-0.5	-0.20	-0.015	-0.09	-0.007	-0.19
+N+L (1997)	vs.	+N+L (2011)	414.3	5.56 ***	2.3	0.91	0.165	1.04	0.028	0.75
+N+L (1997)	vs.	REF (1997)	-25.5	-0.34	-6.0	-2.42	-0.198	-1.24	-0.006	-0.17
+N+L (1997)	vs.	REF (2001)	225.3	3.03	-2.8	-1.11	-0.087	-0.55	<0.001	0.01
+N+L (1997)	vs.	REF (2006)	408.0	5.48 ***	-3.3	-1.31	-0.009	-0.06	0.028	0.76
+N+L (1997)	vs.	REF (2011)	302.0	4.06 *	0.8	0.30	0.438	2.75	0.134	3.57 *
+N+L (2001)	vs.	+N (1997)	-352.5	-4.74 **	-2.8	-1.11	-0.079	-0.5	0.007	0.19
+N+L (2001)	vs.	+N (2001)	3.0	0.04	0.0	0.00	-0.083	-0.52	-0.028	-0.76
+N+L (2001)	vs.	+N (2006)	97.8	1.31	-0.3	-0.10	-0.141	-0.88	-0.053	-1.43
+N+L (2001)	vs.	+N (2011)	-13.3	-0.18	0.8	0.30	0.093	0.58	0.017	0.44

Table A-2 continued

Contrast	Density		Richness		Diversity		Evenness	
	Diff.	t-ratio	Diff.	t-ratio	Diff.	t-ratio	Diff.	t-ratio
+N+L (2001) vs. +N+L (2006)	-32.5	-0.44	-3.3	-1.31	-0.089	-0.56	0.001	0.03
+N+L (2001) vs. +N+L (2011)	-15.0	-0.20	-0.5	-0.20	0.091	0.57	0.036	0.97
+N+L (2001) vs. REF (1997)	-454.8	-6.11 ****	-8.8	-3.53 *	-0.272	-1.71	0.002	0.04
+N+L (2001) vs. REF (2001)	-204.0	-2.74	-5.5	-2.22	-0.161	-1.01	0.009	0.23
+N+L (2001) vs. REF (2006)	-21.3	-0.29	-6.0	-2.42	-0.084	-0.53	0.036	0.97
+N+L (2001) vs. REF (2011)	-127.3	-1.71	-2.0	-0.81	0.364	2.28	0.142	3.79 *
+N+L (2006) vs. +N (1997)	-320.0	-4.30 **	0.5	0.20	0.010	0.06	0.006	0.16
+N+L (2006) vs. +N (2001)	35.5	0.48	3.3	1.31	0.006	0.04	-0.029	-0.79
+N+L (2006) vs. +N (2006)	130.3	1.75	3.0	1.21	-0.051	-0.32	-0.054	-1.45
+N+L (2006) vs. +N (2011)	19.3	0.26	4.0	1.61	0.182	1.14	0.015	0.41
+N+L (2006) vs. +N+L (2011)	17.5	0.24	2.8	1.11	0.180	1.13	0.035	0.94
+N+L (2006) vs. REF (1997)	-422.3	-5.67 ****	-5.5	-2.22	-0.183	-1.15	<0.001	0.01
+N+L (2006) vs. REF (2001)	-171.5	-2.30	-2.3	-0.91	-0.072	-0.45	0.007	0.20
+N+L (2006) vs. REF (2006)	11.3	0.15	-2.8	-1.11	0.006	0.03	0.035	0.94
+N+L (2006) vs. REF (2011)	-94.8	-1.27	1.3	0.50	0.453	2.84	0.141	3.76 *
+N+L (2011) vs. +N (1997)	-337.5	-4.53 **	-2.3	-0.91	-0.170	-1.07	-0.029	-0.78
+N+L (2011) vs. +N (2001)	18.0	0.24	0.5	0.20	-0.174	-1.09	-0.065	-1.72
+N+L (2011) vs. +N (2006)	112.8	1.51	0.3	0.10	-0.231	-1.45	-0.090	-2.39
+N+L (2011) vs. +N (2011)	1.8	0.02	1.3	0.50	0.002	0.01	-0.020	-0.53
+N+L (2011) vs. REF (1997)	-439.8	-5.91 ****	-8.3	-3.33 º	-0.363	-2.28	-0.035	-0.93
+N+L (2011) vs. REF (2001)	-189.0	-2.54	-5.0	-2.02	-0.252	-1.58	-0.028	-0.74
+N+L (2011) vs. REF (2006)	-6.3	-0.08	-5.5	-2.22	-0.175	-1.1	<0.001	0.01
+N+L (2011) vs. REF (2011)	-112.3	-1.51	-1.5	-0.61	0.273	1.71	0.106	2.82
REF (1997) vs. REF (2001)	250.8	3.37 º	3.3	1.31	0.111	0.69	0.007	0.19
REF (1997) vs. REF (2006)	433.5	5.82 ****	2.8	1.11	0.188	1.18	0.035	0.93
REF (1997) vs. REF (2011)	327.5	4.40 **	6.8	2.72	0.636	3.99 *	0.140	3.75 *
REF (2001) vs. REF (2006)	182.8	2.45	-0.5	-0.20	0.078	0.49	0.028	0.75
REF (2001) vs. REF (2011)	76.8	1.03	3.5	1.41	0.525	3.3 º	0.133	3.56 *
REF (2006) vs. REF (2011)	-106.0	-1.42	4.0	1.61	0.447	2.81	0.105	2.82

****$p \leq 0.0001$; ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; º$p < 0.1$

Table A-3. List of differences in number of individuals per 5 m^2 between a simulated assemblage level thinning distribution and observed density (δ) between 1997 and 2001 among REF (R), +N, and +N+L treatments. Bold p-values indicate significant differences based on a sequential Bonferroni test of probability tests.

