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Deer impact and plant resistance traits

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ABSTRACT

DEER IMPACT AND PLANT RESISTANCE TRAITS

By
Xueyang Fan

White-tail deer (*Odocoileus virginianus*), a generalist herbivore, are widely considered to influence ecological communities, ecosystems and human wellbeing by foraging preferentially on certain plant species. Previous research has shown that high deer density can change the relative abundance of tree species in forest communities. Furthermore, some evidence shows that resistance traits of plants can influence plant photosynthetic ability which is an important factor in an ecosystem. The purpose of this experiment is to test whether plant resistance traits can change within species when they are exposed to high levels of deer herbivores. The experiment, established in 1979, enclosed deer within forest stands at high and low densities. Resistance traits of five dominant woody plant species were sampled from individuals that established during the deer density treatments and are now adults. Plant resistance traits (Leaf mass per area, Leaf dry matter content, C:N ratio, and Wood density) were tested and compared between low and high deer density area by using mixed effect statistical models. Leaf mass per area (LMA), leaf carbon–nitrogen ratio (C:N), and wood density did not respond significantly to increasing deer density. However, leaf dry matter content (LDMC) showed a slight but significant increase in response to high deer density. These results indicate that this plant trait may respond to increasing deer density, resulting in potential impacts on ecosystem functioning.

DEER IMPACT AND PLANT RESISTANCE TRAITS

by
Xueyang Fan

A Thesis
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APPROVAL PAGE

DEER IMPACT AND PLANT RESISTANCE TRAITS

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DEDICATION

没有花，微风就无色无形；没有风，花瓣就不会飞舞；花的重量，就是风的归宿。

将我的成果献给我的家人、来自过去的“我”，以及这整个世界。

I dedicate this work to my family, and the “me” of the past, and the Earth.

在一种力量的指引下，如同漂浮在半空中，我们乘着宇宙的风。

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Last but not least, I would like to thank my family and all my friends. Thanks for their faith in me and allowing me to be as ambitious as I wanted. It was under their watchful eye that I gained so much drive and an ability to tackle challenges head on.

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CHAPTER 1

INTRODUCTION

1.1 Generalist Herbivores

Generalist herbivores, especially ungulates that interact strongly with plants via foraging, are recognized to have profound impacts on individual plants, communities and ecosystems (reviewed by Côté et al. 2004).

Among different ungulates, white-tailed deer (*Odocoileus virginianus*) are adaptable and prolific animals. They can reproduce rapidly under suitable conditions (Waller & Alverson 1997). Forest harvestings and extended habitats provide deer with suitable environmental conditions (Diefenbach et al. 1997). Furthermore, human hunting behavior and natural predators have both been reduced across Europe and North America (McShea et al. 1997). Therefore, although overexploitation in the second half of the 19th century reduced the number and range of white-tailed deer, the population of white-tailed deer in the Eastern United States increased greatly to historically high densities during the 20th century (deCalesta & Stout 1997; Binkley et al. 2006; Estes et al. 2011; Karr et al. 1992; Kielland & Bryant 1998; Williams et al. 2004). Late-20th century estimates of density range from 7.7 to 14.8 deer/km^2 in heavily forested areas (deCalesta and Stout 1997)

As a generalist herbivore, white-tailed deer can feed on various food sources. This adaptability is the main explanation for the overabundance of deer. In different seasons, white-tailed can feed on herbaceous plants, fallen fruits and seedlings of woody plants. It makes this deer species an important species in forest ecosystems that can affect forests widely by their browsing behavior (Côté et al. 2004; Hewitt 2011).

The direct and indirect impacts of the overabundance of white-tailed deer on individual plants and on forest ecosystem functioning are strong, causing many researchers and foresters to consider white-tailed a keystone species (McShea & Rappole 1992; Rooney 2001).

On one side, herbivores can affect individual plants by altering their traits. Herbivores' foraging can stimulate plants to produce induced self-protection traits (Agrawal 2011; Côté et al. 2004). Through herbivore selective browsing behavior, natural selection can choose plants that have higher resistance traits (Duncan et al. 2001). On the other side, the environment and community can be changed by overabundance of herbivores. The species that is preferred by herbivores can be decreased more than other species. Competitive relationships and nutrient cycling can also be disturbed by herbivores. The strength of these mechanisms can be very widely affected by herbivore density (Côté et al. 2004).

1.2 Plant Resistance Traits

The development of resistance traits is one of the important mechanisms for plants to reduce herbivore impacts. For example, chemical defense and morphological defense are resistance traits developed in plants. Leaf toughness and wood density can become barriers to allow plants to escape from herbivores, especially in the seedling period.

There are several ways that the browsing of overabundant herbivores can affect plant resistance traits. Initially, plant defense traits can be favored by natural selection through pressure of herbivores' predation.

Plants that have a high level of resistance traits can have more chances to escape from herbivores. Plants that have lower digestible content or higher morphological defenses can avoid the natural selection (Côté et al. 2004). Individual plants with stronger resistance traits will thus have higher fitness. This type of trait can be heritable and thus can evolve over time in populations exposed to chronic herbivore pressure.

Another pathway for herbivores to change traits of plants is that some plants can generate resistance traits in response to damage from herbivores in a short time period after being attacked. These induced responses to herbivory may subside if and when herbivory activity subsides, or they may remain for the life of the individual (Mithen,Raybould & Giamoustaris 1995; Agrawal, Gorski & Tallamy1999; Lankau 2007; Bidart-Bouzat & Kliebenstein 2008). Although the ability to respond plastically to herbivory may be heritable, the trait state itself will not be directly heritable. For example, resistance traits can be reduced after plants grow beyond the range of herbivore browsing (Bryant & Raffa 1995).

Finally, herbivores can affect plant traits indirectly. For example, the overabundance of certain herbivores species can interrupt the balance of food net by competing with other species or reduce other plant species. It can affect some traits of plants that make them more competitive (Stewart 2001; Baines et al. 1994). Herbivores' abundance can also disturb their habitat by altering vegetation structure or nutrient cycling, which can also change the plant traits, especially the trait involved in both nutrient and defense function. For example, canopies of trees in forest are affected by herbivores and change the growth of understory (McInnes et al. 1992). Other researches showed that white-tailed deer can affect tree seedlings by favoring grasses, ferns and other species that are related to seedlings (Stromayer & Warren 1997).

1.3 Impact of Plant Resistance Traits on Ecosystem Functioning

Resistance traits of plants are not only related to deer browsing but also related to their photosynthetic efficiency which is a critical parameter in ecological research. Previous experiments indicated that the photosynthesis efficiency was reduced with the increasing plant resistance responses. For example, research showed that low LDMC (leaf dry mass content) of leaves of domesticated cassava translated into higher water content. It suggests that they had higher water-soluble photosynthetic enzymes per unit dry mass, which could contribute to their higher photosynthetic rate per unit dry mass (Beno î et al. 2008). Other research indicated that high LMA (leaf mass per area) was associated with more structural tissue, lower mass-based chlorophyll and nitrogen concentrations, and lower mass-based photosynthesis (Foteini et al. 2010).

