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The impacts of white-tailed deer (Odocoileus virginianus) herbivory on the forage quality of forest vegetation

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THE IMPACTS OF WHITE-TAILED DEER (*Odocoileus virginianus*) HERBIVORY ON THE FORAGE QUALITY OF FOREST VEGETATION

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Jonathan David Becker ENTITILED The impacts of white-tailed deer (*Odocoileus virginianus*) herbivory on the forage quality of forest vegetation BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Becker, Jonathan David. M.S. Department of Biological Sciences, Wright State University, 2017. The impacts of white-tailed deer (*Odocoileus virginianus*) herbivory on the forage quality of forest vegetation.

White-tailed deer (*Odocoileus virginianus*) are abundant across North America. Deer impact ecosystems, both directly and indirectly. These impacts are driven by the foraging preferences of deer. The energy, protein, mineral, fiber, and secondary metabolite content of plants are important factors that inform the selective herbivory of deer. I examined the interactions between forage quality and deer impacts in northern Wisconsin using deer exclosures. I examined the forage quality of four focal species (*Acer saccharum, Maianthemum canadense, Dryopteris intermedia* and *Carex pensylvanica*) in both control and exclosure plots. Forage quality parameters measured were energy, protein, ash, phosphorus, silica, fiber, and saponins. I found that deer herbivory did not uniformly decrease the forage quality within individual species. This study provides preliminary support for a predicted increase in low forage quality plants in response to heavy deer herbivory. Further research is necessary to support this trend, including a focus on defensive secondary metabolites.

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INTRODUCTION

History of deer in North America

White-tailed deer (*Odocoileus virginianus,* hereafter "deer") were abundant (23-34 million) in North America prior to European settlement. The deer population was severely reduced during 1850-1900 to 300,000 – 500,000 individuals. Unregulated market hunting was largely responsible for this reduction in population. At this time, state regulations on deer harvest were not strictly enforced and were ultimately unsuccessful in maintaining a stable population of deer. Eventually, the scarcity of deer reduced the importance of deer products in the marketplace. The Lacey Act of 1900 prevented the sale and interstate transport of wild game, bringing an end to the widespread market hunting of deer. Stricter regulations on deer hunting were put in place in response to reduced market demands and public realization of the scarcity of deer (McCabe and McCabe 1984; McCabe and McCabe 1997). Current deer populations in North America are likely in excess of 28.5 million (Crete 1999). Modern land use changes such as habitat fragmentation, human development, and agricultural practices have contributed to the increasing abundance of deer (Roseberry and Woolf 1998; Waller and Alverson 1997). Extirpation of natural predators such as wolves also played a role in the rebound of deer populations (Callan et al. 2013). Continuing changes to the landscape across North America, recovering wolf populations, climate change, and debate over hunting regulations are factors that continue to shape the deer population in North America.

Deer impacts on ecosystems

Because of their abundance, deer have a large impact on ecosystems. These impacts can be both direct and indirect (Côté et al. 2004; Rooney and Waller 2003). Deer can be considered keystone herbivores, as they exert a disproportionate impact on plant communities and other trophic levels (Waller and Alverson 1997).

Direct impacts

A large body of scientific research has documented the direct impacts of deer on the plants they consume. Aldo Leopold conducted pioneering work in this area, compiling reports of deer damage on crops and forests across the United States (Leopold, Sowls, Spencer 1947). Since Leopold's time, deer numbers have continued to increase along with instances of deer damage to vegetation. Deer consume a large amount of woody plant material, directly impacting individual trees and forest communities. High deer densities have been shown to reduce the development and density of tree species such as red maple (*Acer rubrum*) and American beech (*Fagus grandifolia*). The removal of such species is associated with increases in the grass, sedge, and fern communities in the same area (Horsley, Stout, DeCalesta 2003). Analysis of long term plant community data collected by the US Department of Agriculture indicates that deer density is a major factor in determining the abundance of tree seedlings and composition of the forest understory. These data also suggest that deer density is directly correlated with invasive plant abundance (Russell et al. 2017). Deer exclosure studies have shown deer herbivory

strongly reduces the recruitment of native hardwood trees (Shelton et al. 2014). Deer impacts are also observed in understory forbs such as the genus *Trillium*. High deer densities are associated with smaller plants and lower flowering rates in *Trillium.* High levels of deer browsing observed on species in this genus may result in extirpation from an area if deer density is not reduced (Augustine and Frelich 1998). Heavy browsing pressure on preferred plants may reduce their ability to compete for nutrients. Mycorrhizal activity was depressed significantly by moose (*Alces alces*) browsing in an exclosure study. Since mycorrhizal fungi supply plants with important nutrients, this reduction may decrease the competitive ability of heavily browsed plants (Rossow, Bryant, Kielland 1997).