	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
Taxon	Mean	SE	Mean	SE	Mean	SE			
Acer pensylvanicum	-4.57	3.10	-0.93	1.85	-0.02	1.04	0.1621	0.0732	0.3807
Acer rubrum	-7.17	5.11	-0.47	1.22	-0.37	1.33	0.0854	0.0846	0.4975
Acer saccharum	2.59	0.19	0.90	0.15	6.00	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Actaea pachypoda	-4.75	4.19	0.00	0.00	0.00	0.00	0.3148	0.3148	1.0000
Actaea racemosa	1.32	0.64	0.00	0.00	-0.41	0.43	0.0479	0.0252	0.3702
Ageratina altissima	0.33	0.19	-1.03	0.99	0.00	0.00	0.0425	0.1365	0.3200
Amaranthus spp.	-0.17	0.19	0.00	0.00	0.00	0.00	0.4786	0.4786	1.0000
Amelanchier arborea	0.00	0.00	0.00	0.00	-0.20	0.20	1.0000	0.4073	0.4073
Aralia nudicaulis	0.00	0.00	0.00	0.00	-1.20	1.07	1.0000	0.3148	0.3148
Arisaema triphyllum	1.69	0.40	0.42	0.14	1.25	0.00	0.0091	0.2077	**<0.0001**
Aristolochia spp.	-0.16	0.19	0.00	0.00	-1.24	1.18	0.4871	0.3278	0.3176
Aster spp.	4.45	0.76	0.00	0.00	-0.12	0.18	**<0.0001**	**<0.0001**	0.6430
Athryum filix-femina	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Betula alleghaniensis	-1.62	0.90	0.03	0.22	-0.51	0.54	0.0432	0.1782	0.2571
Betula lenta	7.24	0.29	0.65	0.70	7.09	0.55	**<0.0001**	0.5055	**<0.0001**
Boehmeria cylindrica	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Cardamine angustata	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Carex spp.	-7.51	3.12	2.01	1.05	-0.94	0.76	0.0005	0.0173	0.0228
Carya cordiformis	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Caulophyllum thalictroides	0.25	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Chamerion angustifolium	-0.94	0.33	-0.18	0.19	-0.14	0.17	0.0519	0.0357	0.6423
Circaea lutetiana	0.00	0.00	0.00	0.00	-0.10	0.15	1.0000	0.6489	0.6489
Clematis virginiana	0.00	0.00	0.25	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Collinsonia canadensis	-0.16	0.19	0.00	0.00	0.00	0.00	0.4867	0.4867	1.0000
Convallaria majuscula	0.25	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Cornus alternifolia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Dennstaedtia punctilobula	-0.47	0.34	0.00	0.00	-0.42	3.84	0.1710	0.5056	0.4169
Dichanthelium clandestinum	6.32	0.87	-1.65	1.23	0.00	0.00	**<0.0001**	**<0.0001**	0.0981
Dioscorea villosa	-0.17	0.19	-0.10	0.15	-0.66	0.54	0.6233	0.3075	0.2782
Dryopteris carthusiana	2.00	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Fagus grandifolia	0.00	0.00	0.75	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Fraxinus americana	-0.09	0.34	0.50	0.00	1.33	0.22	**<0.0001**	0.0009	0.0087
Galium spp.	0.33	0.19	-0.08	0.13	0.25	0.00	0.1031	0.5235	**<0.0001**
Geranium maculatum	0.00	0.00	0.00	0.00	-0.31	0.34	1.0000	0.4137	0.4137
Goodyera pubescens	0.00	0.00	-0.08	0.14	0.00	0.00	0.7091	1.0000	0.7091

Table A-3 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Graminoid	1.04	0.70	2.04	0.25	0.50	0.00	0.1321	0.2739	**<0.0001**
Hexastylis virginica	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Impatiens pallida	0.50	0.00	0.00	0.00	0.25	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Lindera Benzoin	0.00	0.00	0.00	0.00	0.25	0.00	1.0000	**<0.0001**	**<0.0001**
Liriodendron tulipifera	-20.79	16.96	-12.80	4.56	-11.54	2.80	0.3505	0.3243	0.4183
Lycopodium spp.	0.00	0.00	0.25	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Magnolia acuminata	-0.19	0.51	0.07	0.24	0.89	0.55	0.4410	0.0964	0.1363
Magnolia fraseri	0.12	0.17	2.00	0.00	0.50	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Medeola virginiana	-3.18	1.99	0.00	0.00	-0.36	0.40	0.0623	0.0934	0.4056
Monarda clinopodia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Monotropa uniflora	-0.16	0.18	-0.10	0.15	0.00	0.00	0.6325	0.4873	0.6439
Nyssa sylvatica	0.00	0.00	-0.21	0.25	0.00	0.00	0.4843	1.0000	0.4843
Osmorhiza clatonia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Ostrya virginiana	-0.17	0.19	0.00	0.00	0.00	0.00	0.4832	0.4832	1.0000
Oxalis stricta	0.00	0.00	0.00	0.00	-0.08	0.14	1.0000	0.7019	0.7019
Oxydendron arboreum	0.75	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Parthenocissus quinquefolia	0.00	0.00	0.00	0.00	-0.20	0.20	1.0000	0.4092	0.4092
Phytolacca americana	-29.75	10.96	-31.94	12.89	-38.46	31.91	0.4481	0.4116	0.4469
Podophyllum peltatum	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Polygantum biflorum	-0.51	0.49	0.00	0.00	0.00	0.00	0.3355	0.3355	1.0000
Polygonum spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Polystichum acrostichoides	-0.32	2.02	2.64	0.30	0.08	0.46	0.0778	0.4457	**<0.0001**
Potentilla simplex	-0.97	1.83	0.00	0.00	0.00	0.00	0.3363	0.3363	1.0000
Prenanthes altissima	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Prosartes maculata	0.67	0.33	0.25	0.00	0.00	0.00	0.1647	0.0750	**<0.0001**
prunus pensylvanica	0.84	1.52	2.62	4.07	1.21	2.47	0.3243	0.4493	0.3751
Prunus serotina	-5.80	8.61	-7.17	12.02	12.18	6.24	0.4713	0.0518	0.0790
Pycanthemum virginianum	0.00	0.00	0.00	0.00	-0.80	0.72	1.0000	0.3153	0.3153
Quercus alba	0.25	0.00	0.25	0.00	0.25	0.00	1.0000	1.0000	1.0000
Quercus prinus	-0.47	0.35	0.00	0.00	0.00	0.00	0.1739	0.1739	1.0000
Quercus rubra	-0.74	0.38	0.13	0.30	0.34	0.77	0.0639	0.1379	0.4438
Robinia pseudoacacia	-0.70	0.51	1.47	0.45	-0.14	0.82	0.0016	0.3072	0.0382
Rosa carolina	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Rubus spp.	68.55	5.45	63.51	2.27	56.71	3.15	0.2138	0.0207	0.0345
Rumex spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Sambucus canadensis	-1.62	0.73	-1.28	1.04	-0.06	0.12	0.4047	0.0168	0.1571
Sassafras albidum	-2.96	7.46	-7.94	5.67	-9.64	10.42	0.2993	0.3170	0.4698
Smilax ecirrhata	0.58	0.19	0.42	0.14	0.00	0.00	0.4112	0.0152	0.0345
Smilax rotundifolia	-2.08	1.14	3.37	0.84	6.53	0.75	0.0002	**<0.0001**	0.0023

