INFLUENCE ON BIODIVERSITY ON CANOPY PROCESS IN A HARDWOOD PLANTATION FOREST ECOSYSTEM

by

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I dedicate this to my friends and family, and my beautiful cat Ripley.

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ABSTRACT

Increased biodiversity generally enhances terrestrial ecosystem productivity. While niche-use efficiency is thought to drive the biodiversity-productivity relationship, the mechanisms within niche-use efficiency are not well understood. A potential mechanism for niche-use efficiency is nutrient-use efficiency. To measure nutrient-use efficiency, we calculated nitrogen-resorption efficiencies (NRE) because nitrogen is an important growth limiting nutrient for forest productivity. We used a plantation implemented as a full factorial design that included two levels of competition, implemented as different planting densities (one- and two-meter planting densities), and three diversity levels (monocultures, two-, and three-species plantings) that included three hardwood tree species (northern red oak (*Quercus rubra*), black cherry (*Prunus serotina*), and American chestnut (*Castanea dentata*). For our nitrogen-resorption efficiency data, we found that NRE increased as diversity and planting density decreased, but the magnitude of the response varied among species. This outcome suggests that while increased diversity likely provides a release from intra-specific competition, different combinations of species will play a critical role in shaping biodiversity-productivity relationships. Forest nutrient cycling can also be influenced by herbivory. To address the effects of forest diversity on herbivory rates, we monitored rates of foliar damage along with foliar nitrogen content. To measure foliar nitrogen content, we collected spectral data from early, midseason, and late season foliar samples. To assess foliar damage, we collected and imaged leaves from two canopy positions in order to measure late season foliar area and estimate pre damaged foliar area. We found that diversity and foliar nitrogen content have a positive relationship, and diversity does influence canopy damage but the effects vary among species and density. Upon further analysis, we found that foliar nitrogen content and canopy damage are correlated. Meaning individual trees showed a release from intraspecific competition, which lead to an increase in available nutrients and higher canopy quality, showing that stands with higher canopy quality experienced higher levels of damage.

CHAPTER 1.   INTRODUCTION

The biodiversity productivity relationship (BPR) is a theory that relates changes in biodiversity to changes in productivity (Tilman and Downing 1994; Tilman et al. 1996; Aarseen 1997; Hector et al. 1999; Loreau 2000; Spehn et al. 2000; Humbert and Dorigo 2005; Smith 2007; Liang et al. 2015). Biodiversity is commonly referred to as species richness, or the number of different species in a community. The term productivity is defined numerous ways depending on the goals of the different studies, but is commonly measured in biomass accumulation, carbon sequestration, or economic return (Keltry 2006).

The BPR describes three main possible outcomes: (1) as biodiversity increases productivity increases, (2) as biodiversity increases productivity stays the same, and (3) as biodiversity increases productivity decreases. The first possibility is the dominant outcome in many terrestrial and aquatic communities (Tillman et al. 1996; Humbert and Dorigo 2005; Smith 2007; Liang et al. 2016). With terrestrial plants, both experimental and natural studies have shown that as diversity increases productivity generally increases as well, and multi-species stands are on average 24% more productive then monocultures (Zhang et al. 2012) and the richness of species present often influences the rate of increase of productivity.

The BPR was initially studied in grasslands, where species composition and richness influence ecosystem process because of the influence of competition (Tilman and Downing 1994; Tilman et al. 1996; Tilman 1996; Tilman et al. 1997). After decades of ongoing studies, forest systems were included in studies of relationships between diversity and productivity, and while the outcomes were largely similar; there are several caveats to note with the comparisons. First, in experimental studies with forest systems, immature trees (2-3 years old) tend to be used for practicality, and maturity for many tree species can take decades. This reliance on young trees is important to note because forest productivity is commonly measured in growth or biomass, and immature trees gain biomass at a higher rate than mature or reproductive trees (Stephenson et al 2014).

While numerous experiments have shown a positive relationship between diversity and productivity, the mechanisms driving these relationships are not well understood. Studies

involving time series of productivity across diversity gradients have suggested that the influence of carbon and nutrient cycling along contribute to a positive BPR (Cardinale et al. 2011; Reich et al. 2012). Though these studies used diversity as a predictor variable, further studies theorized a concept within the BPR (i.e., niche-use efficiency) based on resource partitioning due to niche complementarity (Hector 2011) and theoretical models incorporating niche complementarity as a way to quantify the influence of biodiversity on productivity have supported the idea (Liang et al. 2015).

Niche-use efficiency is a concept that supports niche complementarity as a way to explain the positive relationship within the BPR, specifically looking at intra- and interspecific competition within a community (Tilman et al. 1997, Loreau and Hector 2001). Niche-use efficiency and niche complementarity assume that a greater diversity provides a release from intraspecific competition because of species-specific mechanisms by which organisms use resources (Fridley 2001). Dissimilarities among species allows for a diversity of strategies involving resources acquisition. The influence of competition on BPR is driven by the ability to capture resources and space by either suppressing or tolerating their neighbors within a community. In forest systems, this can manifest as nutrient acquisition or light capture (Williams et al. 2021). Theory predicts that intraspecific competition should be stronger than interspecific competition, and this is true for observational field studies more so than experimental or greenhouse settings (Adler et al. 2018). Intraspecific competition is usually stronger than interspecific competition because each species limits its own population growth (Grossiord 2018; Hodapp et al. 2019), so an area with only one species is competing with conspecifics for the same resources.

Along with changes in diversity, trophic-level interactions (e.g., herbivory) can influence nutrient cycling in forests. Herbivory, in this thesis defined as free-feeding chewing folivory, reduces the foliar tissue, limiting plant photosynthetic ability even outside the damaged area (Nabity et al. 2009) and a reduction in photosynthesis can directly impacts biomass accumulation. The reduction in foliar tissue area has also been shown to decrease seed production in subsequent growing seasons (Pearse et al. 2015).

Herbivory can be categorized into outbreak and background levels. Outbreak levels usually cause high levels of damage. These periodic spikes of stand damage can lead to tree mortality

and because of the potentially negative effects of outbreak herbivory, outbreak levels are more heavily researched then background levels of herbivory, yet shifts in background levels of herbivory, which generally range from 2-20% canopy damage, can have a more negative effect on tree growth and stand productivity than periodic outbreak defoliation (Zvereva et al. 2012; Couture et al. 2015)

Herbivores are generally grouped into either specialists or generalists. Specialists feed on very few plants, usually within a single genus or closely related plants. Generalists can be oligophagous and polyphagous and feed within one or more than one plant families (Ali & Agrawal 2012). Both specialists and generalists can cause outbreak and background levels of herbivory.

Changes in diversity influence herbivore feeding patterns (Nichols et al. 1999; Yamamura 2002; Jactel and Brockerhoff 2007; Abdala-Roberts et al. 2015; Grossman et al. 2017). According to the resource concentration hypothesis, as planting diversities increases, herbivory rates by specialists on targeted plant species will decrease (Root 1973). This hypothesis was derived from the thought that higher diversity levels would cloak individual, focal tee species targeted by specialists. This suggests that monocultures would be at greater risk for increased herbivore feeding by specialist insects because of a concentration of host density. Unlike specialists, generalist herbivores are less influenced by host species diversity (Abdala-Roberts et al. 2015). Rather, generalists are more swayed by leaf traits and species evenness, not just the tree diversity levels (van Schrojenstein Lantman et al. 2018, Muiruri et al. 2018).

To test this concept, we used a preexisting tree stand at Purdue's Martell forest to design an experiment examining foliar herbivore rates (leaf area) and foliar traits (N) along with litter traits (N). My overarching hypothesis for this study is: lower diversity levels will increase intraspecific competition leading to higher nitrogen resorption efficiencies (NREs) as well as concentrating hosts leading to increased herbivory.