However, less research has investigated general changes in resistance trait of plants in forest under high herbivore pressure. The present experiment examined whether the overabundance of white-tailed deer, one of the most common and significant generalist herbivores in North America, can change the resistance traits of abundant woody plant species generally.

1.4 Hypotheses

Much prior research has shown that generalist herbivores can alter the relative abundance of plant species within communities (McShea & Rappole 1992; Rooney 2001; Horsley et al. 2003; Côté et al. 2004), and in doing so alter average resistance traits at the community level. The goal of the present research is to determine whether chronic high densities of can, in addition, alter resistance traits *within* species. To do so, the current research took advantage of a long-standing experiment established in 1979 by Horsley and colleagues (Horsley et al. 2003) that exposed an entire generation of trees to chronic high deer densities during tree establishment. Now adults, these trees are the focus of the present research. The core hypothesis is that chronic high densities of white-tailed deer can alter herbivore resistance traits within the dominant tree species in a forest community.

CHAPTER 2

METHODS

2.1 Study Sites and Deer Treatment

The study site and deer treatment were done by previous experiment. The present sample collection and resistance trait measurement are based on those treatment.

In 1979, Horsely and colleagues (Horsely et al. 2003) established an experiment at Kane experimental forest in northwestern and north-central Pennsylvania. It was located at four sites in the experimental forest. The landscape is dominated by contiguous forest without interspersed agricultural land. Annual precipitation averages 1067 mm with 550 mm received during the growing season. The climate in this area is humid with an average daily temperature of 9 °C (Cronce & Ciolkosz 1983). The four experimental sites include: Wildwood Tower (WW, 41°34'22"N, 78°28'30" W), located at 710 m elevation in Elk County; Fools Creek (FC, 41°38'48" N, 79°08'11" W) and Deadman Corners (DM, 41°34'40" N, 79°06'19" W), both at 550 m elevation, are respectively located in Warren and Forest County; State Game Land 30, GL (41°38'21" N, 78°19'33" W), at 670 m elevation, located in McKean County (Horsley 2003).

Among those four sites, the forest canopy is dominated by black cherry (*Prunus serotina*), red maple (*Acer rubrum*), American beech (*Fagus grandifolia*), pin cherry (*Prinus pennsylvanica*), and birch (*Betula lenta* and *Betula lutea*). In addition, small amounts of white ash (*Fraxinus americana*), eastern hemlock (*Tsuga canadensis*) and other trees species are present.

In previous treatment, 10% of each experimental site was clear-cut and 30% of each experimental site was thinned following the method (Horsley et al. 2003). At each site, a 65-ha exclosure was constructed using 2.4-m woven wire fencing. Exclosures were divided into three 13-ha areas and one 26-ha area. Wild deer were driven from each deer density area, once after fence erection and again after logging, to insure that all wild deer were removed. Captive-raised yearling female deer were placed into these exclosures according to the plan: one deer in 26 ha = 3.8 deer/ km^2; one deer in 13 ha = 7.7 deer/km^2; two deer in 13 ha = 15.4 deer/km^2; four deer in 13 ha = 30.9 deer/ km^2; hereafter these densities are described as 4, 8, 15, and 25 deer/km2. Deer were radio-collared and replaced immediately upon death or escape. Clear cutting and exclosure building were done between 1979 and 1980.

In the present experiment, samples were collected only from the lowest and highest deer density areas. Moreover, all the samples were collected from trees that established after the clearcut and grew to through the sapling stage while the deer treatments were in place. Consequently, all the trees sampled for the present experiment grew through and survived the deer impact imposed by the experimental exclosure treatment.

2.2 Resistance Traits Measurement

To investigate the general impact of herbivores on plant resistance traits, the most common tree species in the forest were sampled for a suite of traits that have been show to confer resistance to herbivores. In addition, sampling the most dominant trees species provided enough sample size to provide sufficient statistical power. Then, according to survey of tree species abundance by Forest Service of U.S., black cherry (*Prunus serotina*) red maple (*Acer rubrum*), american beech (*Fagus grandifolia*), pin cherry (*Prinus pennsylvanica*), and sweet birch (*Betula lenta*) were chosen in the project. The collection and measurement of herbivore resistance traits followed the methods described by Cornelissen and colleagues (2003).

For each deer density (2) and each site (4) per species (5), ten individuals were collected. So in total, there are 400 individual trees included in the experiment theoretically. Because there were not 10 individuals to be found in some treatment X species X site combinations, samples were in fact collected from 325 trees in the field. Samples were collected in July and August 2012 in collaboration with Alex Royo of the Northeast Experiment Station, USDA Forest Service with assistance from his field crew.

2.2.1 Leaf Toughness

Leaf mass per area (LMA) and leaf dry matter content (LDMC) are considered as two physical traits that are related to the modulus of elasticity and leaf toughness. Thus, these two leaf traits were analyzed as characters that influence browsing preference by deer.

To collect samples (following method of Cornelissen et al. 2003), relative young but fully expanded and hardened leaves without obvious symptoms of pathogen or herbivore attack and without substantial cover of epiphylls were collected from two separate twigs of each tree that were most exposed to direct sunlight during the daytime. Samples were collected by shotgun sampling. The total twigs with leaves were rehydrated in a dark space over night until being measured.

For the measurement of LMA, an appropriate number of leaves, based on the size of leaves, from each twig were scanned by a flat-bed scanner after being rehydrated. The total leaf area of each twig was calculated by using Mathematica (Wolfram Research). The fresh weight was measured immediately after the scanning. For each twig, LMA value was calculated by dividing fresh weight of leaves by their area. The LMA value of each individual tree is the mean LMA of the two twigs of each tree.

For the measurement of LDMC, the same leaves from LMA measurement were used. After the fresh weights and area measurement, leaves were removed into oven to be dried. These leaves were dried at 60 ^{o}C for 72 hours and measured for dry mass. LDMC value was calculated by dividing dry mass by fresh mass for each twig. And the LDMC for each tree was the mean value of the two twigs in one tree.

2.2.2 Wood Density

One wood sample was collected at 1.4 m height from every sampled tree with an increment borer (5.15 mm diameter extractor). The length of each wood core was measured immediately in the field. Wood cores were dried at 60 ° C for 72 hours and weighed after drying. The volume of wood core was calculated by using the length of core and wood core diameter. The value of wood density was calculated by dividing dry mass by wood core volume.

