LONG-TERM EFFECTS OF DEER BROWSING ON NORTHERN WISCONSIN FOREST PLANT COMMUNITIES

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

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ABSTRACT

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The effects of excessive white-tailed deer (*Odocoileus virginianus*) browsing on forest understory plant communities are well-documented. However, these studies are usually short-term, and fail to focus on phylogenetic diversity and additional variables that may explain species composition. This study examined ecological and phylogenetic diversity, vegetation structure, and light (% insolation) in short (7 year) and long-term (22 year) exclosures (and paired controls) to identify if competitive exclusion occurs in long-term exclosures. A deer browsing susceptibility index (DBS) was also developed to identify species reliant on exclosures for persistence. Statistical analysis using 2-way ANOVAs showed increased percent cover, vegetation height, and phylogenetic diversity in old exclosures compared to controls, but there were no significant differences in ecological diversity. Ecological and phylogenetic diversity was greatest in old exclosures compared to young, but young exclosures were more ecologically diverse than paired controls. There were no differences in light or biomass for any exclosure age or treatment. Based on these results, competitive exclusion is not occurring in these 22 year exclosures. A comparison of mean pairwise phylogenetic distance to a randomized null model shows phylogenetic clustering in old controls, indicating browsing is a habitat filter selecting for species least susceptible to browsing (i.e. graminoids and club mosses). As expected, browse susceptible species consisted mainly of broadleaf herbs. Highly susceptible species may rely on exclosures for persistence, and although competitive exclusion does not occur, exclosures are not a practical way to promote ecological and phylogenetic diversity in forest understories.
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INTRODUCTION

White-tailed deer populations have increased considerably in the last several decades in the United States. The once endangered species was absent or rare in most of the continental US and deer overabundance was restricted to a few isolated patches in New York, Wisconsin, Michigan, and Utah (Leopold et al. 1947). Today, white-tailed deer are easily found in all but a few western states of the country (Cold 2009) and overabundance is a well-documented widespread problem (Côté et al. 2004; Healy et al. 1997; Rooney 2001; Stromayer and Warren 1997) at the state level. Wisconsin is not an exception, even though in the late 1800's white-tailed deer populations were only found in the northernmost parts of the state (Cold 2009). In 1897, Wisconsin recorded the lowest number of deer harvested to date (2,500 individuals). By contrast, today's deer populations are estimated at 1.6 million, which is third highest in the United States (WDNR unpublished data).

Five factors have contributed to the growth of deer populations to their current densities. First is increased habitat availability due to farm abandonment, suburban sprawl, and habitat fragmentation (Rhoads et al. 2010). These expanded areas provide excellent forage for deer as graminoids and other herbaceous plants recolonize abandoned areas. Secondly, as agricultural land use becomes more efficient (e.g. fertilization, higher planting densities, and removal of hedgerows and fence lines) crops provide an alternative nutrient-rich food source to sustain high deer population densities throughout the year (Smith et al. 2007). Thirdly, better enforcement of game laws and weapon restrictions has encouraged population growth (Waller and Alverson 1997). In Wisconsin, most hunters are gun-hunters and hunting season is limited
to a bucks-only nine day period after Thanksgiving. The short hunting season reflects laws set forth when deer populations were low, and the state is working to identify target densities in an attempt to combat deer overabundance (WDNR 1998). Fourthly, natural predators (e.g. wolves, bobcats, bears, and pumas) have largely been extirpated from the continental United States (Woodroffe 2000), significantly reducing mortality rates in deer populations (Rooney and Waller 2003). Lastly, climate change has reduced winter severity, resulting in increased deer survival (Côté et al. 2004).

Deer Impacts on Forest Communities

Due to their feeding strategy, white-tailed deer (Odocoileus virginianus) overpopulation results in more homogenous and less diverse forest understories (Rooney et al. 2004; Rooney 2009; and Stromayer and Warren 1997). Deer prefer to browse on high quality nutrient-rich plants when possible and only move to lower quality food sources when high quality forage is depleted (Côté et al. 2004). Generally, higher deer densities promote increases in abundance of browse-tolerant and resilient species, while most other plant species decline in abundance (Horsley et al. 2003). Specifically, graminoid species tend to dominate highly browsed areas while forest herbs are more likely to disappear (Webster et al. 2005; Rooney et al. 2004; and Rooney 2009).

Plant species declines are a result of both direct browsing from deer and indirect competition for resources between plants (Rooney and Waller 2003). Exclosure studies have been crucial in understanding how plants respond to browsing pressure and how forest understory composition changes with fluctuating deer densities (Anderson and Katz 1993;
Ruhren and Handel 2003; and Rooney 2009). However, these studies are often short-term (1-10 years) investigations that fail to look at whether or not removal from deer alone is enough to sustain an increase in overall diversity in the long-term (10+ years). Those that are long-term (Marquis 1981; Anderson and Loucks 1979; and Kay and Bartos 2000) usually focus on the growth of woody species and have neglected other important factors such as light availability and species diversity. Furthermore, even fewer studies have examined differences between long-term and short-term exclosures to understand how plants respond over time to reduced browsing pressure from deer (Husheer et al. 2003). These limitations, combined with regular use of exclosures as a method to protect vulnerable plants from deer, highlight the importance of studies on long-term species composition shifts in areas protected from deer. They are crucial to overall recovery efforts of forest plant communities.

**Plant Species Diversity**

Species composition shifts can be identified using different measurements of species diversity. Conventionally, species evenness and richness are used to quantify diversity. Although these metrics are helpful, a measure of phylogenetic diversity provides additional valuable information that cannot be obtained using traditional measures. Phylogenetic diversity is based on the evolutionary history of species (Faith and Baker 2006). It emphasizes the importance of phylogenetic distance (genetic dissimilarity) between species as an indicator of diversity (Vellend et al. 2011). However, ecological diversity indices (i.e. Shannon-Wiener and Simpson's Diversity) determine diversity by the number of species present and their relative abundances.
All species in these metrics are weighted equally, regardless of their degree of genetic difference from one another.

Diversity indices are used for a variety of reasons and evolutionary history may not be of particular interest to all researchers. However, researchers looking at ecological diversity indices have often assumed they are representative of phylogenetic relationships or have ignored them altogether. This is a dangerous assumption. Evolutionary history can only be understood fully by examining phylogenetic relationships of species. These relationships can also help identify conserved functional traits that are important to species persistence and function under specific environmental conditions (Cavendar-Bares et al. 2012; and Pellissier et al. 2012). In this particular case, I am interested in herbivory as an environmental condition that selects for specific plant traits. Closely related species may be more or less preferred by deer depending on their characteristics.

**Competition for Light and Competitive Exclusion**

Light availability and browsing are two main driving forces influencing forest understory composition in deciduous hardwood forests. Different species of plants respond differently to changes in light conditions, and those that are able to recognize shading from neighbors are generally better competitors. Plants have been shown to alter growth patterns based on light conditions using the ratio of red to far red light (F:FR) reaching their photoreceptors. Lower ratios of R:FR indicate shading, and those plants able to maximize their absorption of photosynthetically active radiation will have a competitive advantage (Schmitt and Wulf 1993). However, browsing by white-tailed deer increases the amount of light energy reaching the
forest floor (Rooney and Waller 2003) and allows shade intolerant and browse resistant species to dominate the landscape (Krueger et al. 2009). The R:FR ratio plays less of a role in these communities, and other factors (such as nutrients or browsing pressure) are more likely to influence plant communities.

Removal of plant biomass by deer browsing can cause several changes to plant physiology. Most plants respond by putting out new growth to compensate for lost surface area, and browsing can increase plant defenses against herbivory depending on the plant species being browsed (Davidson 1993; and Karban and Myers 1989). However, these defenses are expensive, and in the absence of deer undefended plants are the superior competitor (Côté et al 2004). Therefore, those plants that increase their resistance to herbivory will dominate heavily browsed landscapes, but lose their competitive advantage when deer browsing pressure is removed.