1. Lower diversity will increase intraspecific competition leading to higher NREs.

2. More diverse communities will be of higher canopy quality causing higher levels of herbivory.

Because of the importance of forests in terrestrial ecosystems, the goal of this work is to understand mechanisms underpinning the biodiversity-productivity relationship and how diversity influences trophic-level interactions. More specifically, this research will address how planting diversity and planting density affect nutrient movement and herbivore feeding. My research had two primary objectives:

1. To quantify the influence of planting diversity and density on nutrient resorption efficiencies of individual trees.

2. To quantify the influence of planting diversity and density on herbivore feeding rates and relating variation in foliar quality with insect community level canopy damage.

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CHAPTER 2.   IMPACT OF STAND DIVERSITY AND INTRASPECIFIC COMPETITION ON NITROGEN-USE EFFICIENCY OF INDIVIDUALS IN A PLANTATION FOREST

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2.1   Abstract

Increased biodiversity in forests generally enhances ecosystem productivity. While niche-use efficiency is thought to drive this positive relationship, the specific mechanisms of niche-use efficiency are largely unknown. One suggested mechanism is nutrient-use efficiency, or the mobilization of nutrients from foliar tissue to storage areas prior to senescence. Nitrogen is an important growth limiting nutrient for forest productivity and it is unclear how nutrient dynamics are influenced by different levels of tree diversity and competition. To test the extent to which biodiversity and competition affect nitrogen resorption, we calculated nitrogen resorption efficiencies (NRE) from individual trees in a forest plantation. The plantation was implemented as a full factorial design that included two levels of competition, implemented as different planting densities (one- and two-meter planting densities), and three diversity levels (monocultures, two-, and three-species plantings) that included three hardwood tree species (northern red oak (*Quercus rubra*), black cherry (*Prunus serotina*), and American chestnut (*Castanea dentata*). We collected both mid-season foliar (August) and senescent litter samples and we determined nitrogen concentrations and calculated NRE for each. We found that NRE increased as diversity and planting density decreased, but the magnitude of the response varied among species. This outcome suggests that while increased diversity likely provides a release

from intra-specific competition, different combinations of species will play a critical role in shaping biodiversity-productivity relationships.

2.2 Introduction

Species diversity is declining globally and is expected to continue to decrease drastically in the future due to increased rates of habitat fragmentation, changes in land-use patterns and climate change (Sax and Gaines 2003). It has been suggested that a continued loss of biodiversity will lead to negative economic and environmental consequences (Chapin et al. 2000; Brooks et at. 2002; McCallum 2015). Declines in species diversity can influence multiple factors of ecosystem functioning that impact ecosystem health and productivity such as water quality, soil health, and atmospheric carbon sequestration (Tilman et al. 1996; Humbert and Dorigo 2005; Smith 2007; Bonan et al. 2008; Jactel et al. 2018). Understanding responses of individuals to different levels of diversity will help us understand how loss of biodiversity will influence population dynamics and community structure, and ultimately ecosystem functioning, in future environments.

The influence of biodiversity on productivity, primarily measured as biomass accumulation, economic return, or carbon sequestration (Reich et al. 1997; Tilman et al. 2001; Keltry 2006), is generally positive across many terrestrial and aquatic systems (Tilman and Downing 1994; Tilman et al. 1996; Aarseen 1997; Hector et al. 1999; Loreau 2000; Spehn et al. 2000; Humbert and Dorigo 2005; Smith 2007; Liang et al. 2015). The positive relationship between biodiversity and productivity has been estimated using theory and mathematical modeling and quantified using experimental approaches (Naeem et al. 1994; Loreau 1998; McCann 2000). Experimental approaches, which use replicate plots with controlled levels of species diversity, have proven useful to uncover mechanisms related with drivers of biodiversity-productivity relationships (Tilman 1996; Isbell et al. 2009; Huang et al. 2018; Mason and Connolly 2020). Although there is considerable evidence supporting a positive biodiversity-productivity relationship, mechanisms underpinning this relationship are not well understood.

Possible mechanisms within the biodiversity-productivity relationship have been debated for decades, and range from complementarity (Loreau 2000) and facilitation effects to random chance based on selection effects (van Ruijven and Berendse 2005). Many studies have focused on the effects of species richness and evenness on nutrient dynamics, which shed light on the

theorized mechanism of niche-use efficiency within the BPR framework (Naeem et al. 1994; Turnbull et al. 2013; Liang et al. 2015; Liang et al. 2016; Grossman et al. 2017; Niklaus et al. 2017). Conceptually, niche-use efficiency is thought to be driven by species performance in mono- and poly-cultures and how these interactions are affected by light interception, precipitation patterns, nutrient availability, soil quality, and other abiotic factors that affect the uptake and use of resources. Niche-use efficiency is believed to be driven by a release of intra-specific competition and a more complimentary use of resources as communities sharing resources become more diverse (Wang et al. 2005; Craine and Dybzinski 2013; Hodapp et al. 2019). An important limiting resource in closed forest systems is nutrient availability (Fernández-Martínez et al. 2014), and competition can influence nutrient dynamics. Individuals experiencing interspecific competition better utilize available resources compared with individuals experiencing intraspecific competition in forests (Hooper et al. 1998; Svanbäck and Bolnick 2007). While niche-use efficiency is thought to drive a positive biodiversity-productivity relationship, the physiological processes contributing to this relationship are not well characterized.

Nutrient availability is a factor that contributes to niche-use efficiency, and part of nutrient availability is the resorption and storage of nutrients before dormant periods. Perennial plants can resorb up to 60% of foliar nitrogen pre senescence (Vergutz et al. 2012), which can be used for future plant growth especially since nitrogen is a growth limiting nutrient. By resorbing nitrogen, the plant is less dependent on soil nutrient content, which is heavily competed for by neighboring plants and species (Borer et al. 2015; Fay et al. 2015). Moreover, species richness influences nutrient resorption rates, where higher levels of species richness causes plants to more efficiently use foliar nutrients to produce biomass (Lu et al. 2019).

In this study, we examined the influence of diversity and competition on nutrient dynamics by comparing nitrogen resorption efficiencies (NRE) grown in a plantation in a factorial design of mono- and polycultures. We hypothesized that intraspecific competition, both as species diversity and proximity to another individual will influence nitrogen resorption efficiency of individual trees. Specifically, we predicted that lower diversity (i.e., monocultures) levels will increase intraspecific competition, leading to increased NRE of individuals. Also, we predicted that increased competition, regardless of intra- or interspecific, implemented as lower planting

densities, will lead to higher NRE of individuals. Outcomes of this work will advance our understanding of specific mechanisms that drive niche-use efficiency and help to better understand the mechanistic processes driving the biodiversity-productivity relationship in forest systems.

2.3   Methods

2.3.1   Experimental Design

This study was conducted in a mixed-species forest plantation located at the Martell Forest research station (40.4°N, -87.0°W) near Purdue University. The site was planted in 2007 and included three blocks planted in a full-factorial combination of three different tree species: northern red oak (*Quercus rubra* (authority?)), black cherry (*Prunus serotina*), and American chestnut (*Castanea dentata*) planted at three different densities (one, two, and three m^2 spacing). Within each block there are six single-species plots, six two-species plots, and two three-species plot per planting density. Because of substantial tree mortality in the three-m^2 density plots in one of the replicate blocks of the field site, we excluded the three m^2 density treatment from field collections and analyses. The total number of plots within each block was 14 and the total number of plots used at the site was 42. Each plot within each block consisted of 56 trees, 26 of which were used as border row trees to separate plots and minimize edge effects. No sampling was conducted in these border trees. Further details about the plantation can be found in (Gauthier et al. 2013).

2.3.2   Canopy foliar collections

In 2017, canopy foliar samples were collected at the end of midseason (August 30- September 9) using pole pruners. Within all plots for each block, three trees of each species within the plot were sampled. Leaves were collected from the upper and lower thirds of canopies of individual trees. Seven leaves from each canopy position were sampled from northern red oak and American chestnut trees; to collect a comparable mass of leaf tissue, twenty leaves were sampled per canopy position in black cherry trees. The total number of trees sampled from each plot varied depending on species combinations. In single-species plots three trees were sampled, in two-species plots six trees (2 species x 3 from each species = 6 trees) were sampled, and in three-

species plots nine trees (3 species x 3 from each species = 9 trees) were sampled. The total number of foliar samples collected was 432 ((([6 single-species plots per block x 3 trees per plot] + [6 two-species plots per block x 6 trees per plot] + [2 three-species plots per block x 9 trees per plot]) x 2 canopy position) x 3 blocks = 432). After foliar collections, samples were flash frozen in liquid nitrogen then oven dried at 90° C until samples reached a constant mass. Dried samples were ball milled and then processed for chemical analyses.