Table A-3 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Streptopus lanceolatus	3.32	1.32	-0.53	0.72	0.38	0.18	0.0116	0.0315	0.1482
Thelypteris noveboracensis	0.43	2.68	3.53	0.43	5.66	0.60	0.1449	0.0341	0.0081
Tilia americana	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Tussilago farfara	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Urtica dioica	9.01	12.26	5.41	3.67	0.84	13.62	0.3666	0.3396	0.3836
Viburnum acerifolium	0.00	0.00	-0.12	0.18	-0.06	0.12	0.6470	0.7858	0.7039
Viola spp.	-9.04	17.48	-15.53	13.04	-22.97	14.66	0.3913	0.2811	0.3674
Vitis spp.	-5.93	15.47	-9.04	4.90	-15.81	2.57	0.4300	0.2774	0.1175
Zanthoxylum americanum	-0.19	1.36	3.19	0.27	0.36	0.41	0.0004	0.4037	**<0.0001**

Table A-4. List of differences in number of individuals per 5 m^2 between a simulated assemblage level thinning distribution and observed density (δ) between 1997 and 2006 among REF (R), +N, and +N+L treatments. Bold p-values indicate significant differences based on a sequential Bonferroni test of probability tests.

	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
Taxon	Mean	SE	Mean	SE	Mean	SE			
Acer pensylvanicum	-0.24	1.53	-0.63	1.66	-1.22	1.07	0.4437	0.3097	0.4081
Acer rubrum	-5.84	3.95	-0.56	1.22	1.45	0.79	0.0889	0.0037	0.0852
Acer saccharum	0.92	0.13	1.42	0.13	1.25	0.00	0.0251	**<0.0001**	0.2791
Actaea pachypoda	-2.21	2.00	0.00	0.00	0.00	0.00	0.3143	0.3143	1.0000
Actaea racemosa	0.25	0.51	0.50	0.00	-0.18	0.25	0.4541	0.2768	**<0.0001**
Ageratina altissima	0.41	0.14	-0.57	1.01	0.00	0.00	0.3227	0.0449	0.3749
Amaranthus spp.	0.62	0.17	0.00	0.00	0.00	0.00	0.0071	0.0071	1.0000
Amelanchier arborea	0.00	0.00	0.00	0.00	-0.06	0.12	1.0000	0.7819	0.7819
Aralia nudicaulis	0.00	0.00	0.00	0.00	-0.36	0.41	1.0000	0.4010	0.4010
Arisaema triphyllum	6.19	0.27	5.92	0.14	2.00	0.00	0.2864	**<0.0001**	**<0.0001**
Aristolochia spp.	-0.08	0.13	0.00	0.00	-0.65	0.68	0.7299	0.3708	0.3437
Aster spp.	0.88	0.41	0.75	0.00	-0.06	0.13	0.4161	0.0499	**<0.0001**
Athryum filix-femina	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Betula alleghaniensis	-0.24	0.52	-0.18	0.35	-0.26	0.26	0.5559	0.5503	0.5269
Betula lenta	3.63	0.18	4.82	0.60	5.03	0.25	0.0543	0.0007	0.4803
Boehmeria cylindrica	0.00	0.00	0.25	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Cardamine angustata	0.75	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Carex spp.	1.33	1.68	8.50	1.05	4.92	0.47	0.0001	0.0194	0.0047
Carya cordiformis	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Caulophyllum thalictroides	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Chamerion angustifolium	-0.54	0.32	-0.17	0.19	-0.12	0.16	0.2505	0.2029	0.6499
Circaea lutetiana	0.00	0.00	0.00	0.00	-0.04	0.10	1.0000	0.8378	0.8378
Clematis virginiana	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Collinsonia canadensis	-0.07	0.13	0.00	0.00	0.00	0.00	0.7341	0.7341	1.0000
Convallaria majuscula	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Cornus alternifolia	0.00	0.00	0.25	0.00	1.75	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Dennstaedtia punctilobula	0.54	0.23	0.00	0.00	-0.83	1.26	0.0522	0.1813	0.3443
Dichanthelium clandestinum	0.07	0.47	0.87	1.17	2.00	0.00	0.2801	**<0.0001**	0.2091
Dioscorea villosa	0.43	0.13	0.42	0.14	0.04	0.25	0.7816	0.1330	0.1428
Dryopteris carthusiana	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Fagus grandifolia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Fraxinus americana	-0.19	0.24	0.50	0.00	0.07	0.23	**<0.0001**	0.3070	**<0.0001**
Galium spp.	3.62	0.17	7.92	0.14	0.00	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Geranium maculatum	0.00	0.00	0.00	0.00	-0.13	0.20	1.0000	0.6279	0.6279
Goodyera pubescens	0.00	0.00	-0.08	0.14	0.00	0.00	0.7064	1.0000	0.7064