When browsing pressure is removed, fast-growing and light-loving species should fill in the canopy and shade out other competitors. However, these species must be present within the area and have dispersal capabilities to reach exclosures. Initially, I expect increases in diversity since heavily browsed species are no longer limited by deer. However, through competitive exclusion those species which are better suited for harnessing the environment’s limited resources (including light and nutrients) will "win" and drive other competitors extinct (Gause et al. 1934). These competitive dominants are generally a few species that, once established, reduce diversity by preventing germination of new species (Hautier et al. 2009). Specifically, I am comparing levels of insolation (incoming solar radiation) and species
composition of browsed plots, short-term exclosed plots, and long-term exclosed plots so I can identify species lost from reduced light levels (competition) over time. I can also identify those species particularly susceptible to deer browsing that may rely on exclosures for persistence.

**OBJECTIVES**

The goal of this study was to determine whether removal of browsing pressure would increase diversity in the forest understory over the long-term. Specifically, I focused on 8 main objectives using data collected from short-term (7 year) exclosures, long-term exclosures (22 year), and paired unexclosed controls at a single study site. Using these data I was able to identify temporal trends and the current state of diversity in each area. I was also able to catalog and compare environmental conditions between each group as an explanation for these trends.

**Objective #1: Identify differences in vegetation structure between exclosures and controls.** Vegetation structure directly influences forest dynamics. Specifically, it affects light attenuation and the availability of resources for other plants (Rooney and Waller 2003). High levels of deer browsing reduce vegetation complexity and allow greater amounts of light to reach the forest floor. Browsing also promotes higher nutrient availability for browse-tolerant species, since more digestible plants are unable to fully utilize available resources and are returned to the soil as organic matter by deer (Pastor and Cohen 1997). By removing browsing pressure and tracking vegetation structural responses I was able to identify the impacts of deer browsing on plant growth, and determine if they directly influenced diversity.
Objective #2: Identify differences in insolation between exclosures and controls.

Insolation is directly influenced by vegetation structure. Areas with more vertically complex plant species generally have reduced insolation because taller (and usually mature) vegetation attenuates more light than species at the ground layer (Aubin et al. 2000). In addition, shading from taller vegetation promotes the growth of a shade-tolerant herbaceous layer, which generally consists of broadleaved plants. These plants further shade out the area beneath their leaves and reduce the amount of light energy reaching the forest floor. Browsing counteracts this, and promotes the growth of shade-intolerant plants (particularly graminoids), which usually do not have broad leaves (Rooney 2009). By comparing species composition and insolation in browsed and exclosed areas I determined how light energy relates to species diversity.

Objective #3: Identify differences in above-ground photosynthetic biomass in exclosures and controls. Above-ground photosynthetic biomass is a good indicator of how much forage is available for deer. Deer do not easily digest the non-green parts of plants and browse on green vegetation whenever it is available (Harris 1945). Some evergreen species, like Tsuga canadensis, are particularly browse sensitive because they are an important overwintering food source for deer (Anderson and Loucks 1979). Furthermore, above-ground photosynthetic biomass can be used as basic indicator of resource availability in the absence of herbivory, since the energy stored in above-ground biomass has come from existing stores. In the absence of browsing, areas with greater biomass have increased vegetative output due to an increase in environmental stability and ability to utilize available resources. By comparing biomass, I
determined if browsing contributed to overall declines in available forage. I also identified whether plant resource use increased or decreased with browsing, since any increase in biomass must come from a utilization of available resources in exclosures. Therefore, increases in biomass are indicative of higher resource use in these areas.

**Objective #4: Identify changes in phylogenetic diversity through time and between exclosures and controls.** Phylogenetic diversity metrics provide a way to evaluate the evolutionary history of species within an area. Combined with ecological diversity, researchers use these metrics to obtain valuable information about the total diversity (species and genetic) at a site. It also provides information about habitat filtering of species traits under specific environmental conditions like browsing (Kraft et al. 2007). By analyzing the phylogenetic diversity between exclosures and controls, I determined whether browsing is a habitat filter and identified particular characteristics of species selected or avoided by deer. Furthermore, by tracking phylogenetic diversity through time, I identified evolutionary history losses under certain environmental conditions.

**Objective #5: Use ecological diversity indices to identify changes in diversity through time and between exclosures and controls.** Ecological diversity metrics are important tools for researchers, since they are an easy and effective way to compare diversity between sites. They also can be directly compared through time to indicate changes to species diversity under different environmental conditions. In addition, each is weighted for abundance as a measure of how evenly distributed species are within an area. However, these metrics do not provide information about selected species traits or evolutionary history since relatedness within
species is not measured. Despite these limitations, I used ecological diversity metrics as a supplement to phylogenetic diversity to fully explore differences in diversity between browsed and unbrowsed areas. I also utilized them to identify overall trends of diversity through time.

**Objective #6: Compare diversity metrics to identify similarities and differences.** As mentioned above, phylogenetic and ecological diversity metrics are important tools for determining diversity of a site. However, these metrics calculate diversity values based on different data and may not be consistent. There are two commonly used ecological diversity metrics (Shannon-Wiener diversity and Simpson’s diversity), but they are calculated slightly differently using species abundance or presence/absence data. As a result, these two ecological diversity metrics may also differ some, but their overall trends should be consistent. Using the calculations obtained from both ecological diversity metrics, I compared their performance to measures of phylogenetic diversity to identify the similarities and differences between them. The goal was to determine whether ecological diversity metrics were good predictors of phylogenetic diversity, since many researchers have assumed ecological diversity metrics are indicative of phylogenetic relationships (Vellend et al. 2011).

**Objective #7: Identify exclosure age as a factor influencing diversity, vegetation structure, and insolation.** Exclosure age is an important factor influencing vegetation dynamics within exclosures. Young exclosures recently removed from browsing are structurally very different from those that have been long-term exclosed since they lack mature vegetation. This will have impacts on insolation and diversity. By tracking changes to diversity over time and comparing diversity and insolation between old and young exclosures, I identified the impact of
removing browsing in the short-term and long-term. I also identified if competitive exclusion was taking hold and what impact it had on those variables.

**Objective #8: Create a deer browsing susceptibility index to determine if any species are reliant on exclosures for persistence or benefit from deer herbivory.** Plant tolerance to herbivory is crucial in determining plant species composition in areas with deer browsing. Some species contain secondary metabolites to deter herbivory, while others have growth patterns that tolerate it. Other species cannot persist at high levels of herbivory, while some species even benefit (Augustine and McNaughton 1998). Furthermore, species susceptible in one area may not be equally susceptible in other areas with different weather or soil conditions. To assess all species under my specific site conditions, I developed a deer browsing susceptibility index to evaluate each individual species presence inside and outside exclosures. With this index I identified species reliant on exclosures for persistence and also identified if closely related species were preferred (or avoided) by deer.

In summary, I investigated the impacts of removal from browsing on forest plant communities in both long-term and short-term exclosures by tracking plant species diversity through time. I also utilized light data as a possible indicator of resource limitation, and used biomass as an indicator of overall resource availability. Together, I used these variables to answer the question of whether species diversity increases are sustainable after long-term removal from deer herbivory. I also identified specific mechanisms influencing these plant communities. Comparisons between long-term and short-term exclosures are important in
understanding how plants respond over time to reduced browsing pressure. They provide insight on whether deer reduction strategies will increase diversity in the understory.

HYPOTHESIS AND PREDICTIONS

Overall, I expect diversity to initially increase over time in exclosures, and then decline as competitive exclusion takes hold. As a result, I expect the following predicted responses of vegetation to deer herbivory. Firstly, I predict increased vegetation height and percent cover in exclosures because deer are no longer removing biomass from these areas. As a result, there will be increased availability of forage for deer inside exclosures because plants inside are not being browsed. I also predict increased insolation in controls and decreased insolation in exclosures since deer browse taller vegetation, remove structural complexity, and permit more energy to reach the forest floor.

Secondly, I expect vegetation responses to affect phylogenetic and ecological diversity. There will be increased phylogenetic diversity in exclosures compared to controls because browse-intolerant species will no longer be limited by deer. Browsing acts as a habitat filter and will promote the growth of browse-tolerant graminoid species that are all closely related. Intolerant species vary in their species characteristics and their positions across the phylogenetic tree, so the growth of these species in exclosures will increase diversity. However, over time diversity will decline in exclosures because competitive dominants will outcompete other inferior competitors for limited resources. In addition, ecological diversity will be greater in exclosures compared to controls because species will establish. However, diversity will also decline over time in exclosures because of competitive exclusion. Both of these diversity metrics
will show the same overall trends (higher diversity inside exclosures compared to outside), but trends between years will be less consistent because phylogenetic diversity metrics are more sensitive to gains or losses of specific (more distantly related) species.