2.3.3   Litter foliar collections

In late September 2017, at the start of leaf abscission, leaf litter was collected weekly through December. Litter was collected using buckets with a collection area of 0.13 m^2 (diameter = 0.41 m). The number of buckets used was adjusted to represent a similar area in each plot: one bucket in the one-meter planting densities and four buckets in the two-meter planting densities. After litter collections, litter was sorted based on species, planting diversity, planting density, and collection date. Samples were ball milled, and then processed for chemical analyses.

2.3.4   Foliar chemical analysis

Foliar carbon and nitrogen were analyzed using a Thermo Finnigan Flash 1112 Elemental Analyzer (San Jose, CA, USA). Atropine was used as a standard.

2.3.5   Statistical Analyses

Midseason foliar nitrogen concentrations were analyzed using a 4-way analysis of variance (ANOVA) following the model $y_{ij} = \mu + B_i + S_k + D_j + P_l + BS_{ik} + BD_{ij} + BP_{il} + SD_{kj} + SP_{kl} + DP_{jl} + BSD_{ikj} + BDP_{ijl} + BSP_{ikl} + SDP_{kjl} + BSDP_{ikjl} + e_{ikjl}$. In this model, μ represents the mean, B represents planting diversity $_i$, S represents tree species $_k$, D represents planting density $_j$, P represents canopy position $_l$, and e represents the error term. Litter nitrogen concentrations were analyzed using an ANOVA similar as the one used for analysis of midseason nitrogen concentrations, except we substituted time (t_x) for canopy position as a fixed effect. Examination of residuals confirmed that both midseason and litter nitrogen concentrations followed normal distributions.

Nitrogen resorption efficiency was calculated by dividing litter nitrogen content by foliar nitrogen content and then multiplying by 100. NRE was analyzed using ANOVA and we excluded sampling period as a fixed effect in analysis of NRE because the main effect of sampling period, and all interactions, were not statistically significant. NRE data were log+1 transformed to meet assumptions of normality. We considered p values < 0.05 as significant and $0.05 \leq p \leq 0.10$ as marginally significant. All statistical analyses were performed using JMP v.14 (SAS Institute, Cary, NY 2018).

2.4   Results

2.4.1   Mid-season foliar nitrogen levels

Canopy nitrogen content varied among tree species, but the responses depended on diversity level, planting density, and canopy positions (Table 1; SI Table 1). Overall, oak trees had ~20% more foliar nitrogen than cherry or chestnut trees and were ~17% greater in the upper, compared with lower, canopies across all tree species (SI Table 1; Fig. 1). The influence of diversity on foliar nitrogen levels varied among species and planting density (Table 1; Fig. 1). Cherry and chestnut trees had increased nitrogen levels as diversity increased, while for oaks trees nitrogen levels decreased with increase diversity (SI Table 1; Fig. 1). Foliar nitrogen levels tended to increase in the larger planting density, but the response varied among species and diversity levels (Table 1; Fig. 1). We found a marginally significant interaction between diversity, species, planting density, and canopy position, suggesting that the influence of diversity and density on canopy nitrogen levels was largest in the upper canopies, but the magnitude of this response varied among species (Table 1; Fig. 1).

2.4.2   Litter nitrogen levels

Foliar litter nitrogen levels varied among tree species and collection periods (Table 2; SI Table 2; Fig. 2). Litter nitrogen levels were generally higher in oak, compared with cherry and chestnut, and lower levels of nitrogen occurring in the earlier, compared with later, collection periods (Fig. 2) We also found a marginally significant interaction suggesting that the variation among tree species and collections periods depended on the diversity level (Fig. 2), with higher diversity

levels showing great concentrations of nitrogen in later collection periods as diversity levels increased.

2.4.3   Nitrogen resorption efficiencies

NRE was influenced by diversity level, with highest NRE values in the monocultures and NRE decreasing by 7% in two-species combinations and 9% in three-species combinations (Table 3; SI Table 3; Fig. 3). The influence of diversity levels on NRE, however, depended on planting density and this response varied among tree species (Table 3; Fig. 3). On average, the one-meter planting density monocultures had ~18% greater NRE than polycultures; this response, however, was much less pronounced in the two-meter planting density.

2.5   Discussion

An increase in ecosystem productivity as plant diversity increases is thought to be sustained by more efficient uses of limited resources (Liang et al 2015). As biodiversity increases, individuals within a community would more efficiently use the available nutrients, water, and light due to a reduction in interspecific competition for resources (Grossiord 2018; Hodapp et al. 2019), and expand available niche space to accommodate a more diverse set of individuals. While niche-use efficiency has been promoted as a driver of the biodiversity-productivity relationship, specific mechanisms facilitating niche-use efficiency are not well characterized. Here, we demonstrated that intraspecific competition affects the efficiencies of nitrogen resorption in trees, providing a physiological mechanism for the complementarity effect of niche-use efficiency.

We found that as species richness increased, so did midseason foliar nitrogen content, and this response was more pronounced in closer planting densities. This outcome suggests that intraspecific competition (i.e., monocultures) influences nutrient uptake during the growing season. While litter nitrogen content was also influenced by species richness, the magnitude of the response was minimal ($< 2\%$). Our findings contrast with those of others, where species richness had a negative relationship with green leaf and litter nitrogen content (Fornara et al. 2009; Lu et al. 2019). One possible explanation for this contrast is the relatively small number of species (i.e., three) included in our study, whereas other studies considering nutrient dynamics have included between ten and 16 (Tillman et al 2001; Lu et al. 2019). When considering only

the lower number of species in many of these studies, the trends disappear or are opposite, suggesting that consideration of the biodiversity-productivity relationship is context dependent, either with the number of species included or potentially the relatedness of niche space of the species considered.

Previous studies have focused on the roles of intra- and interspecific competition on the biodiversity-productivity relationship as related with nutrient dynamics (Weber and Deutsch 2012; Carnicer et al 2015; Lu et al. 2019). We found that each of our species varied in foliar and litter nitrogen content across the diversity and density gradients, suggesting that species specific responses can influence biodiversity-productivity relationships. Indeed, the concept of niche overlap among different species has the potential to generate differential abilities of species to coexist and can influence a positive biodiversity-productivity relationship.

We found that as stand diversity increased, NRE decreased. This relationship is opposite of findings of Lu et al. (2019), who found that NRE increased as diversity increased. One potential reason for differences between our findings and those of Lu et al. (2019) could be ecotype or functional group differences. Lu et al. (2019) was conducted in grassland setting with multiple functional groups, while we are considering forest plantations, potentially suggesting that different mechanisms driving niche-use efficiency may be system specific. Regardless of this difference, our findings lend support to the concept of niche-use efficiency. A release from intraspecific competition should relax NRE because the competition for deposited nitrogen via species-specific uptake mechanisms through the litter pathway will be reduced and highlight the influence of complementarity in the contribution of niche-use efficiency on biodiversity-productivity relationships.

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Table 2.1: Summary of F and P values for midseason foliar collections using a full factorial ANOVA with block as a random effect. Df, degrees of freedom (numerator, denominator). P values <0.05 are bolded and P values 0.05<P<0.10 are italicized.

Treatments and interactions	*Df*	F	*P*
Diversity	2, 412	2.03	0.133
Density	1, 412	0.20	0.656
Diversity x density	2, 412	2.44	*0.089*
Species	2, 412	14.36	**<0.001**
Diversity x species	4, 412	2.19	*0.069*
Density x species	2, 412	0.80	0.452
Diversity x density x species	4, 412	3.03	**0.018**
Canopy position	1, 412	18.24	**<0.001**
Diversity x canopy position	2, 412	0.92	0.105
Density x canopy position	1, 412	2.65	0.105
Diversity x density x canopy position	2, 412	0.77	0.466
Species x canopy position	2, 412	0.58	0.560
Diversity x species x canopy position	4, 412	0.40	0.809
Density x species x canopy position	2, 412	0.06	0.943
Diversity x density x species x canopy position	4, 412	2.07	*0.084*

Table 2.2: Summary of F and P values for leaf litter collections using a full factorial ANOVA with block as a random effect. Df, degrees of freedom (numerator, denominator). P values <0.05 are bolded and P values 0.05<P<0.10 are italicized.