Table A-4 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Graminoid	-0.75	0.53	0.09	0.22	0.00	0.00	0.0895	0.1251	0.4407
Hexastylis virginica	7.75	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Impatiens pallida	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Lindera Benzoin	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Liriodendron tulipifera	-26.69	13.93	-26.33	12.48	-7.09	4.13	0.5025	0.0755	0.0581
Lycopodium spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Magnolia acuminata	-0.07	0.32	-0.53	0.50	0.32	0.24	0.2933	0.2203	0.0683
Magnolia fraseri	-0.06	0.12	0.00	0.00	0.50	0.00	0.7765	**<0.0001**	**<0.0001**
Medeola virginiana	2.81	1.19	0.75	0.00	1.31	0.26	0.0667	0.1381	0.0691
Monarda clinopodia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Monotropa uniflora	-0.08	0.13	-0.08	0.14	0.00	0.00	0.7825	0.7280	0.7105
Nyssa sylvatica	0.00	0.00	-0.16	0.22	0.00	0.00	0.5515	1.0000	0.5515
Osmorhiza clatonia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Ostrya virginiana	0.17	0.13	0.00	0.00	0.00	0.00	0.2709	0.2709	1.0000
Oxalis stricta	0.00	0.00	0.00	0.00	-0.09	0.14	1.0000	0.6867	0.6867
Oxydendron arboreum	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Parthenocissus quinquefolia	0.00	0.00	0.00	0.00	-0.06	0.12	1.0000	0.7762	0.7762
Phytolacca americana	-17.62	8.52	-37.32	10.79	-21.32	13.49	0.0789	0.4304	0.1779
Podophyllum peltatum	0.00	0.00	0.75	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Polygantum biflorum	-0.38	0.40	0.00	0.00	0.00	0.00	0.3695	0.3695	1.0000
Polygonum spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Polystichum acrostichoides	6.27	1.39	8.18	0.28	2.28	0.29	0.1049	0.0031	**<0.0001**
Potentilla simplex	-1.23	0.92	0.00	0.00	0.00	0.00	0.1019	0.1019	1.0000
Prenanthes altissima	0.00	0.00	0.75	0.00	0.50	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Prosartes maculata	1.60	0.21	2.25	0.00	0.50	0.00	**<0.0001**	0.0005	**<0.0001**
prunus pensylvanica	0.87	1.21	-0.20	3.18	-0.92	1.04	0.4188	0.1541	0.3941
Prunus serotina	-6.64	6.04	-17.44	10.99	-6.45	4.93	0.2050	0.4913	0.1895
Pycanthemum virginianum	0.00	0.00	0.00	0.00	-0.24	0.30	1.0000	0.4803	0.4803
Quercus alba	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Quercus prinus	-0.31	0.29	0.25	0.00	0.50	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Quercus rubra	-0.15	0.33	0.17	0.29	0.39	0.41	0.3167	0.2074	0.4072
Robinia pseudoacacia	-0.12	0.32	-0.43	0.44	-0.32	0.50	0.3754	0.4867	0.4846
Rosa carolina	3.00	0.00	3.25	0.00	9.75	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Rubus spp.	-8.49	4.47	0.90	4.17	12.10	4.85	0.0624	0.0005	0.0491
Rumex spp.	0.00	0.00	0.25	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Sambucus canadensis	-0.78	0.60	-1.40	1.04	-0.03	0.09	0.3701	0.1549	0.0759
Sassafras albidum	-2.58	3.47	-5.06	6.00	-2.26	3.08	0.3680	0.4898	0.3483
Smilax ecirrhata	-0.09	0.15	-0.08	0.14	0.00	0.00	0.7596	0.6765	0.7093
Smilax rotundifolia	3.53	0.65	4.15	0.79	8.04	0.38	0.3113	**<0.0001**	**<0.0001**

Table A-4 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Streptopus lanceolatus	1.83	0.81	-0.23	0.73	0.94	0.13	0.0432	0.1696	0.0285
Thelypteris noveboracensis	-2.46	1.60	0.01	0.37	-0.38	0.63	0.0727	0.1310	0.3949
Tilia americana	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Tussilago farfara	0.25	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Urtica dioica	3.69	9.08	23.96	3.81	-5.74	6.00	0.0041	0.1939	**<0.0001**
Viburnum acerifolium	0.00	0.00	-0.35	0.34	-0.03	0.09	0.3581	0.8810	0.3821
Viola spp.	47.98	14.94	51.74	8.45	3.12	16.93	0.4213	0.0147	**<0.0001**
Vitis spp.	-25.56	6.03	-34.65	18.37	-10.97	3.02	0.3442	0.0134	0.0934
Zanthoxylum americanum	3.12	0.73	4.01	0.24	1.13	0.37	0.1715	0.0185	**<0.0001**

Table A-5. List of differences in number of individuals per 5 m^2 between a simulated assemblage level thinning distribution and observed density (δ) between 1997 and 2011 among REF (R), +N, and +N+L treatments. Bold p-values indicate significant differences based on a sequential Bonferroni test of probability tests.