2.2.3 Carbon: Nitrogen Ratio (C: N)

Low N content and high C: N ratio in leaves indicates lower quality food for herbivores (Cornelissen et al. 2003), and as such can affect herbivores’ choice of plants. The analysis of C: N were conducted by using Costech Analytical Elemental Combustion System 4010 (ECS 4010, Costech Analytical Technologies, Valencia, CA). Leaves were first ground to 40 meshes in a Wiley Mill (Thomas Scientific, Swedesboro, NJ). In this analysis, the leaf sample is broken down into its elemental components. Then the samples enter the Gas Chromatography (GC) separation column and detected by the Thermal Conducttivety Detector (TCD). At last, the ECS software compares the elemental peak to known standard material. Data then was generated for each element on a weight basis and was converted to percent of sample.

2.3 Statistical Analysis

To test the general hypothesis that the deer density treatment affected plant resistance traits, linear mixed models were applied, treating deer density as a fixed effect and both species and site as random effects. This approach tests the hypothesis that deer density affects traits generally without respect to specific sites or specific species. The models were implemented by using restricted maximum likelihood in the lme4 package (Bates & Maechler, 2009) in R (R Development Core Team, 2009). Residuals were visually inspected for normality and homoscedasticity, and response variables were log-transformed as appropriate. The formulation of the model is:

$$\begin{aligned}\text{Variable} \sim \text{Fixed effect}_1 + \text{Fixed effect}_2 + \ldots + \text{Fixed effect}_n \\ + \text{Random effect}_1 + \text{Random effect}_2 + \ldots + \text{Random effect}_n\end{aligned} \tag{2.1}$$

To identify the models with random effects that best fit the data, the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used for model comparison. The model with the lowest AIC or BIC number is the best fitted model. The reason is that the formula of AIC is:

$$\text{AIC}=-2\text{Loglik}+2N \tag{2.2}$$

Where Loglik is the logged likelihood value, and N is the number of parameters in the model. AIC balances the explanatory power of the model against the number of parameters in the model. The model with the lowest AIC indicates is considered the most parsimonious.

In addition, to test the hypothesis that deer density significantly affects the trait in question, Markov Chain Monte Carlo (MCMC) methods were used to calculate p-values for the fixed effect (Bates & Maechler, 2009).

CHAPTER 3

RESULTS

3.1 Data Distribution

In the experiment, the distribution of trees was unbalanced in some sites because some species were rare in some site by treatment combinations and consequently less than 10 individuals could be located. American Beech and the Red Maple were hard to find at the Game Land site. American Beech was also rare at the Wild Wood site. Besides, Red Maples and Sweet Birches were not found in high deer density zone at the Wild Wood site. However, because species and sites are random effects in the analysis and the lme4 package in R does not require balanced data to do the analysis, the data still can be analyzed through this approach (Bates & Maechler, 2009).

For wood density, 175 and 145 individuals were sampled from low and high deer density crossing all four sites and five species. For LDMC, LMA, and C: N ratio, 175 and 148 measurements were done in low and high deer density, respectively.

Preliminary boxplot (Figures 3.1 to 3.4) and plots with standard error bars (Figures 3.5 to 3.8) showed that for each species, only LDMC showed a slight generally increase trend from low deer density to high deer density.

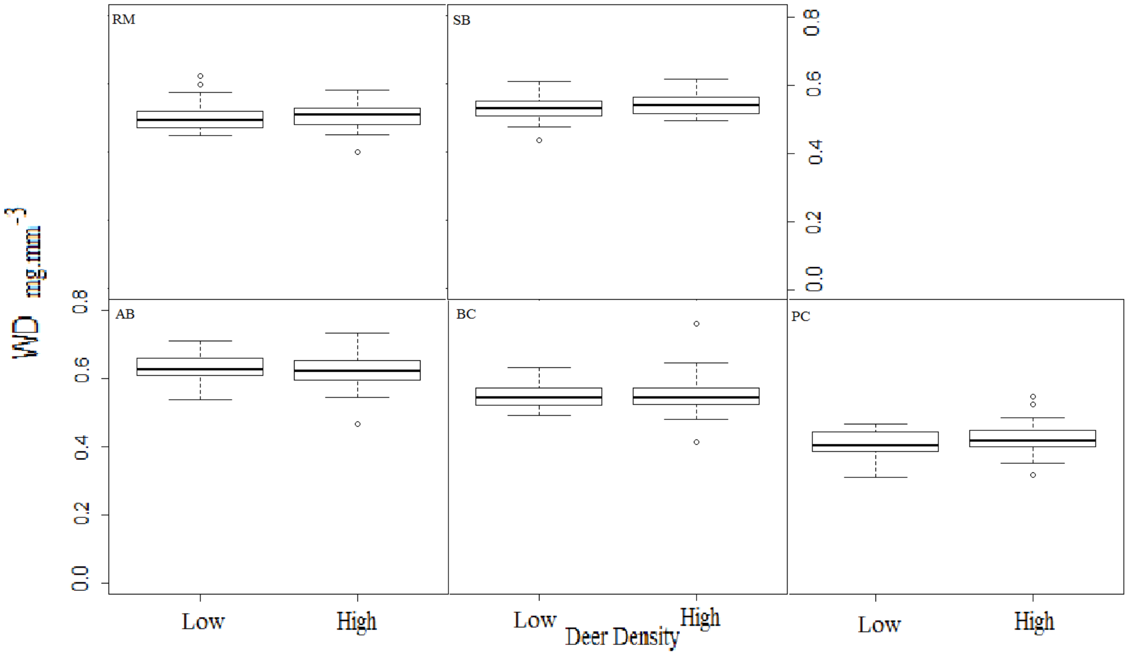

Figure 3.1 Boxplot of Wood Density (WD) data among different species (AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities.