Thirdly, species susceptibility will play a crucial role in determining species composition inside and outside exclosures. Graminoids, ferns, and club mosses will be browse-tolerant and will predominantly be found in controls, while evergreens and many broadleaf herbaceous plants will be browse-susceptible and will persist mainly in exclosures. Lastly, I expect exclosure age to significantly influence vegetation structure and composition. Older exclosures will have decreased diversity, taller vegetation, and less insolation than younger exclosures because of competitive exclusion

**METHODS**

*Study Site*

This study was conducted at The Dairymen’s Club near Boulder Junction, Wisconsin. It is a privately owned 6,175 acre game reserve established in the 1920’s and is characterized by many different land types including lakes, marshes, sedge meadows, and hardwood forest (Rooney 2006). The climate is continental with a temperature range of -20°C in winter to 32°C in summer, and annual precipitation ranges between 550 to 780mm (Rooney 2009). In 1990, four long-term deer exclosures were constructed on the property, and 2 short-term exclosures were constructed 15 years later in 2005. Control (unexclosed) areas were established adjacent to both long-term and short-term exclosures for direct comparison in the same years. Each exclosure varies in size from 190 to 720m². Details on each exclosure age and size are listed in Table 1.
Data on above-ground biomass, vegetation height, insolation, and species diversity was collected from June 9 – June 12 2012. 2012 percent cover data and plant samples for genetic analysis were collected during the same time frame. All percent cover data from 2011-2006 (excluding 2007) was collected from permanent transects in the first week of June of the year sampled by Dr. Thomas Rooney. Percent cover data was collected using the line-intercept method, where cover of all the vegetation ≤1m above the ground was recorded. A measuring tape was laid on the ground beneath the vegetation, and each time a leaf or stem above the tape intercepted the transect, the length (to the nearest cm) of the tape covered and species identity was recorded. Percent cover for the $i$th species in a plot was calculated as $(\sum n_i)/1,500$ where $n$ is the length of the tape covered by each occurrence of species $i$ along that transect. The denominator is the number of cm in three 5m transects. Because multiple species can intercept the line transect at the same segment at different heights, total percent cover can exceed 100%. Percent cover of the $i$th species across all 4 plots within a treatment is $(\sum n_i)/6,000$, where denominator is the number of cm in twelve 5m transects (Rooney 2009).

**Measurements and Analysis of Light Availability**

Forest canopy data was collected using Solar Pathfinder™ (Iron City, TN) along three permanently established 10m transects which run through each exclosure/control pair. Each transect is 5m apart, and extends 5m into and outside each exclosure (Rooney 2009). Light measurements were taken 0.5m off the ground at 2.5m intervals across each transect and classified as either control ($n = 36$) or exclosure ($n = 36$) based on their location. Some measurements were on the fence line, and those were classified as between ($n = 18$). To sample
insolation, the Solar Pathfinder™ dome was placed and leveled at each transect interval, and the compass was adjusted toward north. A minimum of two photos were taken of the reflective dome using a digital camera, and photos were analyzed using the program Solar Pathfinder™ 2.0. This program estimates percentages of monthly insolation from the proportion of open canopy at each data point. Percent insolation was summed from May to August (the growing season of this summer guild of plants), and mean percent insolation for each transect interval was calculated based on all photos of that site. The coefficient of variation was used as a measure of total variability of the estimates of percent insolation. I conducted a 2-way ANOVA of mean light values within and between individual control/exclosure pairs (n = 6), as well as a spatial auto-correlation (Moran's I) across all transects (n = 90) to identify any changes in percent insolation between treatment and control areas.

Measurements and Analysis of Vegetation Structure

I compared vegetation height and percent cover inside and outside exclosures to characterize vegetation structure. To classify plant community structure at each site, maximum plant height (up to 2m) was also recorded at each interval. Vegetation taller than 2m was classified as advanced growth, anything >50cm but <2m was considered intermediate growth (usually dominated by tree saplings), and vegetation <50cm was considered to be a short (usually herbaceous) layer. From there, I graphed height of vegetation against mean percent insolation to compare the effect of vegetation structure on incoming light energy. Percent cover of the herbaceous layer was calculated as an average from each of the 3 permanent 5m
transects in each exclosure/control site using the line-intercept method described in Rooney (2009).

2-way ANOVAs were used to test for significant differences between controls and exclosures for percent cover (factors = exclusion, year) and height of vegetation data (factors = exclusion, age). Firstly, I pooled all controls (n = 6) and compared them to all pooled exclosures (n = 6). Secondly, I pooled by age and compared all 22 year exclosures (n = 4) and controls (n = 4). Thirdly I pooled again by age, but instead compared all 7 year exclosures (n = 2) to 7 year controls (n = 2). Linear regressions were used to test for significant trends of decreasing percent insolation with increasing present cover and vegetation height.

Analysis of Above-ground Photosynthetic Biomass

To sample above-ground photosynthetic biomass, a new 10m transect was established in each exclosure and control area. A 20x20cm quadrat was then randomly placed on the ground within 10 paces along that transect, and all plant material (up to 1m tall) within that quadrat was clipped to the ground and bagged. This process was repeated for each of the 8 samples collected from all transects. All plant material was returned to the lab at Wright State University, where any woody/non-photosynthetic plant material was discarded from each bag prior to drying. All remaining material was placed into a paper bag, where it was dried at 100°C for 1hr and at 70°C for an additional 24hrs to obtain dry weight. After each bag was dried it was weighed to the nearest 0.001g on a lab balance.
Differences in above-ground photosynthetic biomass were analyzed separately for each exclosure/control pair using log response ratios as a measure of effect size. I used the following equation:

\[
\ln R = \ln \left( \frac{\overline{X}^E}{\overline{X}^C} \right)
\]

where \(\overline{X}^E\) is the mean biomass value in the exclosure (n = 8) and \(\overline{X}^C\) is the mean biomass value of the control (n = 8). I plotted the 95% confidence interval using the standard error of the response ratio to determine if the effect was significant (p <0.05). In this analysis, a positive confidence interval reflects an exclosure with significantly more above-ground photosynthetic biomass than its paired control, a negative confidence interval indicates a control with significantly more above-ground photosynthetic biomass than its paired exclosure, and a confidence interval that spans the x-axis (positive to negative) indicates no significant difference between the two.

**Diversity Metrics and Analysis**

**Phylogenetic Diversity**

A site-specific rooted phylogenetic tree was created using DNA sequences of three gene regions. One of these, chloroplast *rbcL* gene, is highly conserved across angiosperms and has been the workhorse of broad-scale phylogenetics across higher plants (e.g., Chase et al. 1993). This gene aligns unambiguously across green plants and provides solid information on genetic relationships across the samples I studied. The other two genes are more rapidly evolving genes
used widely in fine-scale phylogenetics in flowering plants: the chloroplast trnL–trnF region (Taberlet et al. 1991), and the nuclear ribosomal internal transcribed spacer (ITS) regions, including the embedded 5.8S gene (Baldwin et al. 1995). Sequences for numerous taxa were available from the National Center for Biotechnology Information (U.S. National Library of Medicine) database (Benson et al. 2010). For each gene I used both existing sequences from GenBank® and sequences obtained from the Morton Arboretum in collaboration with Dr. Andrew Hipp. All species with unavailable sequences from Genbank® were collected and shipped to Dr. Hipp at the Morton Arboretum (Lisle, IL) for sequencing (Tables 1-3).

Data were aligned using Muscle v 3.8.31 (Edgar 2004a, b) by Dr. Hipp. The rbcL data were globally aligned without ambiguities, including all taxa. However, global alignment of all taxa simultaneously for the ITS and trnL–trnF regions produced alignments that were riddled with ambiguities. To address this, data matrices were exported by order to first produce multiple alignments for each order. Then, profile alignments were utilized, in which the alignment within each order is held fixed but nucleotide positions are allowed to shift among orders. Profile alignments were conducted among most closely related orders, moving progressively up the tips to the root of the green plants tree of life, using the Angiosperm Phylogeny Group tree (2009) as updated in APG Web (Stevens 2001 onwards).