Treatments and interactions	*Df*	F	*P*
Diversity	2, 208	0.38	0.685
Density	1, 208	0.09	0.769
Diversity x density	2, 208	0.23	0.795
Species	2, 208	24.57	**<0.001**
Diversity x species	4, 208	0.97	0.426
Density x species	2, 208	0.05	0.956
Diversity x density x species	4, 208	0.66	0.622
Collection period	2, 208	4.76	**0.010**
Diversity x collection period	4, 208	0.61	0.654
Density x collection period	2, 208	0.13	0.878
Diversity x density x collection period	4, 208	0.10	0.981
Species x collection period	4, 208	1.88	0.116
Diversity x species x collection period	8, 208	1.87	*0.069*
Density x species x collection period	4, 208	0.42	0.796
Diversity x density x species x collection period	8, 208	1.19	0.307

Table 2.3: Summary of F and P values for nitrogen resorption efficiencies using a full factorial ANOVA with block as a random effect. Df, degrees of freedom (numerator, denominator). P values <0.05 are bolded and P values 0.05<P<0.10 are italicized.

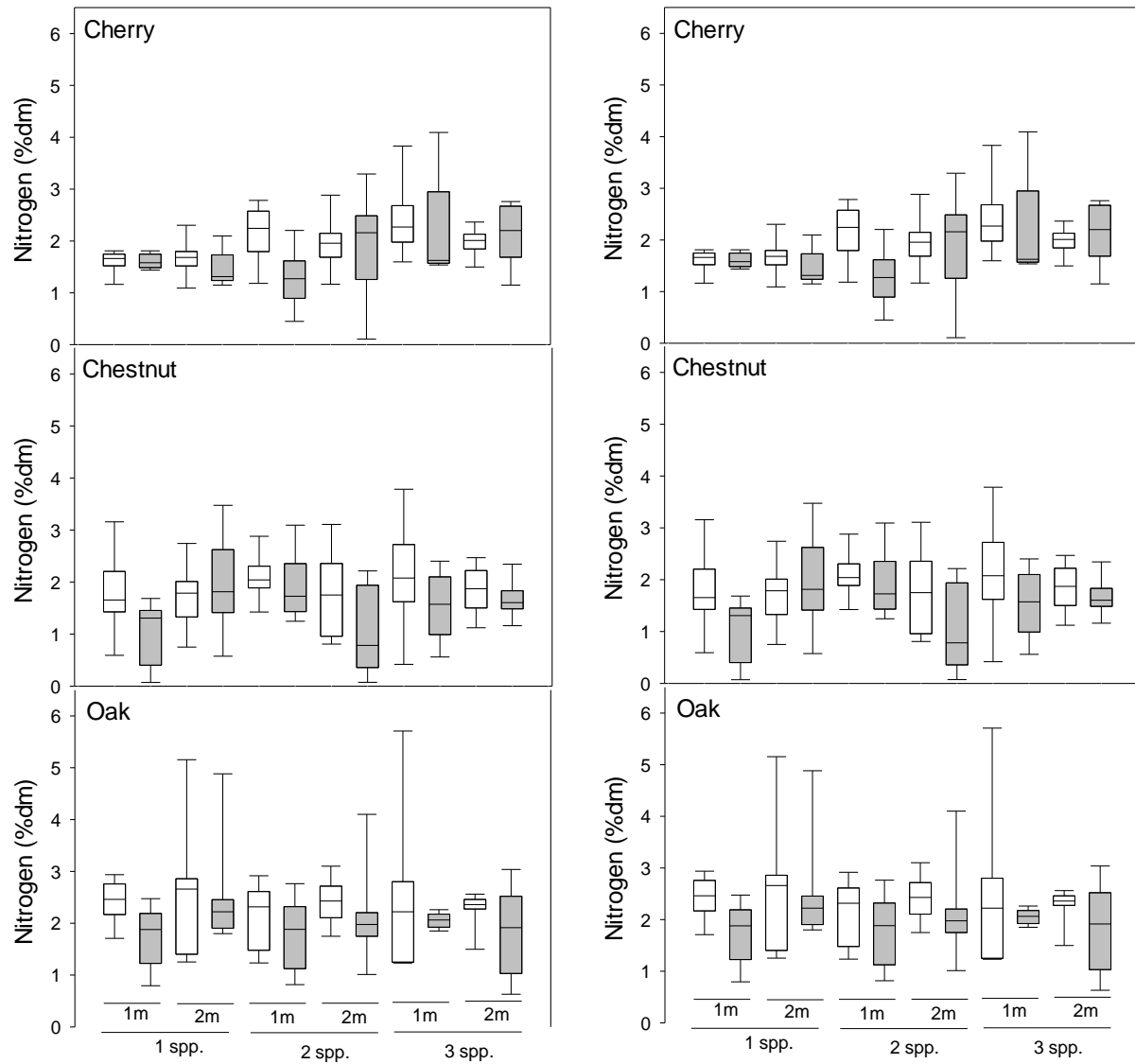| Treatments and interactions	*Df*	F	*P*
Diversity	2, 216	3.53	**0.031**
Density	1, 216	0.62	0.432
Diversity x density	2, 216	3.33	**0.038**
Species	2, 216	1.18	0.311
Diversity x species	4, 216	1.61	0.174
Density x species	2, 216	0.54	0.583
Diversity x density x species	4, 216	4.98	**0.001**