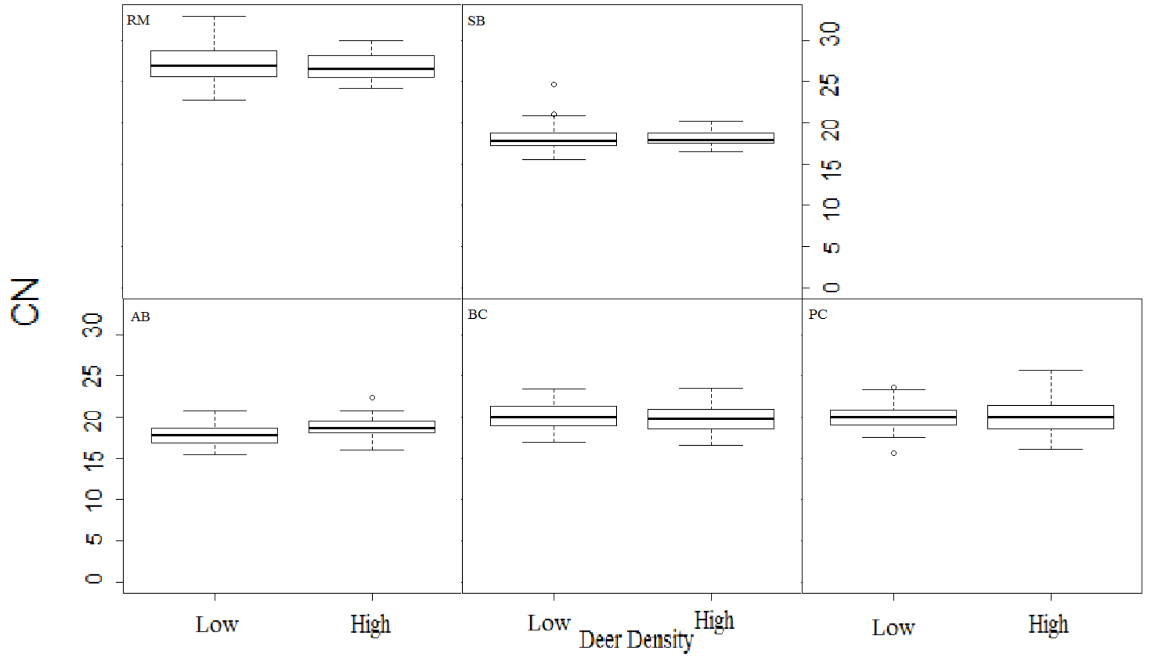

Figure 3.2 Boxplot of C:N ratio (CN) data among different species (AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities.

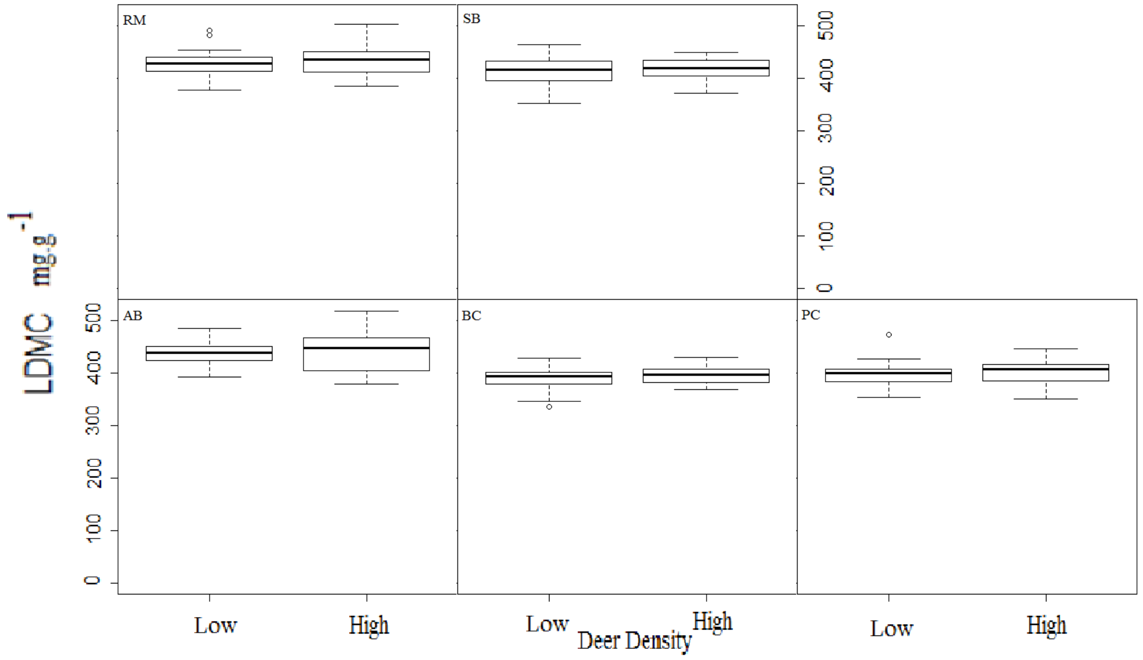

Figure 3.3 Boxplot of Leaf Dry Matter Content (LDMC) data among different species (AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities.

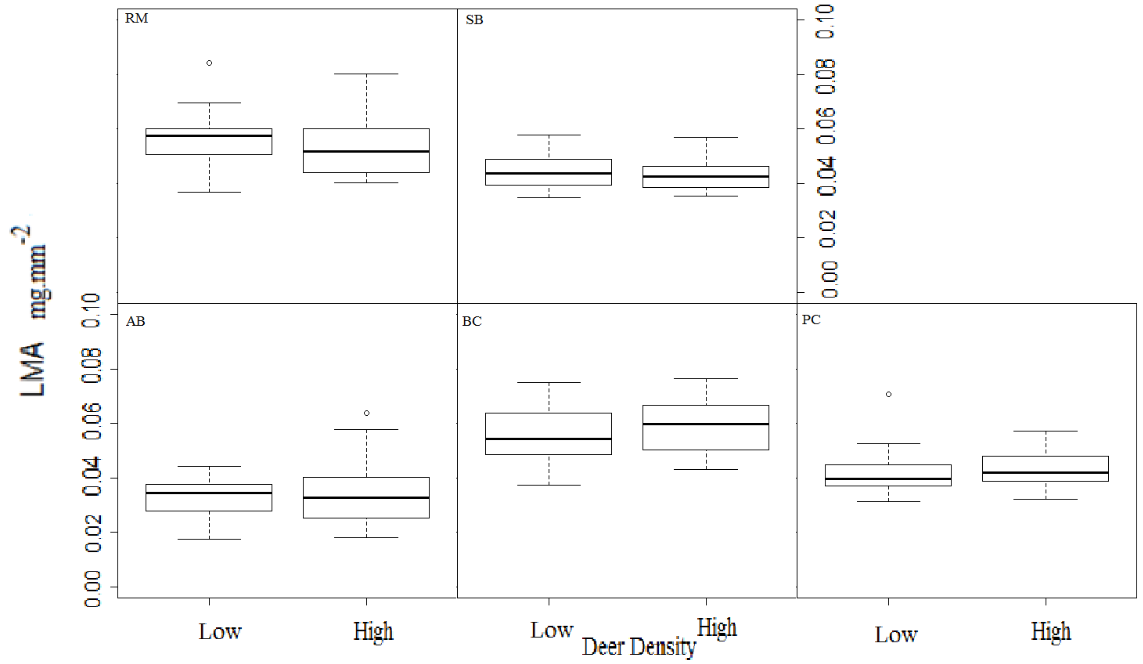

Figure 3.4 Boxplot of Leaf Mass per Area (LMA) data among different species (AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities.