Long term deer herbivory can result in decreased species richness and diversity in plant communities. This loss in diversity occurs as species poor at regenerating after browsing are extirpated (Begley-Miller et al. 2014; Perea, Girardello, San Miguel 2014). Deer also influence plant communities in ways not indicated by species richness and diversity. Deer herbivory has been shown to result in biotically homogenous plant communities. Such communities largely consist of plants that are unpalatable and tolerant of deer herbivory such as grasses and sedges (Rooney 2009). Communities dominated by such unpalatable plants reflect a legacy of the filtering effect of deer browsing (Begley-Miller et al. 2014). Trends of increasing abundances in grasses and sedges are supported by fifty years of survey data in northern Wisconsin and Michigan (Wiegmann and Waller 2006). These long term changes in forest plant communities appear to be correlated with

local level impacts of deer herbivory, observed using exclosure experiments. This analysis indicates that deer herbivory is one of the most influential factors shaping the composition of forests in this region (Frerker, Sabo, Waller 2014).

Heavy browsing pressure by deer favors plants with herbivory tolerance and/or defense strategies. Browsed plant communities are shown to have significantly higher expression of physical defenses such as spines and thorns (Takada, Asada, Miyashita 2001). Browsed jewelweed (*Impatiens capensis*) communities exhibit increased fruit production as a tolerance response to heavy deer browsing pressure (Martin, Agrawal, Kraft 2015). Such changes in the defense profiles of plant communities alter interactions with deer and other herbivores that interact with these plants (Stinchcombe and Rausher 2001).

Indirect impacts

Deer also impact ecosystems indirectly. Indirect impacts are characterized by one species impacting another organism through one or more mediating species (Strauss 1991). In the case of deer herbivory, this is largely observed in the form of deer impacting plant communities through their herbivory with cascading impacts on other taxa. The direct impacts of deer on ground vegetation cover and plant community composition can lead to a variety of impacts on habitats and other taxa. For example, deer browsing appears to be linked to disruption of pollinator activity. This is driven by deer browsing of plant species associated with specific pollinators. In some species, deer

appear to preferentially browse certain plants with showy flowers, consuming the flower directly (Augustine and Frelich 1998). Such flowers are typically associated with insect pollinated plants. Field exclosure studies support the idea of reduced insect pollinated plants in areas with deer browsing pressure (Rooney 2009). The modification of plant communities has fitness consequences for pollinators as well. Sika deer (*Cervus nippon*) browsing activity is associated with changes in the structure and toxicity of Japanese stinging nettle (*Urtica thunbergiana*). Butterfly larvae have slower development and a lower growth rate when feeding on *U. thunbergiana* leaves obtained from areas with high deer browsing pressure compared to leaves obtained from areas with negligible deer presence (Kohyama et al. 2017). Removal of certain flowering plants by deer herbivory is associated with reduced visitation by pollinators such as the bumblebee (*Bombus*) (Sakata and Yamasaki 2015).

Deer browsing alters the vertical structure of the shrub layer in forests. Low shrubs are particularly targeted by deer along with palatable saplings such as maple (*Acer* spp.) (Meier et al. 2017). Disruption of habitat in this manner impacts fauna that use this niche such as arthropods and small mammals. Deer herbivory has been experimentally shown to result in decreased arthropod activity (Bressette, Beck, Beauchamp 2012). Web-building spiders appear to be particularly sensitive to deer presence, as deer herbivory is associated with removal of anchor points critical for the construction of webs (Roberson et al. 2016). Small mammals appear to significantly prefer the habitat of deer exclosure plots rather than control plots (Shelton et al. 2014).

Indirect impacts driven by deer are not limited to faunal interactions. Deer herbivory pressure is often associated with increased light availability. Deer herbivory can alter soil nutrient levels and arbuscular mycorrhizal fungi activity by compacting the soil and changing decomposition rates. These changes in the environment also impact plant communities that deer do not feed on (Bressette, Beck, Beauchamp 2012; Sabo et al. 2017).

Deer impacts on humans

The direct and indirect impacts of deer on the environment have implications for human activities including agriculture, forestry, and horticulture (Fargione, Curtis, Richmond 1991; Reimoser 2003; Stewart, Mcshea, Piccolo 2007). Abundant deer populations also negatively impact humans by deer-vehicle collisions (Fagerstone and Clay 1997). These combined impacts are difficult to quantify, but have been estimated to cost in excess of two billion dollars annually in the United States (Conover 1997).