Multiple alignments were then concatenated and analyzed using likelihood in RAxML v7.2.6 (Stamatakis 2006), using the GTRCAT nucleotide substitution model, using the multithreading option on a 4-core Intel processor (Stamatakis and Ott 2008). Analysis was conducted using 200 bootstrap replicates. Branch lengths were optimized on the resulting tree
using penalized likelihood (Sanderson 2002) as implemented in the ape package (Paradis et al. 2004) of R v. 2.13.1 (R Development Core Team 2011). Smoothing parameters from 10 to 0.001 were tried and found to have no appreciable effect on the branch lengths on the tree. The reported tree (Fig. 1) utilizes a smoothing parameter of 1.0. All new (previously unavailable) sequences from material sent to the Morton Arboretum will be submitted to GenBank®, and the site-specific phylogenetic tree will be submitted to TreeBASE.

There are several metrics of phylogenetic diversity. Here, I used mean pairwise phylogenetic distance (MPD), a built in metric from picante in R (Kembel et al. 2010), as a measure of phylogenetic diversity. MPD averages phylogenetic distances of all pairs of taxa in each control and exclosure across the entire tree and weights them by abundance (percent cover). I compared controls and exclosures by year for all pooled data (n = 6) and data separated by age (classifications were old (n = 4) and young (n = 2)) using a 2-way ANOVA (factors = exclusion, year). I also created a null model to test for standardized effect size of the phylogenetic diversity metric. I created a null model by randomly shuffling the taxa labels of the tree and comparing observed MPD to that model using 999 runs. I then compared different subsets of those data to the null model to identify which years and which exclosure/control pairs were most influenced by phylogenetic diversity. I tested MPD against a null model for two reasons: 1) to determine if there is any phylogenetic information to be gathered from these data and 2) to identify if phylogenetic clustering or overdispersion is occurring at the study site. A MPD value of 1 indicates species evenly spread across the phylogenetic tree, while values
significantly less than 1 indicate phylogenetic clustering (a loss of phylogenetic diversity). Values significantly greater than 1 indicate phylogenetic overdispersion.

**Ecological Diversity Indices**

Ecological diversity indices were calculated using both Simpson’s and Shannon-Wiener diversity metrics. For both, percent cover data from each control and exclosure was used for abundance. The equation for Simpson’s Diversity is:

\[ D = 1 - \left( \frac{\sum (PC_{sp}(PC_{sp}-1))}{(PC_{TPC}(PC_{TPC}-1))} \right) \]

Where:

- \( PC_{sp} \) is the percent cover of an each individual species, and \( PC_{TPC} \) is the total percent cover of all species.

The equation for Shannon-Wiener Diversity is:

\[ H' = -\sum (RPC_{sp}(\ln(RPC_{sp}))) \]

Where:

- \( RPC_{sp} \) is the relative percent cover of each individual species.

To check for significant trends, I ran a 2-way ANOVA (factors = exclusion, year) on exclosure and control values pooled by age (old, \( n = 4 \), and young, \( n = 2 \)).

**Comparisons of Ecological and Phylogenetic Diversity and Exclosure Age**

For comparisons of ecological diversity to phylogenetic diversity, I graphed both ecological and phylogenetic metrics for 2012 and visually inspected them for any differences. I graphed 22 year and 7 year exclosures/controls separately. Additionally, I compared the
influence of exclosure age by running a 2-way ANOVA (factors = age, year) on old (n = 4) and young (n = 2) exclosures for both ecological and phylogenetic diversity metrics. I also ran the same analysis on old (n = 4) and young (n =2) controls, to check for unexpected spatial variation in diversity.

**Deer Browsing Susceptibility**

To evaluate species susceptibility to deer browsing and those reliant of exclosures for persistence, I developed a deer browsing susceptibility index (DBS) in collaboration with Dr. Andrew Hipp that compares species presence inside to species presence outside exclosures. It is not based on any other susceptibility indices, and was developed independently. It scales from 0 to 1 for each species, and represents the fraction of that species present inside versus outside the exclosure. A score of 0 indicates exclusive species presence outside of the exclosure, while a score of 1 indicates species presence only inside exclosures. To exclude rare species that would skew DBS calculations, I only included species present in more than two exclosures or controls in two or more years.

DBS was calculated separately for each year using the following equation:

\[
DBS = \left( \sum_{s=1}^{n} PC_i \right) / \left( \sum_{s=1}^{n} PC_i + \sum_{s=1}^{n} PC_o \right)
\]

Where:

- \( PC_i \) is the percent cover inside the exclosure, \( PC_o \) is the percent cover outside the exclosure, and
- \( \sum(s=1, n) \) is the sum of \( PC_i \)(or \( PC_o \)) across all exclosures (or controls) for each species present.
Susceptibility classifications were determined by the susceptibility index. Species in the low susceptibility category were species with an index score less than 0.4 (<40% of the time, they are found inside exclosures). Those classified as medium susceptibility were species with scores greater than 0.4 but less than 0.85. Species classified as highly susceptible had an index score greater than 0.85 (>85% presence inside exclosures).

RESULTS

Vegetation Structure

Vegetation height was not significantly different between exclosure and control areas when samples from old and young exclosures were pooled together (Fig. 2). However when separated out by age, vegetation was 3 times taller in old exclosures than in controls. Young exclosure/control pairs did not show the same pattern (Fig. 2). Based on my classification of vegetation structure, older control areas were dominated by a short vegetation layer (<50cm), while controls and young exclosures were dominated by intermediate growth (>50cm, but <2m in height). Intermediate growth also dominated old exclosures (Fig. 2). Percent cover data collected from 2006 to 2012 (excluding 2007), showed significantly more coverage in exclosures than in controls, regardless of age (Fig. 3 and Fig 4). Percent cover significantly increased over time in young exclosures, but did not change in paired controls (Fig. 4). There were no significant changes to percent cover over time in old exclosures or controls (Fig. 3).

Insolation

There were no significant differences in mean percentage of insolation between exclosures and controls for the growing season (May-August, Fig. 5). I also did not detect
significant differences in percent insolation when comparing exclosure/control pairs of different ages (Fig. 5). Furthermore, regressions of percent insolation as a function percent cover and vegetation height did not yield significant results (Fig. 6 and Fig 7). A spatial auto-correlation using Moran’s I did not identify significant spatial decay in percent insolation between any control and exclosure pairs (p≥0.60).

*Above-ground Photosynthetic Biomass*

Differences in above-ground photosynthetic biomass (biomass) were detected using log response ratios as a measure of effect size. In 5 of the 6 exclosures, biomass was not significantly different when compared to controls. However, one exclosure had significantly more biomass than its paired control (Fig. 7).

*Phylogenetic Diversity*

Tables 2-4 show sources of gene sequences used for phylogenetic analysis. The phylogenetic tree in Fig. 1 (rooted from the genus *Lycopodium*) is based on the gene sequences listed in Tables 2-4. It characterizes the genetic relationships of all 36 species at the study site and all phylogenetic analyses are based off of these relationships.

*Mean Pairwise Phylogenetic Distance Comparisons*

Data was grouped by exclosure age (old or young) and MPD was calculated separately for exclosures and controls from 2006 to 2012 (excluding 2007). This analysis showed old controls were less diverse than old exclosures in every year sampled (Fig. 9). However, MPD was not significantly different in old control or exclosure areas between years (Fig 9). Comparisons of
young exclosure/control pairs showed no difference in MPD between them or between years (Fig. 10).

To expand the amount of information provided by MPD comparisons in the above section, I ran tests of MPD from 2006 to 2012 (excluding 2007) against a randomized null model. These data are represented in Figs. 11-14. Again, data was grouped by age. All exclosures (young and old) and young controls were not significantly different from the null model (Fig. 11, Fig. 12, and Fig.13), but old controls were significantly different (more phylogenetically clustered) than the null (Fig. 14). I tracked changes in MPD compared to the null model over time to identify if at a certain age diversity differences become significant. I was unable to identify a point at which diversity shifts in this way, even though young controls may become more phylogenetically clustered over time (Fig. 13).