Fig. 2.1: Influence of diversity, species, density, and canopy position on foliar nitrogen concentration. Box and whisker plots for the midseason 4-way interaction between planting diversity (1spp., 2spp., 3spp.), planting density (1 meter, 2 meter), canopy layer (upper third of canopy, white bars; lower third of canopy, grey bars), and tree species (cherry, top box; chestnut, middle box; oak, bottom box). % dm, percent dry mass.
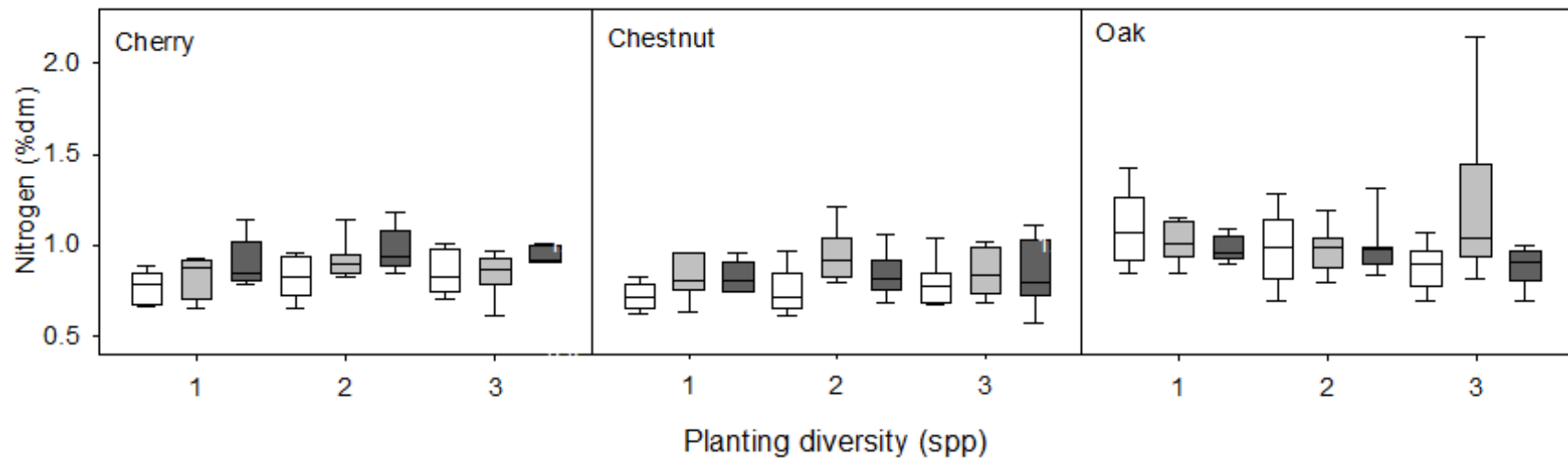
Fig. 2.2: Influence of diversity, species, and collection period on leaf litter nitrogen concentration. Box and whisker plots for the 3-way interaction between planting diversity (1spp., 2spp., 3spp.), collection periods (Sept 11-29, white bars; Oct 1-20, grey bars; Oct 22-Nov 10, black bars), and species (cherry, top box; chestnut, middle box; oak, bottom box). % dm, percent dry mass.
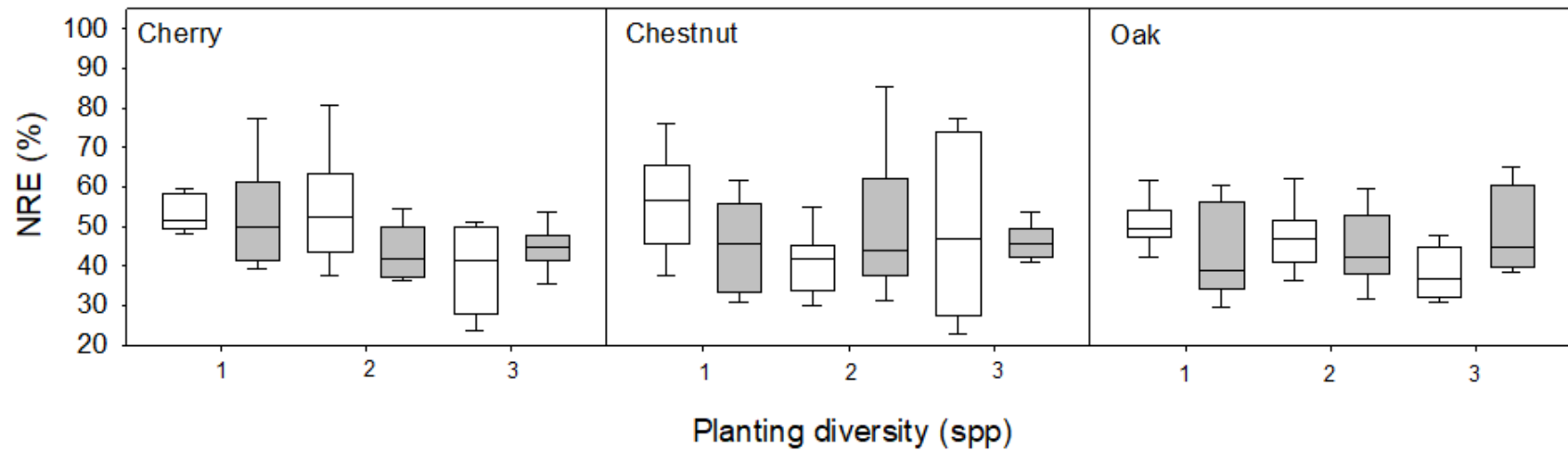
Fig. 2.3: Influence of diversity, species, and density on nitrogen resorption efficiencies. Box and whisker plots for the nitrogen resorption efficiencies 3-way interaction between diversity (1spp., 2spp., 3spp.), density (1 meter, white bars; 2 meter, grey bars), and species (cherry, top box; chestnut, middle box; oak, bottom box).

Table S2.1

Species	Canopy Position	Diversity (spp) by density (m)					
		1 spp		2 spp		3 spp	
		1m	2m	1m	2m	1m	2m
Cherry	Upper	1.6±0.3	1.7±0.2	2.1±0.2	2.0±0.2	2.4±0.2	2.0±0.3
	Lower	1.6±0.3	1.5±0.2	1.3±0.2	1.9±0.2	2.2±0.2	2.1±0.3
Chestnut	Upper	1.8±0.2	1.7±0.2	2.1±0.2	1.8±0.2	2.1±0.2	1.8±0.2
	Lower	1.0±0.2	2.0±0.2	1.9±0.2	1.1±0.2	1.5±0.2	1.7±0.2
Oak	Upper	2.4±0.2	2.5±0.2	2.2±0.2	2.5±0.2	2.4±0.2	2.3±0.2
	Lower	1.7±0.2	2.4±0.2	1.8±0.2	2.1±0.2	2.1±0.2	1.8±0.2

Table S2.2

Species	Collection Period	Diversity (spp) by density (m)					
		1 spp		2 spp		3 spp	
		1m	2m	1m	2m	1m	2m
Cherry	September	0.8±0.1	0.7±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.9±0.1
	October	1.0±0.1	0.8±0.1	1.0±0.1	0.9±0.1	0.8±0.1	0.9±0.1
	November	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.1	0.9±0.2	1.0±0.1
Chestnut	September	0.7±0.1	0.8±0.1	0.8±0.1	0.7±0.1	0.8±0.1	0.7±0.1
	October	0.8±0.1	0.9±0.1	1.0±0.1	1.0±0.1	0.8±0.1	0.9±0.1
	November	0.8±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.9±0.1	0.8±0.1
Oak	September	1.2±0.1	1.0±0.1	1.0±0.1	1.0±0.1	0.8±0.1	1.0±0.1
	October	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.3±0.1	1.1±0.1
	November	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	0.8±0.1	0.9±0.1

Table S2.3

Species	Diversity (spp) by density (m)					
	1 spp		2 spp		3 spp	
	1m	2m	1m	2m	1m	2m
Cherry	55.2±5.0	52.3±4.3	55.4±3.6	43.3±3.5	38.5±5.0	44.7±4.3
Chestnut	55.4±4.3	45.3±4.3	40.9±3.5	50.7±3.8	51.2±4.3	46.1±4.3
Oak	50.7±4.3	43.8±4.3	47.0±3.5	44.5±3.5	37.8±4.5	50.3±4.3

CHAPTER 3.   INSECT COMMUNITY CANOPY DAMAGE VARIES ACROSS TREE DIVERSITY LEVELS IN A FOREST PLANTATION

Abstract

Biodiversity generally increases productivity in terrestrial ecosystems. While niche efficiency has been thought to drive biodiversity-productivity relationships, specific mechanistic processes within this framework remain uncertain. Forest ecosystem processes, such as herbivory and nutrient cycling, likely play an important role on the relationship between biodiversity-productivity and herbivorous insects can affect many of these processes. To address relationships of tree biodiversity on insect-mediated canopy damage, we monitored rates of foliar damage along with foliar nitrogen content throughout two growing seasons in a fifteen year-old mixed forest planting. The plantation was implemented as a full factorial design that included two levels of competition, implemented as different planting densities (one- and two meter planting densities), and three diversity levels (monocultures, two-, and three-species plantings) that included three hardwood tree species (northern red oak (*Quercus rubra*), black cherry (*Prunus serotina*), and American chestnut (*Castanea dentata*). To measure foliar nitrogen content, we collected spectral data from early, midseason, and late season foliar samples. To assess foliar damage, we collected and imaged leaves from two canopy positions in order to measure late season foliar area and estimate pre damaged foliar area. We found that diversity and foliar nitrogen content have a positive relationship, and diversity does influence canopy damage but the effects vary among species and density. Upon further analysis, we found that foliar nitrogen content and canopy damage are correlated. Meaning individual trees showed a release from intraspecific competition, which lead to an increase in available nutrients and higher canopy quality, showing that stands with higher canopy quality experienced higher levels of damage.