	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
Taxon	Mean	SE	Mean	SE	Mean	SE			
Acer pensylvanicum	-2.59	1.99	0.62	1.37	-1.28	0.97	0.0942	0.3177	0.1437
Acer rubrum	48.27	3.53	22.32	1.24	26.79	2.49	**<0.0001**	**<0.0001**	0.0712
Acer saccharum	0.11	0.17	-0.08	0.13	0.00	0.00	0.3411	0.4436	0.7253
Actaea pachypoda	-3.05	2.71	0.00	0.00	0.00	0.00	0.3146	0.3146	1.0000
Actaea racemosa	0.77	0.49	0.00	0.00	-0.47	0.48	0.1065	0.0529	0.3531
Ageratina altissima	3.54	0.20	1.45	1.00	0.00	0.00	**<0.0001**	**<0.0001**	0.1090
Amaranthus spp.	-0.12	0.16	0.00	0.00	0.00	0.00	0.6029	0.6029	1.0000
Amelanchier arborea	0.00	0.00	0.00	0.00	-0.12	0.16	1.0000	0.5957	0.5957
Aralia nudicaulis	0.00	0.00	0.00	0.00	-0.72	0.69	1.0000	0.3208	0.3208
Arisaema triphyllum	1.38	0.32	0.17	0.14	2.50	0.00	0.0042	**<0.0001**	**<0.0001**
Aristolochia spp.	-0.14	0.17	0.00	0.00	-2.33	2.09	0.5563	0.3158	0.3157
Aster spp.	0.24	0.52	0.25	0.00	0.03	0.26	0.6368	0.3973	0.4669
Athryum filix-femina	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Betula alleghaniensis	-1.19	0.72	-0.25	0.25	-0.59	0.38	0.1521	0.2922	0.3287
Betula lenta	1.83	0.22	2.89	1.05	4.53	0.38	0.1933	**<0.0001**	0.0622
Boehmeria cylindrica	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Cardamine angustata	0.50	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Carex spp.	-4.66	3.55	4.69	1.06	-0.87	1.30	0.0029	0.1718	0.0003
Carya cordiformis	0.25	0.00	0.25	0.00	0.00	0.00	1.0000	**<0.0001**	**<0.0001**
Caulophyllum thalictroides	0.75	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Chamerion angustifolium	-0.76	0.30	-0.25	0.21	-0.19	0.20	0.1453	0.1064	0.5969
Circaea lutetiana	0.00	0.00	0.00	0.00	-0.12	0.16	1.0000	0.6097	0.6097
Clematis virginiana	0.00	0.00	0.25	0.00	0.00	0.00	**<0.0001**	1.0000	**<0.0001**
Collinsonia canadensis	-0.14	0.17	0.00	0.00	0.00	0.00	0.5557	0.5557	1.0000
Convallaria majuscula	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Cornus alternifolia	0.00	0.00	0.00	0.00	0.25	0.00	1.0000	**<0.0001**	**<0.0001**
Dennstaedtia punctilobula	-0.36	0.31	0.00	0.00	-1.91	2.36	0.2633	0.3188	0.3133
Dichanthelium clandestinum	-0.60	0.60	-1.98	1.19	0.00	0.00	0.1708	0.3371	0.0336
Dioscorea villosa	-0.14	0.17	0.42	0.13	-0.47	0.38	0.0183	0.3452	0.0063
Dryopteris carthusiana	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Fagus grandifolia	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Fraxinus americana	3.82	0.39	0.50	0.00	0.33	0.22	**<0.0001**	**<0.0001**	0.5517
Galium spp.	0.63	0.16	-0.08	0.14	0.00	0.00	0.0051	0.0071	0.7138
Geranium maculatum	0.00	0.00	0.00	0.00	-0.35	0.37	1.0000	0.3875	0.3875
Goodyera pubescens	0.00	0.00	-0.08	0.14	0.00	0.00	0.7171	1.0000	0.7171

Table A-5 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Graminoid	4.44	0.54	1.66	0.34	1.75	0.00	**<0.0001**	**<0.0001**	0.5658
Hexastylis virginica	0.25	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Impatiens pallida	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Lindera Benzoin	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Liriodendron tulipifera	-36.23	11.81	-18.34	4.72	-13.64	6.11	0.0797	0.0427	0.2748
Lycopodium spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Magnolia acuminata	-0.48	0.39	0.24	0.31	-0.11	0.38	0.1038	0.3087	0.2964
Magnolia fraseri	-0.08	0.14	0.00	0.00	0.00	0.00	0.7025	0.7025	1.0000
Medeola virginiana	3.33	2.45	1.50	0.00	5.09	0.65	0.2569	0.2922	**<0.0001**
Monarda clinopodia	0.00	0.00	0.00	0.00	2.00	0.00	1.0000	**<0.0001**	**<0.0001**
Monotropa uniflora	-0.14	0.17	-0.17	0.19	0.00	0.00	0.6603	0.5558	0.4763
Nyssa sylvatica	0.00	0.00	-0.34	0.34	0.00	0.00	0.3657	1.0000	0.3657
Osmorhiza clatonia	0.25	0.00	0.00	0.00	0.00	0.00	**<0.0001**	**<0.0001**	1.0000
Ostrya virginiana	-0.14	0.17	0.00	0.00	0.00	0.00	0.5556	0.5556	1.0000
Oxalis stricta	0.00	0.00	0.00	0.00	-0.08	0.14	1.0000	0.7111	0.7111
Oxydendron arboreum	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Parthenocissus quinquefolia	0.00	0.00	0.00	0.00	-0.12	0.16	1.0000	0.5989	0.5989
Phytolacca americana	-23.65	7.14	-33.78	10.60	-51.63	36.27	0.2207	0.3014	0.3442
Podophyllum peltatum	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Polygantum biflorum	-0.36	0.38	0.00	0.00	0.00	0.00	0.3902	0.3902	1.0000
Polygonum spp.	0.00	0.00	0.75	0.00	54.75	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Polystichum acrostichoides	1.24	2.22	2.44	0.28	3.73	0.74	0.3443	0.1644	0.0897
Potentilla simplex	-2.00	1.55	0.00	0.00	0.00	0.00	0.1051	0.1051	1.0000
Prenanthes altissima	0.00	0.00	0.00	0.00	0.25	0.00	1.0000	**<0.0001**	**<0.0001**
Prosartes maculata	-0.27	0.29	0.00	0.00	0.00	0.00	0.4096	0.4096	1.0000
prunus pensylvanica	-2.66	1.12	-4.82	6.66	2.99	1.70	0.3517	0.0017	0.1506
Prunus serotina	-20.03	9.02	-20.13	10.70	-11.70	6.89	0.5051	0.2385	0.2654
Pycanthemum virginianum	0.00	0.00	0.00	0.00	-0.49	0.49	1.0000	0.3429	0.3429
Quercus alba	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Quercus prinus	-0.32	0.29	0.00	0.00	0.25	0.00	0.2950	**<0.0001**	**<0.0001**
Quercus rubra	-0.32	0.39	1.25	0.37	-0.56	0.92	0.0047	0.4823	0.0261
Robinia pseudoacacia	0.04	0.38	-0.85	0.62	-1.90	1.37	0.1425	0.0783	0.2944
Rosa carolina	2.25	0.00	10.50	0.00	7.00	0.00	**<0.0001**	**<0.0001**	**<0.0001**
Rubus spp.	-17.25	2.66	18.83	3.21	12.86	3.66	**<0.0001**	**<0.0001**	0.1179
Rumex spp.	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Sambucus canadensis	-1.16	0.65	-1.04	1.05	-0.11	0.16	0.4674	0.0771	0.2939
Sassafras albidum	-7.51	4.67	-14.53	9.22	-10.25	6.00	0.2686	0.3725	0.3681
Smilax ecirrhata	-0.21	0.20	-0.08	0.14	0.00	0.00	0.5053	0.3816	0.7208
Smilax rotundifolia	3.39	0.92	6.23	1.29	10.49	0.79	0.0485	**<0.0001**	0.0006