Selective browsing behavior of deer

Deer are selective herbivores, preferring to browse high quality forage to maximize their energy intake. This selective behavior is directed by forage availability, competition, seasonality, and dietary needs (Burney and Jacobs 2013). The selective herbivory of deer is informed by a learning model, as individuals correlate the taste, smell, appearance, and post-ingestion feedback with forage types (Bailey et al. 1996).

Profile of preferred forage

Forage quality as it relates to deer is determined by the levels of certain plant constituents. High quality forage can be characterized by high energy, protein, and nutrient content, and low levels of silica, fiber, and defensive secondary metabolites (Belovsky 1981; Berteaux et al. 1998; Lashley et al. 2015; Nisi et al. 2015; Parker et al. 1999 Shimojo and Goto 1989; Van Soest 1981).

Energy

Deer selectively feed on forages high in energy, particularly seeking out such forages during the winter when forage availability is low (Parker et al. 1999). The ability for deer to selectively browse forages high in digestible energy is a trait under strong selection. It is important for deer to maintain a positive energy balance in harsh conditions such as winter and to compensate for the energy cost of lactation (Berteaux et al. 1998; Sadleir 1982).

Protein

Studies of deer herbivory on prairie legumes indicate that deer prefer to feed on species with high nitrogen content (Nisi et al. 2015). Observations of the feeding habits of radiocollared Sitka blacktail deer (*Odocoileus hemionus sitkensis*) indicate that deer prioritize consumption of forage with high available protein (Parker et al. 1999). Although deer prioritize the consumption of protein, multiple feeding studies with deer

and other herbivores suggest that protein intake is regulated in respect to other dietary constraints to avoid excess consumption (Berteaux et al. 1998).

Ash

The ash content of forages refers to a broad measure of the inorganic mineral content of a plant. These minerals include many of dietary nutrients required by deer. A study of the preferred forages of moose (*Alces alces*) found that there may be a relationship between plant nutrient content (measured as ash content combined with protein content) and digestibility. This general relationship between digestibility and nutrient content is supported by feeding experiments with captive deer (Belovsky 1981).

Phosphorus

The level of phosphorus in forage is another important factor in deer forage selection. Observations of deer foraging activities indicate that deer selectively browse plants high in phosphorus. Lashley and colleagues (2015) found that forages selected by deer across a range of plant types had significantly higher phosphorus content relative to non-selected forage.

Silica

The second most abundant element in the earth's crust, silicon, is also an important factor in forage quality. Silica (SiO_2) is found in a number of plants, notably grasses and can serve a structural role in these plants. Silica level in forages is largely

related to silica content of the soils present (Van Soest 1994). *In vitro* studies on the digestion rate of plant material in rumen fluids suggest that high silica content is associated with decreased digestibility of organic materials (Shimojo and Goto 1989; Van Soest 1981).

Fiber

Fiber content of forage is another important component of deer forage selection as fiber represents the largely indigestible portion of the plant material. Although some fiber is necessary for proper digestive function, high fiber forages have lower available energy and require a greater energy input to break down (Van Soest 1994). Red deer (*Cervus elaphus scoticus*) preferentially browse on forages with lower concentrations of fiber, specifically fiber quantified using the acid detergent fiber (ADF) method (Forsyth, Richardson, Menchenton 2005). The lignin content of forages is included in popular measures of fiber such as ADF and neutral detergent fiber (NDF). Lignin is associated with reduced digestion of cellular carbohydrates. Lignin forms complexes with these cellular carbohydrates, protecting up to 1.4 times its weight of carbohydrates from digestion (Van Soest 1981).

Secondary metabolites

Another important aspect of forage quality is the secondary metabolites contained in the plant. There is a wide range of such compounds found in plants, many associated with defense from herbivory. These compounds can be classified into several broad

categories including alkaloids, phenolics, terpenenes, glucosinolates, and cyanogenic glucosides (Bennett and Wallsgrove 1994). Secondary metabolites can be part of a plant's constitutive or induced defensive strategies (Herms and Mattson 1992). When chemical defenses are induced in a plant in response to stress, many plants have the mechanisms to allocate defensive chemicals to target locations (Kaplan et al. 2008). Deer can detect secondary metabolites and select forage to minimize their intake of noxious chemicals. This has been observed experimentally as blacktail deer (*Odocoileus hemionus columbianus*) prefer forages with low monoterpene content (Burney and Jacobs 2012; Kimball, Russell, Ott 2012).