Introduction

Forest diversity has been shown to generally have a positive relationship with productivity (Liang et al. 2016). Much of the theory behind a positive biodiversity-productivity relationship is based in niche-use efficiency (Lu et al. 2019), or the more efficient exploitation of resources in more diverse communities due to a release of intraspecific competition (Adler et al. 2018). While

a release from competition can benefit productivity, the impacts on trophic level interactions are less well understood and previous findings have shown that more productive forest stands may be subjected to greater levels of insect herbivory, potentially limiting over yielding (Couture et al. 2015). The responses of insects to diversity in forest stands has been studied with an emphasis on specialist insects, with a general consensus that diversity dilutes the apparency of individual trees, thus providing protection and reducing herbivory (Nichols et al. 1999; Yamamura 2002; Jactel and Brockerhoff 2007; Abdala-Roberts et al. 2015; Grossman et al. 2017). Less consideration, however, has been given to forest damage by communities of insects, and levels of damage caused by polyphagous insects have been shown to be variable over different levels of diversity (Jactel and Brockerhoff 2007).

Insect herbivores can dramatically alter forest productivity and functioning. Outbreak levels of defoliation can alter forest functioning episodically, with consequences for energy flow and carbon and nutrient cycling (Lovett and Ruesink 1995; Lovett et al. 2002; Frost and Hunter 2004; Townsend et al. 2004; Hicke et al. 2013). Defoliation directly decreases transpiration and photosynthesis, tree growth, and seed production as well as increases plant mortality, organic material deposition, canopy light penetration, and water drainage (Stephens et al., 1972; Cook et al. 2008; Cunningham et al., 2009; Hicke et al. 2013; Meehan et al. 2014; Couture et al. 2015), Many of these outcomes influence the amount and quality of nutrients relocated from the canopy to the forest floor, where the fate of nutrients can be variable, including resorption by trees, assimilated by soil microbes, or removed from the system by rain events (Lovett et al. 2002; Frost and Hunter 2004, 2007; Meehan et al. 2014) . While most work to date has focused on the influence of canopy damage on ecosystem functioning under outbreak conditions, less attention has been paid to background levels of herbivory, which generally range from 2-15% damage (Turcotte et al., 2014; Kozlov et al., 2015; Kozlov and Zvereva 2017). Yet, shifts in background levels of defoliation can have a more pronounced, long-term negative impact on the growth of individual trees and stand productivity (Zvereva et al. 2012; Couture et al. 2015).

While diversity of forested systems influences patterns of herbivory, mechanisms driving these patterns are not well established. Nitrogen, in the form of protein, is generally considered the rate limiting substrate for insect growth and development (Mattson 1980). While numerous other factors influence insect herbivore canopy damage, the concept of niche-use efficiency suggests

that trees in more diverse forest stands will be of higher nutritional quality (Muiruri et al. 2018) and able to potentially support a larger herbivore populations and increasing the level of damage caused by insect herbivore communities.

In this study, we 1) assessed the influence of stand diversity on community-level canopy damage caused by insect herbivores and 2) relate stand nutritional quality with canopy damage. We hypothesized that species diversity will influence canopy quality and, in turn, affect patterns of canopy damage. Specifically, we predict that as diversity increases, canopy nutritional quality will increase, influencing rates of canopy damage.

3.1 Methods

3.1.1 Experimental design

This study was conducted in a mixed-species forest plantation at Purdue University Martell Forest (40.444789 N, -87.029833 W) that was planted in 2013. These plots consist of three different tree species (northern red oak [*Quercus rubra*, N], black cherry [*Prunus serotina*, B], and American chestnut [*Castanea dentata*, C]) planted as monocultures, two species pairs, and polycultures at one-, two-, and three-meter densities within three replicate blocks in a complete randomized block design. Because of significant mortality of one tree species in the three-meter plots in one of the three replicate blocks, in this study only the one meter and two meter densities were used. Further details about the plantation can be found in (Gauthier et al. 2013).

3.1.2 Canopy sample collections and damage estimations

In 2018 and 2019, foliar samples were collected in June, August, and October using pole pruners. In each plot, seven leaves from both the upper and lower canopy were sampled from three northern red oak and three American chestnut trees. To collect a comparable amount of leaf tissue from black cherry as oak and chestnut, twenty leaves were sampled from both the upper ad lower canopy.

We estimated canopy herbivory by scanning all foliar samples collected each October on a flatbed scanner. Images were uploaded into the ImageJ software. Images were altered so the missing leaf tissue was filled in following Couture et al. (2015). Damage was calculated by

comparing the pixels in pre and post damaged leaves and the percent of missing area was calculated using the following formula: ((pre herbivory – post herbivory)/pre herbivory) x 100.

3.1.3   Estimating foliar quality

Hyperspectral data was collected on all collected leaves using a full-range spectroradiometer (SVC 1024i, Spectra Vista Corporation, Poughkeepsie, NY). Spectral data were collected on a subset of three leaves per collection. Existing chemometric models (Serbin 2012) were used to estimate foliar nitrogen levels.

3.1.4   Statistical approach

To determine the influence of species diversity, competition, canopy position, tree species, and year on canopy damage rates, we ran a five-way full factorial ANOVA, treating diversity, planting density, canopy position, tree species, and year as fixed effects and block as a random effect using the following model: $y_{ij} = \mu + B_i + S_k + D_j + P_l + M_n + BS_{ik} + BD_{ij} + BP_{il} + BM_{in} + SD_{kj} + SP_{kl} + SM_{kn} + DP_{jl} + DM_{kn} + PM_{ln} + BSD_{ikj} + BSP_{ikl} + BSM_{ikn} + BDP_{ijl} + BDM_{ijn} + BPM_{iln} + SDP_{kjl} + SDM_{kjn} + SPM_{kln} + DPM_{jln} + BSDP_{ikjl} + BSDM_{ikjn} + BSPM_{ikln} + BDPM_{ijln} + SDPM_{kjln} + BSDPM_{ikjln} + e_{ikjln}$. In this model, μ represents the mean, B represents planting diversity $_i$, S represents tree species $_k$, D represents planting density $_j$, P represents canopy position $_l$, M representing year $_n$, and e represents the error term. Data were log transformed in order to meet assumptions of normality and homogeneity of variance. Because of the smaller size of the plots we used, and the spatial proximity to one another, we developed a covariate to account for spatial autocorrelation and included it in the model. This covariate was the average of all damage rates of plots surrounding an individual focal plot.

We ran a similar five-way, full factorial ANOVA to analyze foliar nitrogen levels, treating diversity, density, species, year, and collection period as fixed effects and block as a random effect. Canopy position was removed from this analysis because for black cherry, some upper canopy position samples were not available in October. We then averaged canopy damage and foliar nitrogen to the plot level for each year (n = 82) to examine the correlation between variation in foliar nutritional quality, determined as nitrogen, an index of protein and generally considered the limiting nutrient for insect growth and development (Mattson 1980).

3.2 Results

3.2.1 Canopy Damage

Canopy damage varied among tree species, but variation among species depended on year, canopy position, planting density, and planting diversity (Table 1). Overall, oak leaves had ~27% more damage than cherry or chestnut leaves, and there was ~21% more damage on the upper compared to lower canopy (Fig. 1). Canopy damage also varied among tree species, but the responses varied between planting densities and across years (Table 1). The influence of diversity on canopy damage varied among tree species and between planting densities (Table 1; Fig. 1). Canopy damage was generally greater in more spacious planting densities, but the magnitude, and even direction, of the response varied among species and across diversity levels (Table 1; Fig. 1). Surrounding plot damage was not related to the damage rate measured from individual plots.

3.2.2 Foliar nitrogen content and relationship with canopy damage

Foliar nitrogen varied among tree species but the magnitude of response varied between planting densities, across diversity levels, and within and across years (Table 2). Overall, oak trees had ~7% higher nitrogen content than cherry and chestnut trees, there was ~6% more nitrogen in higher diversity levels compared with monocultures. There was also ~13% more nitrogen during earlier, compared with later, collection periods (Fig. 2). Cherry and chestnut showed similar trends in foliar nitrogen content within years, planting diversities, and planting densities, while oak had the opposite trend. Foliar nitrogen levels tended to increase in the larger planting density, but the response varied among species and diversity levels (Table 2; Fig. 2). Average foliar nitrogen was positively correlated with canopy damage (n = 82, r = 0.24, P = 0.031).