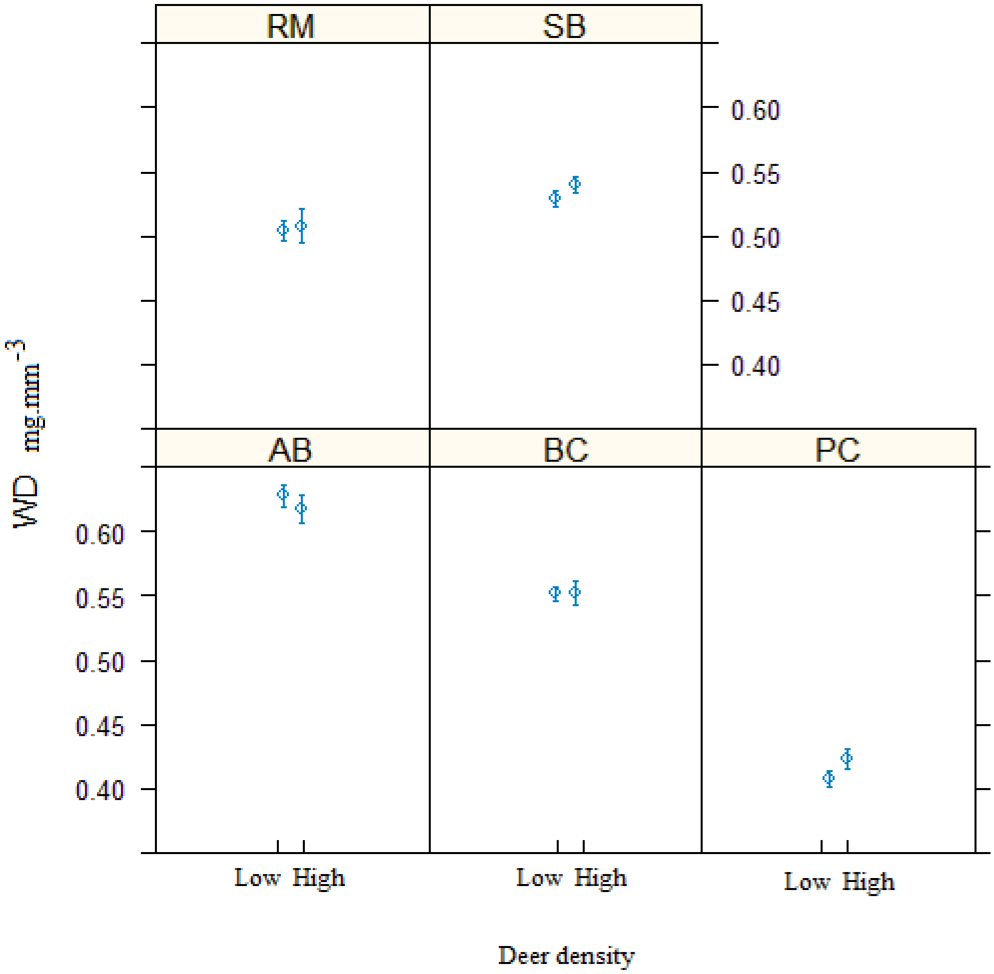

Figure 3.5 Wood Density (WD) changes among different species (AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities with standard error bar.

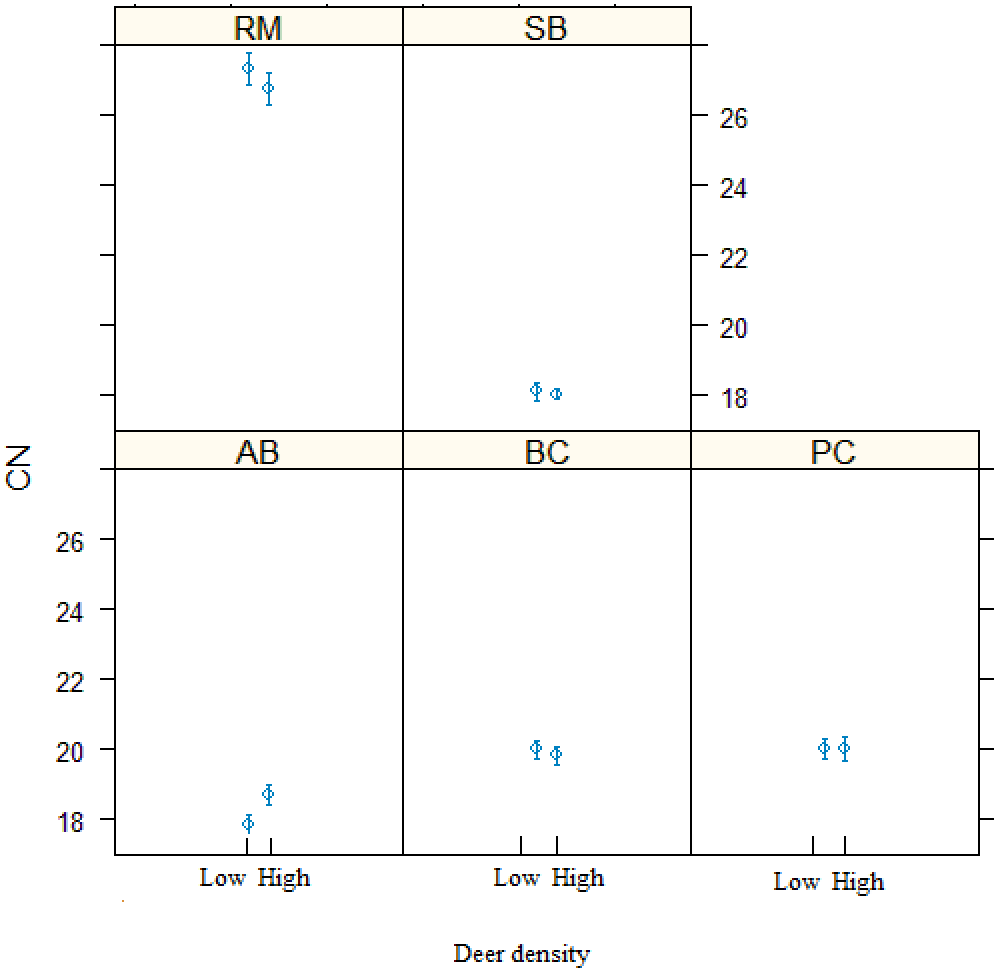

Figure 3.6 Carbon: Nitrogen ratio (C: N) changes among different species(AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities with standard error bar.