Saponins

Saponins are a category of secondary metabolites found in plants. Saponins create a thick foam when shaken in water, a characteristic that is responsible for their name. Molecularly, saponins consist of an aglycone (either steroidal or triterpenoid) connected to a sugar chain. Saponins have been reported in approximately 100 plant families. This broad group of chemicals has properties ranging from herbivory defense to purported human health benefits (Price, Johnson, Fenwick 1987). Saponins can function in both the induced and constitutive defense systems of plants (Szakiel, Paczkowski, Henry 2011). Saponin defenses affect a wide range of herbivores. Herbivorous insect larvae have poor development when feeding on plant material high in saponins (Agrell et al. 2003). Interactions between large herbivores and saponins have been well-documented as the

important agricultural crop alfalfa (*Medicago sativa*) contains various saponins. Such saponins have been associated with bloat in domestic animals such as sheep. The lethal condition is characterized by a severe reduction in rumen bacteria caused by certain saponins (Lu and Jorgensen 1987). Saponins are also associated with a bitter taste, irritation of the mouth and digestive tract, and decreased absorption of nutrients (Sen, Makkar, Becker 1998). Of particular interest to this study, saponins have been previously reported as present in *Maianthemum canadense* and specific saponins have been identified in other members of the genus (Dickerson 1959; Liu et al. 2012; Sibiga, Sendra, Janeczko 1986)

Nutritional ecology at a landscape scale

Variation in these forage quality factors on a landscape scale is related to the fitness of deer populations. Landscape nutrition models developed for elk (*Cervus elaphus*) indicate that low forage quality is associated with lower pregnancy rates. Disturbances often drive changes in forage quality in an area (Proffitt et al. 2016). Heavy browsing pressure by overabundant deer populations could serve as such a disturbance, reducing the forage quality in a given area.

Research questions

In this study, I examined forage quality parameters of browsed and unbrowsed plant communities to answer two research questions. (1) Does deer herbivory lower the

overall forage quality of a plant community? (2) What forage quality characteristics define plant species preferred by deer?

To test question 1, I compared the gross energy, protein, ash, phosphorus, silica, fiber, and saponin content of four plant species protected from deer browsing to the same four species with active deer browsing. Focal species are *Acer saccharum*, *Maianthemum canadense*, *Carex pensylvanica*, and *Dryopteris intermedia*. I hypothesized that the plants protected from deer browsing will have higher average forage quality, characterized by high energy, protein, and phosphorus content and low silica, fiber, and saponin content. There are two potential explanations that could cause a significant difference in forage quality between these plots. First, deer can exert a filtering effect on the plant communities, selectively removing high quality plants (Begley-Miller et al. 2014). Second, changes in the plant community are possible after release from browsing pressure by deer exclusion. Evidence of such patterns of changes in plant quality after release from herbivory by ungulates has been published regarding the moose population on Isle Royale. In this case, plant basal area and nitrogen availability responded to decreased moose herbivory (De Jager and Pastor 2009).

For question 2, I correlated the forage quality data I collected with plant survey data indicating the abundance of the four focal species in both control and exclosure plots. I predicted that the species significantly more abundant in the exclosure plots relative to the control plots (*Maianthemum canadense* and *Acer saccharum*) will have

higher forage quality than the species with lower evidence of deer browsing impacts (*Carex pensylvanica* and *Dryopteris intermedia*). I expected that the lower abundance of certain species in control plots relative to exclosure plots indicates that such a species is more susceptible to deer browsing and is characterized by high forage quality as defined previously.

METHODS

Study site

We collected the plant samples at a private property located in Vilas County, WI. The 2,500 ha property was purchased in 1925 by Dairymens, Inc. and is managed for recreation by members of Dairymens. This property is marked by a legacy of deer impacts. Classified as a game preserve in 1926, hunting is not permitted on the property. Additionally, a tradition of deer feeding was maintained by members for decades. These factors resulted in remarkably high deer populations on the property. Deer density on the property was sustained in excess of 16 deer/km^2, with concentrations of up to 100 deer/km^2 centered around feeding sites. These densities are far in excess of the carrying capacity of 8 deer/km^2 suggested by the Wisconsin Department of Natural Resources. Deer populations significantly declined on the property in response to a ban on supplemental feeding in 2000 (Rooney 2006; Rooney 2009). Four deer exclosures were constructed on the Dairymen's property in 1990. These exclosures were created with 1.8

meter tall wire mesh fence. The exclosures range in size from 196 m^2 to 720 m^2 (Rooney 2009).