3.3 Discussion

In this study, we demonstrated that canopy damage by a community of insect herbivores is influenced by tree diversity in forest stands. We also show that stand quality, measured as foliar nitrogen, increases as stand diversity increases, and that increased stand quality is related with higher levels of canopy damage. Previous work has reported that stand diversity increases, less damage was reported (Jactel and Brockerhoff 2007; Grossman et al. 2017). These studies,

however, focused mainly on focal tree species and largely specialist insect herbivores (Grossman et al. 2017). When generalist insect herbivores were considered, responses of damage were variable (Jactel and Brockerhoff 2007). Additionally, to our knowledge this is the first study to focus on canopy damage by the herbivorous community as a whole. Our findings suggest that when considered at the stand level, forest diversity might increase rates of canopy damage because of shifts, specifically enhancements, in host quality.

In support of our first hypothesis, stand diversity influenced canopy damage, but the responses depended on planting densities and varied among species and within and across years. As planting diversity increased so did foliar nitrogen content. This outcome is likely a consequence of release from intraspecific competition, and the ability of individual trees to more efficiently uptake of nutrients (Yamamura 2002). We also found a positive correlation between canopy damage and foliar nitrogen, suggesting that while individual trees might experience a release from intraspecific competition, an increase in available nutrients might lead to an increased shift in background levels of herbivory.

A positive biodiversity-productivity relationship is predicated on more efficient use of available resources (Tilman et al. 2001). While this promotes greater productivity in forest systems (Liang et al. 2015), the influence of trophic level interactions, specifically insect herbivore-tree interactions, on productivity will need to be considered as host quality changes. Outbreak insect herbivores can create periodic damage, shifting ecosystem functioning and productivity (Lovett and Ruesink 1995; Lovett et al. 2002; Frost and Hunter 2004; Townsend et al. 2004; Hicke et al. 2013), but changes in background damage levels of herbivorous insects have a greater long-term impact on tree growth and forest system functioning (Zvereva et al. 2012; Couture et al. 2015). As future research examines mechanisms driving positive biodiversity-productivity relationships, trophic-level interactions, especially pests and pathogens should be considered.

3.4   References

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Table 3.1: Summary of F and P values for foliar damage using a full factorial ANOVA with block as a random effect. Df, degrees of freedom (numerator, denominator). P values <0.05 are bolded and P values 0.05<P<0.10 are italicized.

Treatments and interactions	*Df*	F	*P*
Diversity	2, 1664	0.843	0.430
Density	1, 1663	6.401	**0.012**
Diversity x density	2, 1664	0.907	0.404
Species	2, 1664	6.975	**0.001**
Diversity x species	4, 1663	0.737	0.567
Density x species	2, 1664	4.521	**0.011**
Diversity x density x species	4, 1664	2.589	**0.035**
Year	1, 1664	0.977	0.323
Diversity x year	2, 1664	0.092	0.912
Density x year	1, 1665	0.076	0.783
Diversity x density x year	2, 1664	0.780	0.459
Species x year	2, 1663	18.879	**<0.001**
Diversity x species x year	4, 1663	1.681	0.152
Density x species x year	2, 1663	1.022	0.360
Diversity x density x species x year	4, 1663	1.739	0.139
Canopy position	1, 1663	10.227	**0.001**
Diversity x canopy position	2, 1663	0.359	0.700
Density x canopy position	1, 1663	0.209	0.648
Diversity x density x canopy position	2, 1663	2.019	0.133
Species x canopy position	2, 1663	32.329	**<0.001**
Diversity x species x canopy position	4, 1663	1.417	0.226
Density x species x canopy position	2, 1663	0.201	0.818
Diversity x density x species x canopy position	4, 1663	0.468	0.759
Year x canopy position	1, 1663	1.006	0.316
Diversity x year x canopy position	2, 1663	1.471	0.230
Density x year x canopy position	1, 1663	1.170	0.280
Diversity x density x year x canopy position	2, 1663	0.710	0.492
Species x year x canopy position	2, 1663	3.434	**0.032**
Diversity x species x year x canopy position	4, 1663	0.848	0.495
Density x species x year x canopy position	2, 1663	0.871	0.418
Diversity x density x species x year x canopy position	4, 1663	0.606	0.659
Canopy damage cofactor	1, 1664	1.769	0.184

Table 3.2: Summary of F and P values for foliar nitrogen (via spectral measurements) using a full factorial ANOVA with block as a random effect. Df, degrees of freedom (numerator, denominator). P values <0.05 are bolded and P values 0.05<P<0.10 are italicized.

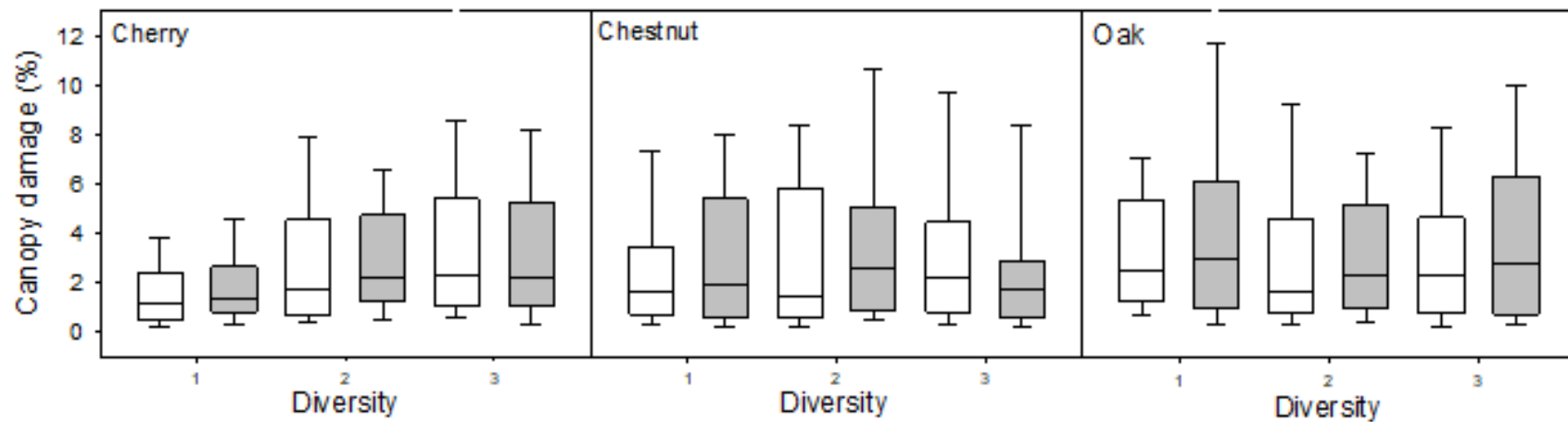| Treatments and interactions	*Df*	F	*P*
Diversity	2,206.2	29.928	**<0.001**
Density	1,206.2	2.268	0.134
Diversity x density	2,206.2	1.270	0.283
Species	2,206.2	33.120	**<0.001**
Diversity x species	4,206.2	21.227	**<0.001**
Density x species	2,206.1	4.081	**0.018**
Diversity x density x species	4,206.2	5.566	**<0.001**
Year	1,206	12.899	**<0.001**
Diversity x year	2,206	2.467	*0.087*
Density x year	1,206	2.635	0.106
Diversity x density x year	2,206	0.079	0.924
Species x year	2,206	8.889	**<0.001**
Diversity x species x year	4,206	3.346	**0.011**
Density x species x year	2,206	3.522	**0.031**
Diversity x density x species x year	4,206	2.907	**0.023**
Collection period	2,206	170.798	**<0.001**
Diversity x collection period	4,206	1.598	0.176
Density x collection period	2,206	0.521	0.595
Diversity x density x collection period	4,206	0.218	0.928
Species x collection period	4,206	0.198	0.939
Diversity x species x collection period	8,206	0.530	0.834
Density x species x collection period	4,206	0.331	0.857
Diversity x density x species x collection period	8,206	0.906	0.513
Year x collection period	2,206	29.850	**<0.001**
Diversity x year x collection period	4,206	0.132	0.970
Density x year x collection period	2,206	1.301	0.275
Diversity x density x year x collection period	4,206	0.316	0.867
Species x year x collection period	4,206	1.743	0.142
Diversity x species x year x collection period	8,206	0.832	0.575
Density x species x year x collection period	4,206	0.530	0.714
Diversity x density x species x year x collection period	8,206	0.824	0.582