*Ecological Indices of Species Diversity*

The two ecological diversity indices showed the same trends when represented graphically. From 2006 to 2012, both Simpson’s and Shannon’s showed no significant differences between old exclosures and their paired controls (Fig. 15 and Fig. 16). In addition, diversity in old exclosures and controls did not significantly change between years sampled in both metrics (Fig. 15 and Fig. 16). In young pairs, both metrics showed greater diversity in controls compared to exclosures from 2006 to 2010, (excluding 2007). However, in 2011 and 2012, both metrics did not show any significant differences in diversity between exclosures and controls (Fig. 17 and Fig. 18).
Comparison of Diversity Metrics

The phylogenetic and ecological diversity indices were inconsistent with one another. Both ecological indices (Fig. 15 and Fig. 16) showed no significant differences in diversity between old controls and exclosures across any year, while the phylogenetic diversity metric (Fig. 9) showed old exclosures to be significantly more diverse than old controls in each year sampled. However, all three graphs consistently showed no changes to diversity across sampling years in exclosures or controls (Fig. 9, Fig. 15, and Fig. 16).

The young exclosure/control pairs were more variable between metrics than were the old ones. Again, the ecological diversity metrics were consistent with one another, but when compared, the phylogenetic and ecological diversity metrics were not. Both ecological diversity indices showed young controls to be significantly less diverse than exclosure areas from 2006 to 2010 (Fig. 17 and Fig. 18), while the phylogenetic metric showed no differences between them in the same years (Fig. 10). Unlike with old exclosure data, all three metrics showed no significant trends of diversity from 2011 to 2012 (Fig. 10, Fig. 17, and Fig. 18). There were no significant differences in phylogenetic diversity between any sampled years for young exclosures or controls (Fig. 10), while Simpson's and Shannon's diversity showed significant differences that ceased to be significant after 5 years of exclusion (Fig. 17 and Fig. 18).

Exclosure Age as a Factor Influencing Species and Phylogenetic Diversity

Each diversity metric showed a different impact of exclosure age on diversity. Phylogenetic diversity was higher in older exclosures compared to younger ones in every year sampled (Fig. 19), however exclosure age comparisons for both ecological diversity metrics
showed no differences in diversity of young and old exclosures after 5 years of exclusion (Fig. 20 and Fig. 21). In addition, phylogenetic diversity was higher in younger controls compared to older ones (Fig. 22), but it was not significantly different when compared using ecological diversity metrics (Fig. 23 and Fig. 24). None of the metrics directly comparing exclosure age showed any significant changes to diversity across sampled years (Fig. 19, Fig. 20, and Fig. 21), but ecological diversity metrics showed smaller differences between old and young exclosures after 2010 (Fig. 20 and Fig. 21). Comparisons of control age also did not show significant changes in diversity measurements across sampled years (Fig. 22, Fig. 23, and Fig. 24).

**Deer Browsing Susceptibility**

All species present in two or more exclosures in more than two years from 2006 to 2012 (excluding 2007) were included in DBS calculations (Fig. 25). The least susceptible species was *Schizachne purpurascens*, a perennial graminoid with an index score of 0.07. There were several species with DBS scores greater than 0.90, but *Polygonatum pubescens*, a perennial broadleaf herb, was the most susceptible species with an index score of 0.99. Other species in the low susceptibility category consisted mainly of graminoids, club mosses, and their close relatives. Intermediately (medium) susceptible species included several species of fern and one woody browse species. Lastly, highly susceptible species included several species of woody browse and broad-leaf herbaceous plants.
DISCUSSION

Vegetation Structure

As expected, vegetation structure (percent cover, vegetation height, and species composition) changed with protection from deer and with increasing time since protection. Protection from deer promoted plant growth, and therefore led to higher percent cover at the ground layer. All control areas had consistently lower percent cover than protected areas due to exposure to deer browsing. Percent cover in young exclosures increased each year, while it remained consistent in old exclosures. These percent cover trends likely indicate a shift from browse tolerance to resource limitation in exclosures. Deer densities have not changed drastically from 2006 to 2012, and browsing pressure has remained relatively consistent at the study site. Browsing pressure explains the percent cover trends seen in controls, since deer can access these areas equally.

In young exclosures, percent cover increased significantly compared to controls after just one year of exclusion. It increased steadily in exclosures each year, and was significantly higher compared to previous years in 2011. This indicates that browsing was the limiting factor in these areas, since its removal significantly increased percent vegetation cover. However, in old exclosures percent cover did not change significantly between years. This is because in the 7 years since exclusion, young controls not yet reached the point in which percent cover increases are no longer possible because there are still 'free' resources available for use. In old exclosures, all limiting resources (e.g. space, light, and nutrients) are being utilized. In exclosures, the main disturbance event (browsing) has been removed, and competition for resources has increased over time as plants have made use of the relatively stable environment.
Vegetation height only becomes significantly greater in exclosures compared to controls after more than 7 years of protection. This is contrary to my prediction, and likely reflects the slow growth and light limitation of taller vegetation (ex. tree saplings) compared to ground layer vegetation. It is also possible that browse-sensitive species are slow to return to exclosures because of short dispersal distances (Ruhren and Handel 2003). Exclosure studies have been used in the past to promote growth of woody species of commercial interest and native slow-growing species (Marquis 1981; Kay and Bartos 2000; and Anderson and Loucks 1979). Deer have been shown to negatively impact tree regeneration, and will likely change canopy species composition in the future if this trend is not reversed. At Dairymen's, Balsam fir and Eastern Hemlock were particularly deer susceptible, and average percent cover of these species was at least 100% greater inside versus outside exclosures, regardless of exclosure age. They are both evergreen species, but they are not equally preferred by deer. Eastern hemlock is a long-lived conifer, and it is preferentially browsed by deer during the winter months (Marquis 1981). However, balsam fir is not a preferred food for deer and it is only consumed after preferential species are no longer available (Beals et al. 1960). Regardless of species preference, deer browsing simplifies vegetation structure and reduces vertical complexity. This change may impact more than just plant species, and may have long-term impacts on forest structure (Côté et al. 2004).

Plants respond differently to removal from deer browsing depending on their growth patterns and nutrient needs. For example, it may be decades before differences in canopy cover are noticeable due to the slow growth of light-limited woody species. However, some above-
ground vegetation responds much more quickly to changes in browsing pressure, despite Dairymen's long history of deer overabundance. *Trientalis borealis* and *Maianthemum canadense* both showed at least a 30% increase in percent cover after just one year of exclusion (2006 data), while *Carex arctata* and *Lycopodium annotinum* declined at least 7%.

**Above-Ground Photosynthetic Biomass**

Vegetation structure is affected by deer browsing, but above-ground photosynthetic biomass is not. In 5 of the 6 pairs, control area biomass is not significantly different from exclosure biomass regardless of age of exclosure. However, Big Gap exclosure biomass was significantly greater than control biomass. These results were unexpected. I hypothesized all exclosures would have increases in above-ground photosynthetic biomass compared to controls because biomass was no longer being browsed by deer. Big Gap is one of the old exclosures and it was constructed underneath a tree-fall gap in 1990, hence its name. It is the only exclosure under a tree-fall gap at the study site. Although the current light environment is not significantly different from the other exclosure/control pairs, this was likely not the case in the immediate years following construction. Light limitation is a key factor influencing sapling regeneration, since most slow-growing tree species require adequate amounts of light to germinate and sustain growth (Poulson and Platt 1989). After a tree-fall event, sapling density increases in response to increased light availability. Eventually, these saplings compete for limited resources, resulting in self-thinning (Runkle 1998). Big Gap's light environment (amount of insolation) was likely much more favorable than the other exclosures because developing tree species did not tower over the rest of the vegetation in the first few years after exclusion. I hypothesize that
this increased insolation combined with a reduction in herbivory promoted a sharp increase in
vegetation growth. While I do not have light or percent cover data from 1990 to 2005 to test
whether this is the case, it is well known that light levels play a crucial role in plant growth (Lin
et al. 2002; King and Antrobus 2005; and Kobayashi and Kamitani 2000). In addition, the Big Gap
exclosure shares all other important characteristics (including age and protection from
browsing) of the other old exclosures. Therefore, light is likely the main variable that explains
these biomass differences in 2012.