Table A-5 continued

Taxon	δ						p-value		
	R		+N+L		+N		R v. NL	R v. N	N v. NL
	Mean	SE	Mean	SE	Mean	SE			
Streptopus lanceolatus	2.69	1.62	3.80	0.72	0.28	0.26	0.3307	0.0856	0.0001
Thelypteris noveboracensis	-0.55	3.18	6.61	0.35	1.67	0.59	0.0021	0.2767	**<0.0001**
Tilia americana	0.00	0.00	0.00	0.00	0.25	0.00	1.0000	**<0.0001**	**<0.0001**
Tussilago farfara	0.00	0.00	0.00	0.00	0.00	0.00	1.0000	1.0000	1.0000
Urtica dioica	1.84	8.65	-3.10	3.71	-18.08	15.67	0.2985	0.1357	0.2258
Viburnum acerifolium	0.00	0.00	-0.18	0.23	-0.11	0.16	0.5276	0.6294	0.6460
Viola spp.	90.75	25.54	46.43	12.68	4.22	15.11	0.0691	0.0025	0.0131
Vitis spp.	-52.88	10.76	-34.83	6.82	-30.32	7.38	0.0865	0.0521	0.3219
Zanthoxylum americanum	-1.75	1.00	0.34	0.34	-0.43	0.42	0.0217	0.1383	0.1205

Appendix B. R code for herbaceous layer simulation

Species Metrics Calculation

```
library(vegan)
#~~~~~~~~~~~~~

##Fork Mountain 1996

plots <- read.csv("fm96_sum.csv")
row.names(plots) <- c("NS1","LIME1","WT1","WT2","NS2",
"LIME2","LIME3","NS3","WT3","NS4","LIME4","WT4")

specnumber(plots)
H<-diversity(plots)
H
J<-H/log(specnumber(plots))
J
abun<-rowSums(plots)

nsr<-mean(rich[c(1,5,8,10)]);lir<-mean(rich[c(2,6,7,11)]);wtr<-mean(rich[c(3,4,9,12)])
nsH<-mean(H[c(1,5,8,10)]);liH<-mean(H[c(2,6,7,11)]);wtH<-mean(H[c(3,4,9,12)])
nsJ<-mean(J[c(1,5,8,10)]);liJ<-mean(J[c(2,6,7,11)]);wtJ<-mean(J[c(3,4,9,12)])

nsr;nsH;nsJ;lir;liH;liJ;wtr;wtH;wtJ
write.csv(H,"H.csv")
write.csv(J,"J.csv")
write.csv(rich,"rich.csv")
write.csv(abun,"abun.csv")
#~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

#END

##Repeat for Fork Mountain 1997; 2001; 2006; and 2011
```