Fig. 3.1: Impacts of diversity, density, and species on herbivory rates. Box and whisker plots for the late season herbivory rates 3-way interaction between diversity (1spp., 2spp., 3spp.), planting density (1m, white bars; 2m, grey bars), and tree species (cherry, top box; chestnut, middle box; oak, bottom box). Herbivory (%) meaning herbivory percent based on leaf area.
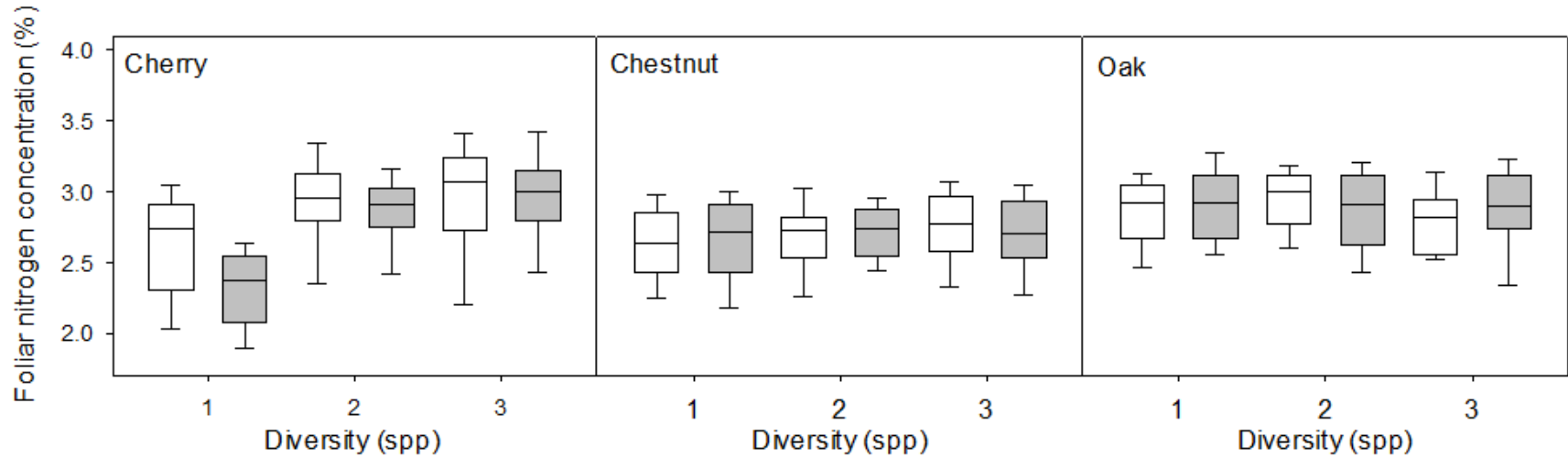
Fig. 3.2: Impacts of diversity, density, and species on foliar nitrogen concentration. Box and whisker plots for the foliar nitrogen 3-way interaction between diversity (1spp., 2spp., 3spp.), planting density (1m, white bars; 2m, grey bars), and tree species (cherry, top box; chestnut, middle box; oak, bottom box). Foliar nitrogen concentration (%) meaning nitrogen present based on spectral data.
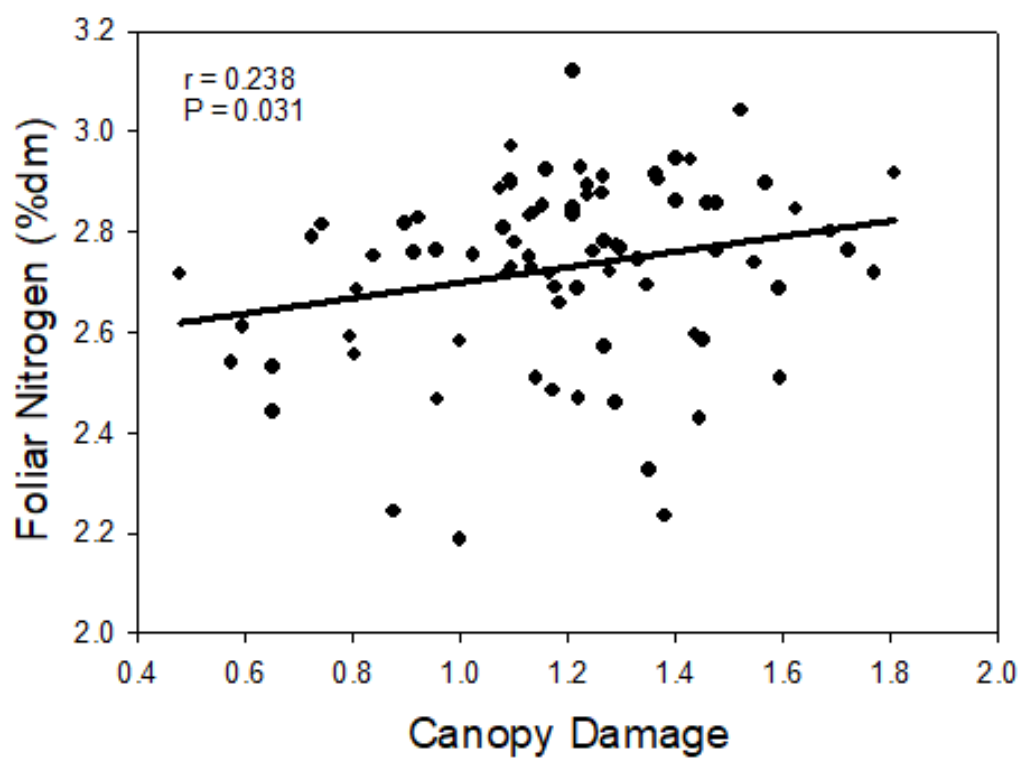
Fig. 3.3: Correlation between canopy damage and foliar nitrogen concentration. % dm, percent dry mass.

CHAPTER 4.   CONCLUSIONS

Declines in global diversity have both economic and environment consequences. By studying changes in community level diversity, we may better understand how diversity changes influence ecosystem productivity. Factors like nutrient resorption, foliar quality, and herbivory rates are influenced by diversity. I tested the influence of diversity on these factors to better understand the physiological components that alter niche-use efficiency within the biodiversity-productivity relationship.

I found that forest diversity, tree density, and species composition influenced nutrient movement, foliar quality, and herbivory rates at the stand level. Higher diversity levels yielded higher mid-season foliar nitrogen content and lowered nitrogen resorption efficiencies (NREs), suggesting a release of intraspecific competition. Higher planting densities also experienced higher rates of herbivore damage, and an increase in damage was related to increased canopy foliar nitrogen levels. We also found both NRE and canopy damage rates varied among species and planting densities, suggesting that different species will respond differently to diversity and competitive interactions to shape forest functioning.

Based on outcomes from my research, I was able to support that increased diversity can release intraspecific competition, based on decreasing nitrogen resorption efficiencies, suggesting a potential mechanism of niche-use efficiency. I also found that canopy damage levels increased as diversity increased, as a result of increased canopy quality. This outcome provides a possible counter-mechanism to niche-use efficiency: as stands more efficiently and completely use available nutrients, they become higher quality and can potentially host larger numbers of insect herbivores, leading to higher levels of canopy damage. How shifts in background levels of canopy damage influence niche-use efficiency is unclear and future research should focus on the role of insect herbivore communities on forest ecosystem functioning in diverse communities.

When considering the biodiversity-productivity relationship in forests, there are a few ways our project could be enhanced for future research. Based on previous research and our experiences during these projects, I would recommend continuing the full factorial design but using 4 or 5 species of trees instead of the 3 we used in order to view a larger portion of the BPR curve. Also,

I would recommend having more replicate blocks, extra border rows, and spacing out the blocks in order to avoid potential issues with spatial autocorrelation. Lastly, I would recommend choosing tree species that are all currently native to the area you are planting in.