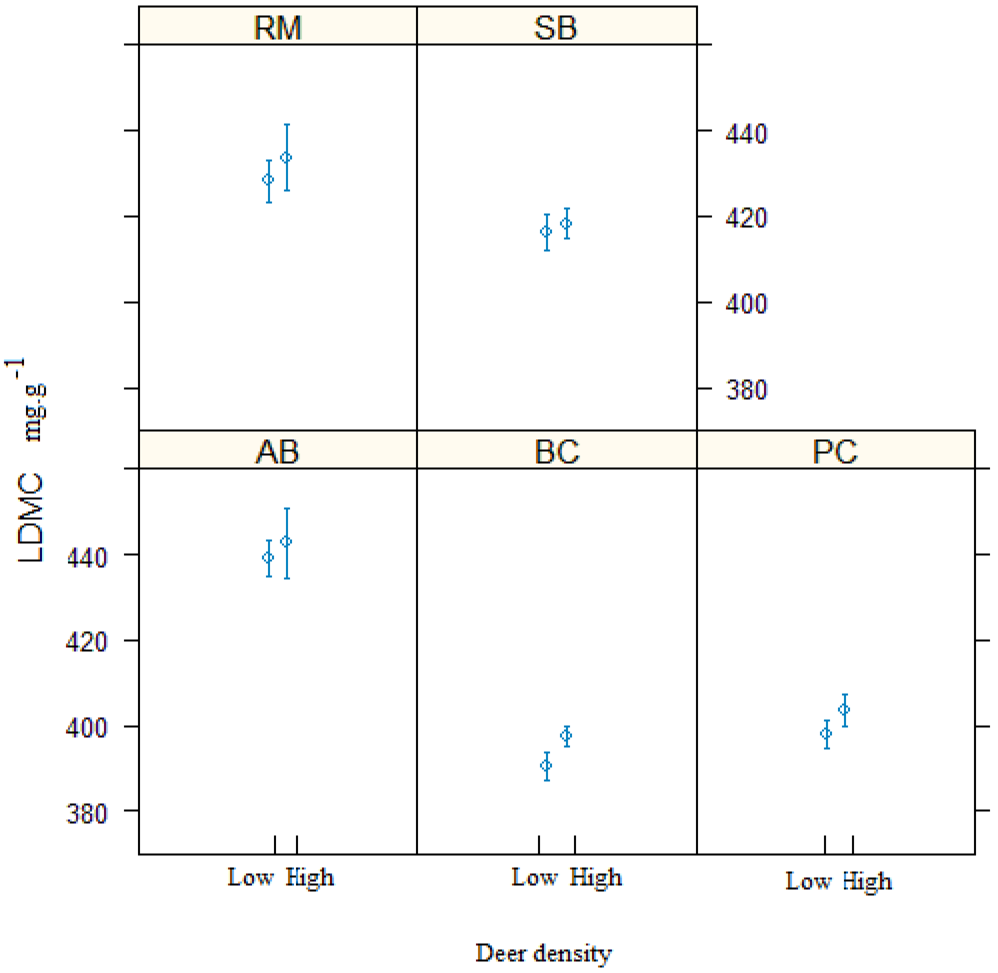

Figure 3.7 Leaf Dry Matter Content (LDMC) changes among different species(AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities with standard error bar.

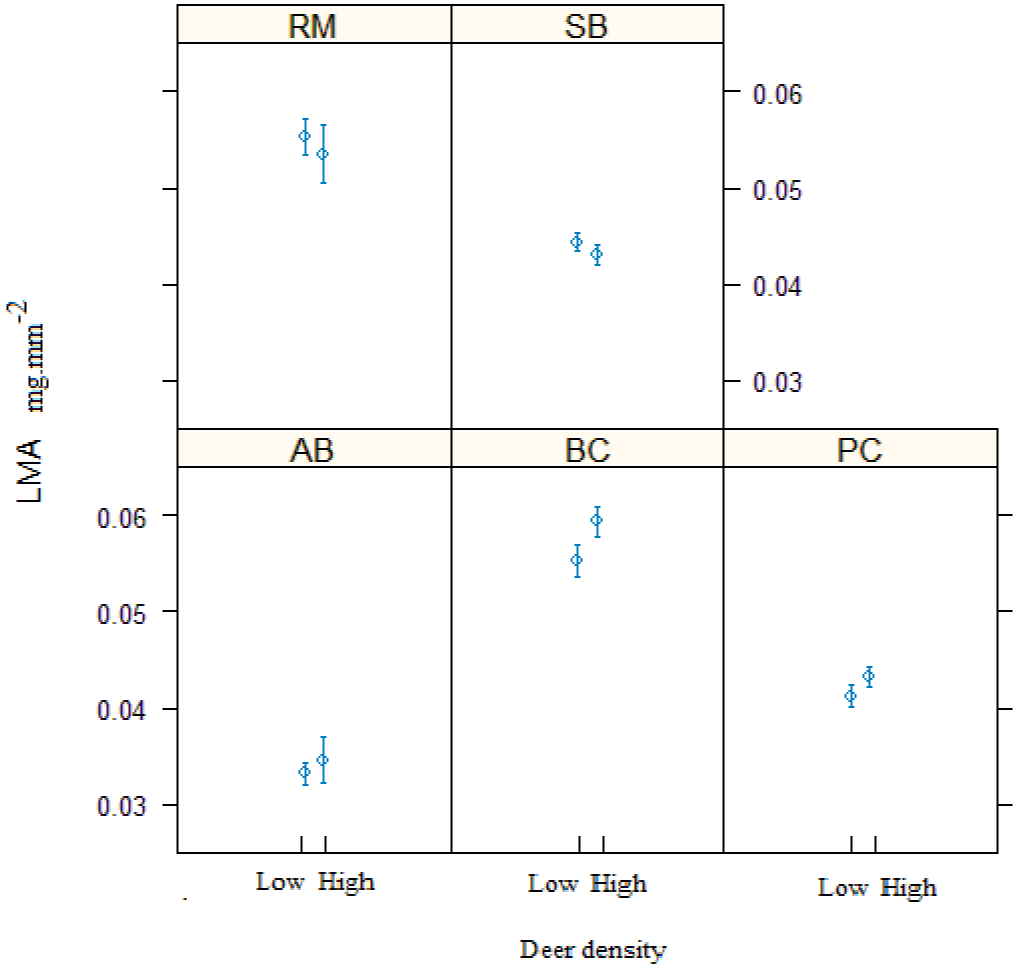

Figure 3.8 Leaf Mass per Area (LMA) changes among different species(AB-American Beech; BC-Black Cherry; PC-Pin Cherry; RM-Red Maple; SB-Sweet Birch) under low and high deer densities with standard error bar.

The assumptions of normality and homoscedasticity were assessed by plotting the residuals against the fitted values. All response variables were log-transformed to meet the assumptions of normality and homoscedasticity. The plot shows that there is a normal distribution for each logged trait value. It means the Mixed-effect model can be used in the analysis which can showed the impact of deer density to resistance traits with the effect of sites and species (Bates et al. 2008).