Bootstrap Thinning Simulation

```
library(vegan)
library(xlsx)
se <- function(x) sd(x)/sqrt(length(x))
#~~~~~~~~~~~~~~

##Fork Mountain 2001 – WT (REF) plots

## Load initial dens data and select only the "WT", "NS", or "LIME"
## plots from the matrix and apply appropriate row names, create a
## vector named "len" that records the number of columns in the
## original matrix
dat <- read.csv("fm97_sum.csv")
  plots <- dat[c(3,4,9,12),]
  row.names(plots) <- c("WT1","WT2","WT3","WT4")
  #plots <- dat[c(1,5,8,10),]
  #row.names(plots) <- c("NS1","NS2","NS3","NS4")
  #plots <- dat[c(2,6,7,11),]
  #row.names(plots) <- c("LIME1","LIME2","LIME3","LIME4")
len <- dim(plots)[2]

## Determine initial densities of each plot and create a matrix
## called "init.dens" and append it to the matrix "plots"
plots <- cbind(plots,as.matrix(rowSums(plots)))
colnames(plots)[len+1] <- "init.dens"

## Load final abundance data and select the "WT", NS, or "Lime" plots from
## the matrix and apply appropriate row names and create new matrices
dat2 <- read.csv("fm01_sum.csv")
  wtplots <- dat2[c(3,4,9,12),]
  row.names(wtplots) <- c("fWT1","fWT2","fWT3","fWT4")
  #nsplots <- dat2[c(1,5,8,10),]
  #row.names(nsplots) <- c("fNS1","fNS2","fNS3","fNS4")
  #liplots <- dat2[c(2,6,7,11),]
  #row.names(liplots) <- c("fLIME1","fLIME2","fLIME3","fLIME4")

## Determine the final densities of each final plot, create a matrix
## of the abundances and append it to the matrix "plots," then
## rename it as either "fin.wt.dense", "fin.ns.dens" or "fin.li.dens"
  wtmat <- as.matrix(rowSums(wtplots))
  colnames(wtmat)[1] <- "fin.wt.dens"
  plots <- cbind(plots,wtmat)
  #nsmat <- as.matrix(rowSums(nsplots))
  #colnames(nsmat)[1] <- "fin.ns.dens"
  #plots <- cbind(plots,nsmat)
  #limat <- as.matrix(rowSums(liplots))
  #colnames(limat)[1] <- "fin.li.dens"
  #plots <- cbind(plots,limat)

## Create empty matrices to store the bootstrapped sample abundance means,
## mean richness, and mean diversity values
avmat <- matrix(nrow=0,ncol=len)
rmat <- matrix(nrow=0,ncol=1)
```

```
hmat <- matrix(nrow=0,ncol=1)
colnames(avmat) <- colnames(plots[1:len])
colnames(rmat) <- c("Richness")
colnames(hmat) <- c("Hprime")

## For loop - 15000 times -- select 4 plots randomly, with replacement, then
## randomly sample the abundance data from each plot, without replacement,
## using the final abundance value as the abundances of "random survival"
for(i in 1:15000){
ran <- plots[sample(nrow(plots),size=4,replace=TRUE),]
rare1 <- rrarefy(ran[1,1:len],ran[1,len+2])
rare2 <- rrarefy(ran[2,1:len],ran[2,len+2])
rare3 <- rrarefy(ran[3,1:len],ran[3,len+2])
rare4 <- rrarefy(ran[4,1:len],ran[4,len+2])
rich <- mean(c(rowSums(rare1 !=0),rowSums(rare2 !=0), rowSums(rare3
        !=0), rowSums(rare4 !=0)))
hprime <- mean(c(diversity(rare1), diversity(rare2), diversity(rare3),
        diversity(rare4)))
x <- list(rare1,rare2,rare3,rare4)
y <- do.call(cbind, x)
y <- array(y, dim=c(dim(x[[1]]), length(x)))
avg <- apply(y,c(1,2),mean)
avmat <- rbind(avmat,avg)
rmat <- rbind(rmat,rich)
hmat <- rbind(hmat,hprime)
}

## Calculate the "observed" average of the final plots - the values which
## you will compare to the simulated distributions, along with the
## "observed" standard error, richness and diversity
  wtav <- t(as.matrix(colMeans(wtplots)))
  wtse <- t(as.matrix(apply(wtplots,2,se)))
  wtav <- rbind(wtav,wtse)
  rownames(wtav) <- c("Mean","SE")
  #nsav <- t(as.matrix(colMeans(nsplots)))
  #nsse <- t(as.matrix(apply(nsplots,2,se)))
  #nsav <- rbind(nsav,nsse)
  #rownames(nsav) <- c("Mean","SE")
  #liav <- t(as.matrix(colMeans(liplots)))
  #lise <- t(as.matrix(apply(liplots,2,se)))
  #liav <- rbind(liav,lise)
  #rownames(liav) <- c("Mean","SE")

## Export simulation and observed average and SE files
h <- cbind(rmat,hmat)
  write.csv(avmat, "wtsim01.csv")
  write.csv(h, "wth01.csv")
  write.csv(wtav, "wtav01.csv")
  #write.csv(avmat, "nssim11.csv")
  #write.csv(h, "nsh11.csv")
  #write.csv(nsav, "nsav11.csv")
  #write.csv(avmat, "lisim11.csv")
  #write.csv(h, "lih11.csv")
  #write.csv(liav, "liav11.csv")

# END
```