*Light Availability*

Incoming solar radiation was not significantly different between any exclosures and
controls even though percent cover, vegetation height, and in one case, biomass, were different
inside versus outside exclosures in 2012. This was unexpected. Vegetation differences between
exclosures and controls should have influenced insolation since plants shade out the area
beneath their leaves (Schmitt and Wulf 1993). I expected increased biomass inside exclosures
due to reduced disturbance and increased growth. Increased growth reduces diversity of plant
species through increased competition for light in the forest understory (Hautier et al. 2009).
However, my measures of insolation were not sensitive to the small changes that occur at the
plant level. Specifically, I measured insolation from the canopy and canopy coverage does not
always change in response to deer herbivory in the short-term. Woody plants that fill in the
canopy are usually slow growing and take years to reach canopy height. Failed sapling
recruitment from deer browsing may not be evident until several years after heavy browsing.
Even then, faster-growing and more browse-tolerant woody species may replace those that
were affected (Whitney 1984). The result is a species composition shift, but not an overall change in the availability of light energy.

Regressions of insolation against percent cover and vegetation height did not show any significant trends. This is further evidence that my metric was not sensitive to changes at the plant level. Percent cover at the understory level does influence the amount of insolation reaching the forest floor (Aubin et al. 2000), but these measurements were not sensitive to changes because of their location ~50cm above the ground. Percent cover measurements were taken right above ground level and below the insolation measurements. Furthermore, vegetation height has a significant impact on available light energy (Hautier et al. 2009; and Messier et al. 1998), and despite the dome's location (sometimes directly underneath immature growth) I did not detect these impacts.

I was also unable to categorize changes in solar isolation even between Big Gap and the other areas I sampled. As mentioned before, Big Gap is under a tree-fall gap which should have positively influenced insolation. This presents a problem, since my method was unable to detect changes in canopy coverage that I know existed. It is possible that tree species have filled in this gap after 22 years, but I should still be able to detect small changes between Big Gap and the other exclosures. In order to detect changes in insolation in the forest understory, a more sensitive light meter must be used and more data points need to be measured. Here, I focused on measurements 5m inside and outside exclosures. It is likely that insolation is not uniform throughout the area, and more measurements would prove useful, especially with a more sensitive meter.
Contrary to my hypothesis, phylogenetic diversity was not always significantly greater in exclosures compared to controls. Old exclosures were significantly more diverse than old controls, but there was no significant difference between young exclosures and controls. In addition, there were no significant changes in diversity between years, suggesting that in the short-term (<10 years) diversity does not decline with exclosure age. However, diversity was significantly greater in old exclosures compared to young ones, suggesting it changes over the long-term (>10 years). These data refute my original hypothesis, as I expected diversity to decline with time and young exclosures to be more diverse than old exclosures. This evidence suggests that phylogenetic diversity does not decline with increasing age of exclosure due to competitive exclusion. Additionally, it may reflect the slow dispersal rates of some browse-susceptible species (e.g. forest herbs), as it can take decades before they recolonize exclosure areas (Ruhren and Handel 2003).

Young controls should have been comparable to old controls since both have been browsed the same length of time. However, young controls were significantly more phylogenetically diverse than old controls when compared. These differences can be attributed to the location of the controls. From the late 1920's to 1999, recreational feeding took place at the Dairymen's lodge, which significantly increased carrying capacity of Dairymen's deer (Rooney 2006). Feeding increases the severity of deer herbivory because more deer are able to be supported per km². The old controls are at a closer proximity to the lodge than the young
controls, and as a result deer herbivory was likely much more severe in old controls than young ones.

Null model comparisons showed no significant differences between observed and expected mean pairwise phylogenetic distances for young exclosures, old exclosures, or young controls. However, old controls were significantly more clustered phylogenetically. This suggests that over the long-term, a habitat filter selected for closely related species in old controls but not in paired exclosures. After plotting species composition of old controls and exclosures for comparison, I determined that browsing was the habitat filter at my study site because the filter was selecting for browse-tolerant species (particularly graminoids and ferns). Graminoid species are browse-tolerant due to their low growing apical meristem and ability to put out compensatory growth following browsing (Rooney 2009). Ferns are more variable in their responses to herbivory, but generally are less preferred by deer because of their chemical composition (Rooney 2009). However, other species without these characteristics are more susceptible to herbivory (see DBS index) and do not put out compensatory growth following browsing as quickly. Eventually, graminoid species competitively exclude less tolerant species, as compensatory growth is much more energetically expensive and slow for intolerant plants (Rooney 2009).

According to my data, phylogenetic clustering does not always occur since young controls are not significantly more clustered than the null. As stated above, control location probably greatly affected my phylogenetic diversity data. Mean phylogenetic distance inside and outside young exclosures is not significantly different, which suggests that habitat filtering is not
occurring in controls. It also suggests that exclusion does not having a significant effect on phylogenetic distance. This is contrary to my hypothesis, since I expected phylogenetic diversity to be greatest inside exclosures and browsing to act as a habitat filter outside exclosures. In addition, I only was able to use two areas for my young exclosure/control comparisons, and more samples are needed throughout the property to determine the impact of exclosure age on phylogenetic diversity.

_Ecological Diversity Indices_

Unexpectedly, ecological diversity trends were opposite my phylogenetic diversity trends in this study. Young controls have greater ecological diversity in 4 of the 6 years sampled, but there are no differences between exclosure and control in old areas. This indicates that young controls have increased species richness and evenness compared to exclosure areas from 2006 to 2010. However, a decline in diversity differences between young exclosures and controls in 2011 and 2012 indicates competitive exclusion in control areas. This is because a decline in ecological diversity indicates either a loss of a species, loss of evenness, or both. A loss of evenness indicates dominance of one or a few species. Old exclosures do not show this trend, and there are no significant differences in species distributions between old exclosures and controls.

Unfortunately, these ecological diversity metrics do not allow me to plot species composition changes through time, so I cannot easily determine which species are being lost or gained in each year. The loss of ecological diversity in controls through time supports my prediction that diversity will decline through time due to competitive exclusion. Comparisons of
exclosure data also support this prediction, since old exclosures are more diverse from 2006 to 2010, but are not significantly different from young exclosures in 2011 and 2012. Over time, young exclosure diversity has increased to levels comparable to old exclosures, while old exclosures remain stable from the entire sampling period. These data indicate that old exclosures have reached a peak of ecological diversity, and I predict that as the old exclosures age further (5-10+ additional years) diversity will decrease and be lower than in young exclosures.

Comparisons of Diversity Metrics

As mentioned above, my ecological diversity and phylogenetic diversity metrics were dissimilar in many ways. However, both ecological metrics were consistent in their overall trends of ecological diversity. Differences between ecological and phylogenetic diversity exist because phylogenetic diversity measures genetic dissimilarity in the form of mean pairwise distance between pairs of taxa. Both types of metrics weight for abundance, so any differences between diversity values are attributed to genetic and evolutionary relationships. Phylogenetic diversity is based on these relationships, not numbers of species, and closely related species are not weighted as heavily as those that are more distantly related. Therefore, an area with fewer, more distantly related taxa will be more phylogenetically diverse than one with several closely related taxa. However, ecological diversity metrics weight all individual species equally, and focus on changes to species numbers and evenness.

Even though phylogenetic and ecological diversity metrics are dissimilar, it does not mean that one is always better than the other. Under certain circumstances ecological diversity
may be sufficient to answer questions about diversity within an area, while in other cases phylogenetic diversity measurements may be needed. For example, if a researcher is interested in preserving an area based on the number of species present, ecological diversity would be an inexpensive and effective way to measure diversity. However, if a researcher is interested in preserving genetic dissimilarity and evolutionary history, phylogenetic analysis is necessary.

In this particular study, relying on ecological diversity metrics alone would not have been a sufficient way to assess diversity. Using phylogenetic diversity, I was able to identify browsing as a habitat filter by plotting species presence inside and outside exclosures across my phylogenetic tree. In previous studies, phylogenetic metrics have been used as a way to assess effects of disturbance (Cavendar-Bares et al. 2012) or environmental gradients (Pellissier et al. 2012) on phylogenetic relationships. This is the first study to identify the effects of deer browsing on phylogenetic diversity, and to specifically detect deer herbivory as a strong habitat filter that selects for closely related browse-tolerant species. This application highlights the importance of utilizing new tools in assessing the effects of deer herbivory, and opens up an avenue for further research in this area. Additionally, it emphasizes the importance of analyzing phylogenetic relationships in making management decisions.