3.2 Model Fitting

The Mixed-effect model is used to analyze the relationship of the deer density and the resistance trait of plants which can not only do the linear system analysis but also consider the effect of some random effects which are not the main variable but can also affect the analysis (such as the site difference and the species difference in the experiment). Among different models, the best simplified model which has the lowest AIC value (Tables 3.1 to 3.4) is:

$$\text{Trait} \sim \text{Deer density} + (1|\text{species}) + (1|\text{site}) \qquad (3.1)$$

It means the deer density effect on plant resistance traits will be checked. Species and site effects will also be considered as a random effect in the analysis. In other words, this model can show the general impact of deer on plants (Bates et al. 2008).

Table 3.1 Models of Wood Density (WD) and AIC (Akaike Information Criterion) Comparison

Model	AIC
WD~dd+sp+sp*dd+(1|sp)	-973.1
WD~dd+sp+(1|site)	-1020
WD~dd+(1|sp)	-1062
WD~dd+(1|site)	-684.8
WD~dd+(1|sp)+(1|site)	-1064

Table 3.2 Models of C:N and AIC (Akaike Information Criterion) Comparison

Model	AIC
C:N ~dd+sp+sp*dd+(1|sp)	-604.1
C:N ~dd+sp+(1|site)	-628.5
C:N ~dd+(1|sp)	-633.7
C:N ~dd+(1|site)	-279.5
C:N ~dd+(1|sp)+(1|site)	-639

Table 3.3 Models of LDMC and AIC (Akaike Information Criterion) Comparison

Model	AIC
LDMC ~dd+sp+sp*dd+(1|sp)	-856.3
LDMC ~dd+sp+(1|site)	-888.5
LDMC ~dd+(1|sp)	-898.8
LDMC ~dd+(1|site)	-784.1
LDMC ~dd+(1|sp)+(1|site)	-905.8

Table 3.4 Models of LMA and AIC (Akaike Information Criterion) Comparison

Model	AIC
LMA~dd+sp+sp*dd+(1|sp)	-136.8
LMA~dd+sp+(1|site)	-157.6
LMA~dd+(1|sp)	-140.6
LMA~dd+(1|site)	49.28
LMA~dd+(1|sp)+(1|site)	-162.9

3.3 Deer Impact

The result showed that LMA, C: N ratio and Wood density do not have significant change between low and high deer density. Nevertheless, result of LDMC showed a slight but significant increase in high deer density area (P=0.0476). (Table 3.5)

Table 3.5 Mean Values and MCMC p-values of Each Trait

Traits	MCMC P-value
Wood Density ($mg.mm^{-3}$)	0.3646
C:N Ratio	0.8834
LMA ($mg.mm^{-2}$)	0.1338
LDMC ($mg.g^{-1}$)	0.0474

CHAPTER 4

DISCUSSION OF RESULTS

The result of this experiment indicates a small but significant relationship between deer activity and plant resistance traits. Leaf dry matter content increased roughly 1%, consistently across all five species (Figure 3.3). The increase in LDMC indicates that the plants that grew up under high deer density have more tissue and have less water in their leaves compared to individuals that grew up exposed to low deer density. This result could be caused by a three distinct mechanisms, discussed below, and which will be the focus of future research. Regardless of the mechanism, these results indicate that chronic high levels of deer herbivores can alter ecosystem functioning both by changing species composition, as was previously known (Côté et al. 2004), but also by changing the traits of species themselves.

These results could be produced by three distinct mechanisms, two of which are direct effects of deer on plants and the third of which is indirect. Each of these mechanisms could be further investigated and tested by additional research.

Herbivore browsing can directly affect the average trait values of a population if deer preferentially browse individuals that have poor resistance traits, thereby decreasing the growth rates and survival of these individuals relative to those individuals with stronger resistance traits. It is important to recognize that the present experiment examined traits of individuals from only a single generation, so no inference regarding heritability and thus, evolution can be made from the data in hand. To test this mechanism, one could grow offspring from individuals sampled from each dear density treatment, and also from the individuals of the surrounding forest that has not been exposed to the deer treatments, as seedlings in a common garden. This natural selection hypothesis predicts that offspring from the deer density treatments will show significantly different resistance trait values, and also that resistance traits will have lower variability among the deer treatments compared to resistance traits of offspring from the larger, unselected forest population. One would need to confirm that maternal effects are not responsible for any differences found.

The second possible mechanism is that intense deer browsing in the high deer density treatment induced responses in individual trees causing them to increase their LDMC. Induction of herbivore resistance traits is a well known phenomenon that occurs in many species and traits (need citations here). While possible, this mechanism is unlikely for two key reasons. First, deer herbivores ceased for these individual trees more than 20 years ago when they grew to heights beyond which deer can reach (~2 m). The deer treatments themselves were also discontinued at the same time. Thus the individuals in question have not been exposed to browsing by deer for a long period of time. Second, unlike some inducible defenses, induction of changes in leaf dry matter content should be fully reversible, as these deciduous trees replace their leaves every year. If it is adaptive to induce higher LDMC in response to deer herbivores, then it should be adaptive to reverse the effect after individuals have grown to heights beyond which deer and other ground-dwelling mammals can reach. Importantly, there are no known browsers which browse the canopies of forest trees in Northern temperate forests. Even though the observed response of increased leaf dry matter content to increased deer density, is unlikely to be due to induced defenses 20 years after herbivores ceased, this possibility cannot be ruled out with the present data. In fact, this mechanism could be eliminated if either 1) LDMC could be experimentally shown to be not inducible, or 2) if induction of increased LDMC could be shown to be reversible. Both of these hypotheses could be tested in a common garden experiment with controlled applications of simulated (or real) herbivores.

The third possible mechanism driving the present results could be due to indirect effects of deer on plant traits via effects on the environment. For example, it is possible that increased deer density could have reduced tree density in the resulting stands, and that this decrease in tree density could increase soil moisture availability, and that this increased soil moisture availability could then decrease LDMC. However, this would predict the opposite result from that found here, where LDMC increased with deer density. In addition, actual tree density was not significantly different among the deer density treatments 10 years after the experiment was implemented, though there was a trend towards lower tree density in the high deer density treatments (Horsley et al. 2003). Another possible indirect effect via environment is that deer could increase nutrient cycling in the high deer density treatment, though it is not clear why this would affect LDMC. While there are many possible mechanisms one could imagine by which deer density could affect plant traits via environmental effects, these mechanisms are not parsimonious. The best approach to rule out such mechanisms would be to explicitly test the direct mechanisms via the experiments described above.

Of these various potential mechanisms of deer impacts on plant species resistance traits in the present experiment, natural selection is the most parsimonious and the most likely. Nevertheless, the present data cannot definitively distinguish amongst these mechanisms. Further observation and experiment will be required to determine the specific mechanism responsible.

In any case, the results presented here show that chronic high levels of deer activity can cause intraspecific shifts in resistance traits. Previous research has shown that deer can change the relative abundance of species and thereby alter ecosystem functioning that is important to human wellbeing such as net primary productivity and carbon sequestration. The results presented here show that ecosystem functioning may additionally be altered by changes within species themselves, and accordingly that efforts to predict the effects of changes in herbivore abundance on ecosystem functioning cannot simply account for changes in species composition.