Probability Tests for Bootstrap Simulation

```
##Fork Mountain 2001

## Read data *WORK YEAR BY YEAR
wtsim <- read.csv("wtsim01.csv")
nssim <- read.csv("nssim01.csv")
lisim <- read.csv("lisim01.csv")
wtob <- read.csv("wtav01.csv")
nsob <- read.csv("nsav01.csv")
liob <-read.csv("liav01.csv")
cname <- c("spp","wtmeano","wtseo","wtmeans","wtses","wtdel","wtp","nsmeano","nsseo",
        "nsmeans","nsses","nsdel","nsp","limeano","liseo","limeans","lises",
        "lidel","lip","wtvndel","wtvnp","wtvnldel","wtvnlp","nvnldel","nvnlp")
results <- matrix(nrow=0,ncol=25)
colnames(results) <- cname

##_____________________________________________________________
## Make a vector of species names for both observed and simulated results (only need to use
## one trmt.) and add a dummy "zero" column to the sim matrices

obname <- colnames(wtob)[-1]
simname <- colnames(wtsim)[-1]
names <- union(obname, simname)

dummy<-matrix(0,nrow=15000,ncol=1)
wtsim<-cbind(wtsim,dummy);nssim<-cbind(nssim,dummy);lisim<-cbind(lisim,dummy)

##_____________________________________________________________
## Note – the following for loops were created around tests that were originally meant to be
## applied manually, but later it they were all automated. Therefore, these loops are not
## optimized for speed.

for(i in names) {

wtspp <- i

if (i %in% names(wtob)) {
        obcol <- which(colnames(wtob)==i)
        wtobsmean <- wtob[1,obcol]; wtobsse <- wtob[2,obcol]
        nsobsmean <- nsob[1,obcol]; nsobsse <- nsob[2,obcol]
        liobsmean <- liob[1,obcol]; liobsse <- liob[2,obcol]
} else {
        wtobsmean <- 0; wtobsse <- 0
        nsobsmean <- 0; nsobsse <- 0
        liobsmean <- 0; liobsse <- 0
}

if (i %in% names(wtsim)) {
        colmn <- which(colnames(wtsim)==i)
        wtcolumn <- colmn;nscolumn <- colmn;licolumn <- colmn
        wtsimmean <- mean(wtsim[,wtcolumn]); wtsimse <- sd(wtsim[,wtcolumn])
        nssimmean <- mean(nssim[,nscolumn]); nssimse <- sd(nssim[,nscolumn])
```

```
        lisimmean <- mean(lisim[,licolumn]); lisimse <- sd(lisim[,licolumn])
} else {
        colmn <- which(colnames(wtsim)=="dummy")
        wtcolumn <- colmn;nscolumn <- colmn;licolumn <- colmn
        wtsimmean <- 0; wtsimse <- 0
        nssimmean <- 0; nssimse <- 0
        lisimmean <- 0; lisimse <- 0
}

## Test 1 - Is the observed mean abundance of a species different than the
## simulated mean abundance?
##-----------

wtdel <- wtobsmean-wtsimmean; nsdel <- nsobsmean-nssimmean; lidel <- liobsmean-lisimmean

## p-value calculation
if (wtobsmean<wtsimmean) {
        wtfreq <- wtobsmean < wtsim[,wtcolumn]
        wtp <- length(wtfreq[wtfreq==FALSE])/15000
} else if (wtobsmean>wtsimmean) {
        wtfreq <- wtobsmean > wtsim[,wtcolumn]
        wtp <- length(wtfreq[wtfreq==FALSE])/15000
} else if ((wtobsmean==0) & (wtsimmean==0)) {
        wtfreq <- wtobsmean < wtsim[,wtcolumn]
        wtp <- length(wtfreq[wtfreq==FALSE])/15000
} else if (wtobsmean==wtsimmean){
        wtp <- 1
}

if (nsobsmean<nssimmean) {
        nsfreq <- nsobsmean < nssim[,nscolumn]
        nsp <- length(nsfreq[nsfreq==FALSE])/15000; nsp
} else if (nsobsmean>nssimmean) {
        nsfreq <- nsobsmean > nssim[,nscolumn]
        nsp <- length(nsfreq[nsfreq==FALSE])/15000; nsp
} else if ((nsobsmean==0) & (nssimmean==0)) {
        nsp <- 1
} else if (nsobsmean==nssimmean){
        nsp <- 1
}


if (liobsmean<lisimmean) {
        lifreq <- liobsmean < lisim[,licolumn]
        lip <- length(lifreq[lifreq==FALSE])/15000; lip
} else if (liobsmean>lisimmean) {
        lifreq <- liobsmean > lisim[,licolumn]
        lip <- length(lifreq[lifreq==FALSE])/15000; lip
} else if ((liobsmean==0) & (lisimmean==0)) {
        lip <- 1
} else if (liobsmean==lisimmean){
        lip <- 1
}

## Test 2 - Is the difference between the observed mean abundance and
## simulated mean abundance different among treatments?
```

```
##----------

wtyi <- wtsim[,wtcolumn]; nsyi <- nssim[,nscolumn]; liyi <- lisim[,licolumn]
wtdi <- wtobsmean-wtyi; nsdi <- nsobsmean-nsyi; lidi <- liobsmean-liyi
wvnbd <- mean(nsdi)-mean(wtdi); wvlbd <- mean(lidi)-mean(wtdi); nvlbd <- mean(nsdi)-mean(lidi)

## p-values
if (mean(nsdi)<mean(wtdi)) {
        wtnsdi <- nsdi < wtdi
        wvnp <-length(wtnsdi[wtnsdi==FALSE])/15000; wvnp
} else if (mean(nsdi)>mean(wtdi)) {
        wtnsdi <- nsdi > wtdi
        wvnp <-length(wtnsdi[wtnsdi==FALSE])/15000; wvnp
} else if ((mean(nsdi)==0) & (mean(wtdi)==0)) {
        wvnp <- 1
} else if (mean(nsdi)==mean(wtdi)) {
        wvnp <- 1
}

if (mean(lidi)<mean(wtdi)) {
        wtlidi <- lidi < wtdi
        wvlp <-length(wtlidi[wtlidi==FALSE])/15000; wvlp
} else if (mean(lidi)>mean(wtdi)) {
        wtlidi <- lidi > wtdi
        wvlp <-length(wtlidi[wtlidi==FALSE])/15000; wvlp
} else if ((mean(lidi)==0) & (mean(wtdi)==0)) {
        wvlp <- 1
} else if (mean(lidi)==mean(wtdi)) {
        wvlp <- 1
}

if (mean(nsdi)<mean(lidi)) {
        linsdi <- nsdi < lidi
        nvlp <-length(linsdi[linsdi==FALSE])/15000; nvlp
} else if (mean(nsdi)>mean(lidi)) {
        linsdi <- nsdi > lidi
        nvlp <-length(linsdi[linsdi==FALSE])/15000; nvlp
} else if ((mean(nsdi)==0) & (mean(lidi)==0)) {
        nvlp <-1
} else if (mean(nsdi)==mean(lidi)) {
        nvlp <-1
}


## Compile and export results
mtrix <- t(as.matrix(c(wtspp,wtobsmean,wtobsse,wtsimmean,wtsimse,wtdel,wtp,nsobsmean,
        nsobsse,nssimmean,nssimse,nsdel,nsp,liobsmean,liobsse,lisimmean,
        lisimse,lidel,lip,wvnbd,wvnp,wvlbd,wvlp,nvlbd,nvlp)))
colnames(mtrix) <- colnames(results)
#rownames(mtrix) <- wtspp
results <- rbind(results,mtrix)

}

write.csv(results, "results11.csv")
```

```
# END
```