If I had based my interpretation of my data on ecological diversity metrics alone, I would have concluded that exclosures are ineffective at promoting diversity over the long-term. However, phylogenetic diversity is actually greater in older exclosures compared to younger ones, suggesting that it takes long-term protection from herbivory to counteract the browsing habitat filter created by deer. I was also able to identify similar characteristics shared by browse-
tolerant species, including poor digestibility and quick compensatory growth (Rooney 2009). However, ecological diversity metrics still provided important information about species evenness and richness in young exclosures, since phylogenetic trends were insignificant. In this case, both metrics were useful, but in different ways.

**Exclosure Age as a Factor Influencing Ecological and Phylogenetic Diversity**

Phylogenetic diversity comparisons between different aged exclosures and controls showed diversity to be higher in old exclosures compared to young ones, and young controls compared to old ones. Higher phylogenetic diversity in old exclosures compared to young exclosures shows long-term exclusion was effective in promoting increased phylogenetic distance (genetic dissimilarity). However, 7 years was not enough time to promote the growth of more distantly related species from those that are browse-tolerant and present outside young exclosures. Exclosures must be left up for more than 15 years in order to significantly increase mean pairwise phylogenetic distance compared to paired controls. Again, this could be attributed to the slow dispersal rates of some herbaceous plants (Ruhren and Handel 2003). Regardless, the effect of browsing as a habitat filter is strong, and it takes years before it begins to dissipate.

As for control comparisons, phylogenetic differences in young controls compared to old controls may be explained by differences in browsing pressure following recreational feeding at the lodge (mentioned above). If browsing pressure was higher in old controls compared to young ones, I can conclude that even following significant deer reductions at Dairymen’s (Rooney 2006) phylogenetic diversity is not increased in the absence of exclosures. Ecological
diversity in old and young controls was not significantly different in any year, suggesting species evenness and richness are comparable between control ages. These data combined with the differences in phylogenetic diversity between controls, suggest an additional habitat filter has affected old controls. I suspect the main influence was increased browsing pressure, but unfortunately I do not have browsing pressure data from this time frame and cannot make any scientifically sound inferences. However, future studies at Dairymen's should analyze current browsing pressure and topography at all exclosure/control pairs to assess the similarity between young and old areas. These data would help determine if there are effects of exclosure location on browsing pressure, and if deer prefer browsing in one area over another.

Ecological diversity comparisons between exclosure and control age show long-term exclosures to be ineffective at promoting diversity over the long-term. Longer-term studies of each exclosure age would help me identify at what point ecological diversity in young exclosures is significantly greater than it is in old exclosures (if current ecological diversity trends continue on the same trajectory). I could also identify a specific age in which ecological diversity is greatest following exclusion, since over time young exclosures should follow the same pattern as old ones. Based on current trends from my ecological diversity metrics, I would expect exclosures to peak in diversity sometime after 7 but before 16 years of age. New exclosure construction and continued data collection in current exclosures would help answer this question.
Deer Browsing Susceptibility Index

Generally, deer browse selectively on digestible plant material that will increase their overall fitness (Horsley et al. 2003). These keystone herbivores have the ability to completely alter plant communities by selecting for unpalatable species (Rooney and Waller 2003), especially those that exhibit structural or secondary compounds that prevent digestibility or deter browsing (Augustine and McNaughton 1998; and Côté et al. 2004). Deer diet changes throughout the year, with graminoids and herbaceous plants comprising most of the diet during the spring and summer months (Rose and Harder 1985). When leafy green (preferred) forage is unavailable, woody browse species (primarily evergreens) comprise most of a deer's diet (Harris 1945). Consequently, both deer preference and plant growth form greatly influence a plant's susceptibility to browsing (Côté et al. 2004).

At Dairymen's, 8 of the 10 plants classified as low susceptibility were graminoid species, which grow close to the ground and quickly compensate growth following browsing. *Lycopodium obscurum* and *Pinus strobus* (white pine) were the other two species in the low susceptibility category. *Lycopodium obscurum* contains structural compounds that make it hard to digest and not a preferred plant for deer. White pine, however, is usually heavily browsed in areas of high deer density (Ross et al. 1970). Generally, my findings are consistent with other studies showing the ability of deer to promote browse-tolerant and unpalatable species (Waller and Alverson 1997; Côté et al. 2004 Horsley et al. 2003; and Rooney 2009). However in this study, DBS is based on species presence inside and outside exclosures, and it does not directly measure a species' susceptibility to deer browsing based on chemical composition or deer
preference. As a result, DBS is also influenced by a species' susceptibility to certain environmental conditions (e.g. shading and competition). Exclosures may represent a more favorable environment for species even if they are not directly browsed by deer.

Despite their presence in heavily browsed areas, browse-tolerant species are not always unpalatable, and specifically, not all graminoids are poor food choices for deer. Some graminoid species may be browse-tolerant, but nutritious enough to continue supporting high densities of deer (Rose and Harder 1985). Therefore, a shift of forest understories to graminoid-dominated plant communities may further prevent deer density reductions, even after the removal of preferred plant species.

Intermediately susceptible plant species included three species of fern and one woody species, *Acer rubrum*. Previously, *Acer rubrum* was not found outside any exclosures at the same study site (Rooney 2009), which indicates either a reduction in browsing pressure in control areas, or an increase in the availability of light outside exclosures since 2006. Red maple is a more light-demanding species, which may respond favorably to increased light energy in heavily browsed areas (Whitney 1984). Again, future studies should reexamine insolation and quantify browsing pressure to additionally explain species composition shifts between exclosures and controls.

The fern species in the intermediate susceptibility category had indices that ranged from 0.66 to 0.83, which suggests ferns, despite their classification, are variable in their browse tolerance and may even be susceptible to browsing in some cases. Additional measured
variables like light and browsing pressure (mentioned above) may explain why some species differ in susceptibility. The term ‘fern' encompasses species in dozens of families across tree of life (Hasebe et al. 1995), and many of these species exhibit different growth characteristics and chemical composition (Rooney 2009). These differences likely explain the differences between my index and the published literature, since some studies list ferns as browse-tolerant (Horsley et al. 2003; and Rooney 2001).

As expected, broadleaf herbs and some woody browse species were classified as highly susceptible. Broadleaf herbs are a preferred food for deer since they are easily digestible, and are outcompeted by browse-tolerant species in heavily browsed areas (Rooney 2009). All 4 broadleaf herbaceous plant species in this category showed susceptibility scores greater than 0.90, indicating they are found in exclosures more than 90% of the time they are present. The two most susceptible herbs, *Polygonatum pubescens* and *Maianthemum candense*, belong to the Liliaceae family *sensu lato*. Species within this family are particularly sensitive to deer browsing, and they are often used to as an indicator of browsing pressure within an area (Balgooyen and Waller 1995; and Rawinski 2010). Although not in the same family, the other two herbaceous species, *Polygala paucifolia* and *Trientalis borealis*, still showed a high susceptibility to browsing. This is either a direct result due to their nutritional content and inability to deter deer, or an indirect response to removal from browsing. Therefore, without significant reductions in current deer densities at Dairymen's, all four of these species are reliant on exclosures for persistence.
The woody species highly susceptible to browsing were two evergreen species, *Tsuga canadensis* (Eastern hemlock) and *Abies balsamea* (balsam fir), and one deciduous species, *Acer saccharum* (sugar maple). As mentioned before, Eastern hemlock is a preferred overwintering browse for deer, so its classification as highly susceptible was expected. However, the literature indicates sugar maple and balsam fir as non-preferred food sources for deer (Beals et al. 1960). Additionally, the sugar maple browse index is a common tool used to assess browsing pressure from deer (Balgooyen and Waller 1995; Rooney and Waller 2003; and Waller and Alverson 1997) because sugar maple is neither actively avoided nor preferred. Since both balsam fir and sugar maple are not first food choices for deer, but were listed as browse susceptible at my study site, I can conclude that deer have consumed most of the preferred browse species and are now browsing on lower-quality forage in this area. However, other variables like soil composition, competition, or insolation may also be influencing species composition.