REFERENCES

Agrawal, A.A. 2011. “Current trends in the evolutionary ecology of plant defence.” *Functional Ecology*, 25: 420-432.

Agrawal, A.A., Gorski, P.M. & Tallamy, D.W. 1999. “Polymorphism in plant defense against herbivory: constitutive and induced resistance in Cucumis sativus.” *Journal of Chemical Ecology* 25: 2285-2304.

Baayen, R.H. 2011. “languageR: Data sets and functions with Analyzing Linguistic Data: A practical introduction to statistics.” http://cran.r-project.org/web/packages/languageR/index.html.

Baines, D., Sage, R.B., Baines, M.M., 1994. “The implications of red deer grazing to ground vegetation and invertebrate community structure of Scottish native pinewoods.” *Journal of Applied Ecology*, 31: 776-783.

Bates, D., & Maechler, M. 2009. “lme4: Linear mixed-effects models using S4 classes.” http://cran.r-project.org/web/packages/lme4/index.html

Benoît, P., Michele, B., Sandie, B., & Doyle, M.2008. “Photosynthesis and Leaf Structure in Domesticated Cassava and a Close Wild Relative: Have Leaf Photosynthetic Parameters Evolved Under Domestication?” *Biotropica*, 40: 305-312.

Bidart-Bouzat, M.G. & Kliebenstein, D.J. 2008. “Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in Arabidopsis thaliana.” *Journal of Chemical Ecology*, 34: 1026-1037.

Binkley, D., Moore, M.M., & Romme, W.H. 2006. “Was Aldo Leopold right about the Kaibab deer herd?” *Ecosystems*, 9: 227-241.

Bryant, J.P., & Raffa, K.F. 1995. *Chemical antiher-bivore defense. In Plant Stems: Physiology and Functional Morphology*. 440. San Diego, CA: Academic.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., & Poorter, H. 2003. “A handbook of protocols for standardised and easy measurement of plant functional traits worldwide.” *Australian Journal of Botany*, 51: 335-380.

Côté, S.E., Rooney, T.P., Tremblay, J.P., Dussault, C., & Waller, D.M. 2004. “Ecological impacts of deer overabundance.” *Annual Review in Ecology, Evolution, and Systematics*, 35: 113-147.

Cronce, R. C., & Ciolkosz, E. J. 1983. *Soils of the Allegheny National Forest Proceedings of the Northeastern Forest Soils Conference*. 8-21. Warren, Pennsylvania, USA.

deCalesta, D.S., & Stout, S.L. 1997. “Relative deer density and sustainability: a conceptual framework for integrating deer management with ecosystem management.” *Wildlife Society Bulletin*, 25: 252-258.

Diefenbach, D.R., Palmer, W.L., & Shope, W.K. 1997. “Attitudes of Pennsylvania sportsmen towards managing white-tailed deer to protect the ecological integrity of forests.” *Wildlife Society Bulletin*, 25: 244-251.

Duncan, A.J., Hartley, S.E., Thurlow, M., Young, S., & Staines, B.W. 2001. “Clonal variation in monoterpene concentrations in Sitka spruce (Picea sitchensis) saplings and its effect on their susceptibility to browsing damage by red deer (Cervus elaphus).” *Forest Ecology and Management*. 148: 259-269.

Estes, J.A., Terborgh, J., Brashares , J.S., Power, M.E., Berger, J., Bond, W.J., & Carpenter, S.R. 2011. Trophic downgrading of planet earth. *Science*, 333: 301-306.

Foteini, H., Michael, R., Martha, L., John, R.E., & Erik, J.V. 2010.”Photosynthesis at an extreme end of the leaf trait spectrum: how does it relate to high leaf dry mass per area and associated structural parameters?” *Journal of Experimental Botany*, 61: 3015-3028.

Hewitt, D.G. 2011. *Biology and management of white-tailed deer*. 87. Boca Raton, FL: CRC press Taylor & Francis Group.

Horsley, S.B., Stout, S.L., & DeCalesta, D.S. 2003. “White-tailed deer impact on the vegetation dynamics of a northern hardwood forest.” *Ecological Applications*, 13: 98-118.

Karr, J.R., Dionne, M., & Schlosser, I. 1992. *Bottom-up versus top-down regulation of vertebrate populations: lessons from birds and fish. Effects of resource distribution on animal-plant interactions.* 244-286. New York, NY: Academic Press.

Kielland, K., & Bryant, J.P. 1998. “Moose herbivory in taiga: effects on biogeochemistry and vegetation dynamics in primary succession.” *Oikos*, 82: 377-383.

Lankau, R.A. 2007. “Specialist and generalist herbivores exert opposing selection on a chemical defense.” *New Phytologist*, 175: 176-184.

McInnes, P.F., Naiman, R.J., Pastor, J., Cohen, Y., 1992. *Effects of moose browsing on the vegetation and litter of the boreal forest, Isle Royale, Michigan, USA. Ecology,* 73: 2059-2075.

McShea, W.J., & Rappole, J.H. 1992. “White-tailed deer as keystone species within forested habitats of Virginia.” *Virginia Journal of Science*, 43: 177-186.

McShea, W.J., Underwood, H.B., & Rappole, J.H. 1997. *The Science of Overabundance: Deer Ecology and Population Management*. 402. Washington, DC: Smithson. Inst. Press.

Mithen, R., Raybould, A.F. & Giamoustaris, A. 1995. “Divergent selection for secondary metabolites between wild populations of Brassica oleracea and its implications for plant-herbivore interactions.” *Heredity*, 75: 472-484.

R Development Core Team 2012. “R: A language and environment for statistical computing.” *R Foundation for Statistical Computing,* Vienna, Austria. http://www.R-project.org/.

Rooney, T.P. 2001. “Deer impacts on forest ecosystems: a North American perspective.” *Forestry*, 74: 201-208.

Stewart, A.J.A., 2001. “The impact of deer on lowland woodland invertebrates: a review of the evidence and priorities for future research.” *Forestry* 74: 259-270.

Stromayer, K.A.K., Warren, R.J., 1997. “Are overabundant deer herds in the eastern United States creating alternate stable states in forest plant communities?” *Wildlife Society Bulletin*, 25: 227-234.

Waller, D.M., & Alverson, W.S. 1997. “The white-tailed deer: a keystone herbivore.” *Wildlife Society Bulletin*, 25: 217-226.

Williams, T.M., Estes, J.A., Doak, D.F., & Springer, A.M. 2004. “Killer appetites: assessing the role of predators in ecological communities.” *Ecology*, 85: 3373-3384.

Wolfram Research, Inc., Mathematica, Version 6.0, 2007, Champaign, IL.