Even though the deer browsing susceptibility index used in this study was consistent with the literature and my predictions, I was unable to determine if susceptibility was due to direct or indirect effects of deer browsing. Additionally, I excluded several species because of their rarity in the data set. Future studies should look at additional variables along with browsing pressure to get a full understanding of the direct and indirect impacts of foraging behavior and environmental conditions on plant species composition inside and outside exclosures. To fully assess susceptibility of all plants to deer browsing within the study site, the sampling area should be expanded to include more individuals of the same species.
CONCLUSIONS

Deer browsing negatively influences forest understory plant communities in two main ways. Firstly, it negatively impacts vertical complexity through reductions in percent cover and vegetation height, and secondly, it impacts species composition by facilitating the dominance of browse-tolerant species. Declines to vertical complexity should increase insolation, since deer open up the forest understory and allow more light to reach the forest floor. Yet, this study did not find any significant changes to percent insolation inside and outside exclosures. Metrics analyzing species composition show browsing acts as a habitat filter and reduces phylogenetic and ecological diversity. Most browse-tolerant species are closely related, which increases genetic similarity and reduces phylogenetic diversity. Additionally, many species are also lost through browsing, which reduces ecological diversity in browsed areas. Over the long-term however, ecological diversity is not different between exclosures and controls, which would suggest competitive exclusion as species richness and evenness are lost. Phylogenetic diversity metrics suggest otherwise, as the impact of habitat filtering is reduced in long-term exclosures.

Contrary to what I expected, long-term exclusion does not result in competitive exclusion from the establishment of competitive dominants. However, percent cover increases decline over time as free resources have been utilized by established plants in long-term exclosures. Long-term exclosures may also be necessary to promote the persistence of highly susceptible browse species, since presence outside exclosures is low for these species. Nevertheless, longer-term studies may not yield the same results, and further research is
needed to assess the impacts of long-term exclusion on plant communities. Competitive exclusion may still be relevant in studies longer than 22 years.

Exclosures are effective at promoting genetic diversity over the long-term, and allowing the persistence of highly susceptible species. However, the dominance of graminoid species is allowing high deer densities to persist over the long-term, since overall forage availability is not impacted by the removal of preferred plant species. In order to promote diversity in forest understories, managers should focus on the reduction of deer densities and not the use of exclosures to promote ecological and phylogenetic diversity.
LITERATURE CITED:


Fig. 1: A rooted phylogenetic tree (using ITS, rbcl, and trnL-F genes) of all species present at the study site.
Figure 2: Mean vegetation height comparisons of all grouped data (pooled) and data separated by age (old and young) between exclosures and controls. Letter changes indicate significance at the p < 0.05 level using 2-way ANOVA within treatment and age. Error bars are ± 1 SD.

Figure 3: Mean percent cover comparisons between old exclosures and controls and across years. Matching letters indicate no significant difference and letter differences indicate significance at the p < 0.05 level using a 2-way ANOVA within and between years. Error bars indicate ± 1 SD.
Figure 4: Mean percent cover comparisons between young exclosures and controls across years. Matching letters indicate no significant difference and letter differences indicate significance at the p < 0.05 level using a 2-way ANOVA within and between years. Error bars indicate ± 1 SD.

Figure 5: Mean percentage of insolation comparisons of all exclosure and control areas using 2-way ANOVA within and between individual exclosure pairs. Matching letters indicate no significant differences at the p<0.05 level. Error bars are ± 1 SD.
Figure 6: Linear regression of all pooled percent cover and insolation values. N.S. indicates the trend is not significant.

Figure 7: Linear regression of all pooled vegetation heights and insolation values. N.S. indicates the trend is not significant.
Figure 8: Log response ratios (as a measure of effect size) comparing above-ground photosynthetic biomass between each individual exclosure and control. An error bar overlapping the x-axis indicates no difference, while a positive error bar indicates more biomass in exclosures compared to controls. Error bars represent 95% confidence intervals. Asterisk indicates significance at the p<0.05 level.
Figure 9: Mean pairwise phylogenetic distance (MPD) comparisons (weighted for abundance) of old exclosure and control areas from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.
Figure 10: Mean pairwise phylogenetic distances (MPD) comparisons (weighted for abundance) of young exclosure and control areas from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.
Figure 11: Observed and expected mean pairwise distances (MPD) of all plant species (weighted for abundance) in the young exclosures from 2006-2012 (excluding 2007). Observed MPD is based on the phylogenetic tree in Fig. 1, while expected MPD is based on a randomized null model. Error bars represent ±1 SD of the expected MPD. Observed MPD values outside ±1 SD are considered significant at the p<0.05 level.

Figure 12: Observed and expected mean pairwise distances (MPD) of all plant species (weighted for abundance) in the old exclosures from 2006-2012 (excluding 2007). Observed MPD is based on the phylogenetic tree in Fig. 1, while expected MPD is based on a randomized null model. Error bars represent ±1 SD of the expected MPD. Observed MPD values outside ±1 SD are considered significant at the p<0.05 level.
Figure 13: Observed and expected mean pairwise distances (MPD) of all plant species (weighted for abundance) in the young controls from 2006-2012 (excluding 2007). Observed MPD is based on the phylogenetic tree in Fig. 1, while expected MPD is based on a randomized null model. Error bars represent ±1 SD of the expected MPD. Observed MPD values outside ±1 SD are considered significant at the p<0.05 level.

Figure 14: Observed and expected mean pairwise distances (MPD) of all plant species (weighted for abundance) in the old controls from 2006-2012 (excluding 2007). Observed MPD is based on the phylogenetic tree in Fig. 1, while expected MPD is based on a randomized null model. Error bars represent ±1 SD of the expected MPD. Observed MPD values outside ±1 SD are considered significant at the p<0.05 level.
Figure 15: Simpson’s diversity index comparisons (weighted for abundance) of old exclosures and controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.

Figure 16: Shannon’s diversity index comparisons (weighted for abundance) of old exclosures and controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.
Figure 17: Simpson’s diversity index comparisons (weighted for abundance) of young exclosures and controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.

Figure 18: Shannon’s diversity index comparisons (weighted for abundance) of young exclosures and controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.
Figure 19: MPD comparisons (weighted for abundance) of young and old exclosures from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.01 level.
Figure 20: Simpson’s diversity comparisons (weighted for abundance) of young and old exclosures from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the $p<0.05$ level.

Figure 21: Shannon’s diversity comparisons (weighted for abundance) of young and old exclosures from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the $p<0.05$ level.
Figure 22: MPD comparisons (weighted for abundance) of young and old controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.01 level.
Figure 23: Simpson’s diversity comparisons (weighted for abundance) of young and old controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.

Figure 24: Shannon’s diversity comparisons (weighted for abundance) of young and old controls from 2006-2012 (excluding 2007) using 2-way ANOVA between and within year. Matching letters indicate no significant differences while different letters indicate significance at the p<0.05 level.
Figure 25: A stacked bar graph representing the calculated browsing susceptibility of each species present in more than one exclosure in two or more years. Light grey bars indicate the proportion the species present in control areas, while dark grey bars indicate the proportion of the species present in exclosures. Species are ordered left to right from the least susceptible (*S. purpurascens*) to most susceptible (*P. pubescens*).
Table 1: Exclosure details including name of exclosure, age of construction, and size for all 6 exclosures used in this study. Each exclosure is paired with an adjacent control (unfenced) area for comparison.

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Table 2: Species names and catalog numbers of ITS gene sequences used for phylogenetic analysis. Those in column 1 were sequenced using PCR in collaboration with Dr. Andrew Hipp at the Morton Arboretum. Sequences from the NIH genetic sequence database (GenBank, column 2) were obtained through the software program Geneious. Numbers to the right of each species name indicate the catalog number of the gene sequence.

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Table 3: Species names and catalog numbers of rbcL gene sequences used for phylogenetic analysis. Those in column 1 were sequenced using PCR in collaboration with Dr. Andrew Hipp at the Morton Arboretum. Sequences from the NIH genetic sequence database (GenBank, column 2) were obtained through the software program Geneious. Numbers to the right of each species name indicate the catalog number of the gene sequence.
Table 4: Species names and catalog numbers of trnL-trnF gene sequences used for phylogenetic analysis. Those in column 1 were sequenced using PCR in collaboration with Dr. Andrew Hipp at the Morton Arboretum. Sequences from the NIH genetic sequence database (GenBank, column 2) were obtained through the software program Geneious. Numbers to the right of each species name indicate the catalog number of the gene sequence.

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