Collection techniques

We collected samples of four focal species (*Acer saccharum, Maianthemum canadense, Dryopteris intermedia,* and *Carex pensylvanica*) from the exclosures and their paired control plots. We collected samples from June 5 to June 9, 2016. We collected samples of at least 20 g of fresh plant material for each species. We conducted random bulk sampling, cutting the terminal 30 cm of larger plants with scissors and collecting the whole plant for smaller plants. We collected twelve replicates for each species in both the control and exclosure plots for a total of 96 samples. We collected replicates as evenly as possible from the four control/exclosure plot pairs as dictated by species abundance. We took care to collect samples representative of the entire area of the plot, moving continuously through the plot, collecting small samples from the plants we encountered. The sampling technique roughly approximated the feeding behavior of deer and avoided a concentration of plant removal. As we collected samples, we added them to plastic bags, and stored completed samples at -20°C.

Energy

I dried the plant samples in an oven at 70°C for 24 hours and ground them using an electric coffee grinder and mortar and pestle. I packed approximately 1 g of dried, ground plant material into two gelatin capsules (Size 0). I loaded these capsules into a

bomb calorimeter (Parr Instrument Company, model 6200). I used four inch ignition thread, and pressurized the bomb with oxygen to 450 psi. I calibrated the calorimeter using 1 g benzoic acid standards at the beginning of each set of samples. In the final calculations, I corrected for the heat of combustion of the fuse, capsules, and nitric acid. I weighed the sample cup after each run to measure the remnant ash content, representing the incombustible portion of the sample. I subtracted ash weight from the sample weight in the final calculations. I calculated gross energy of combustion including the corrections and reported as Joules/gram.

Protein

I measured protein content in the plant samples with the Bradford assay, using a method modified from that of Jones et al. (1989). I combined 0.1 g of frozen plant material with 250 µl of 0.1 M NaOH and ground the plant material for 30 seconds with a small polypropylene pestle in a 1.5 ml microcentrifuge tube. After grinding, I added 750 µl of 0.1 M NaOH to the sample tubes. I agitated the samples using a vortex mixer for 6 seconds and incubated at room temperature for 30 minutes. After the 30 minute extraction period, I vortexed the samples for 6 seconds and centrifuged them for 5 minutes at 12,000 min^{-1}. I removed 250 µl of the supernatant, transferred it to a clean tube and vortexed it for 6 seconds. I prepared Bradford dye reagent (Bio-Rad) by a fivefold dilution with pure water. Additionally, I dissolved 3 mg/ml polyvinylpyrollidone (PVP) in the diluted Bradford reagent. I prepared the colorimetric reaction in a

microplate, mixing 5 µl of the NaOH plant extract with 250 µl of the prepared Bradford dye reagent. I allowed the color to develop for 15 minutes, and measured the absorbance in a spectrophotometer (Molecular Devices SpectraMAX 190) at 595 nm. I used bovine serum albumin as a protein standard, with concentrations ranging from 0.023 – 3 mg/ml. I prepared blank samples using pure water.

This assay was affected by frequent agglutination of the dye-protein complex during the 15 minute color development step. I modified concentrations of the plant extract and ran multiple replicates to minimize this issue. The protein concentrations reported in the results were measured using full strength extract for all species except *D. intermedia*, which had best results at 50% concentration. I corrected for the dilution factor by multiplying by a factor of two. *A. saccharum* had agglutination at multiple concentrations, resulting in three samples from the control plot being unreadable. I found the most consistent results for *A. saccharum* using a full strength extract. I calculated protein concentrations as mg/g by dividing protein content over dry weight of the original sample.

Ash

I determined ash content of the plant samples using a modified version of the Association of Analytical Communities International official method (AOAC International 1995). I dried the plant samples in an oven at 70°C for 24 hours and ground them using an electric coffee grinder and mortar and pestle. I placed 0.5 g of each dried,

ground sample in a pre-weighed porcelain crucible and heated them in a muffle furnace (Barnstead Thermolyne 47900) at 600°C. After 2 hours at this temperature, I removed the crucibles from the furnace, covered them, and allowed them to cool for 5 minutes. I then transferred the crucibles to a desiccator for 15 minutes. After this period, I weighed the crucibles to determine ash content. I calculated ash as % (w/w) by dividing final weight of ash over the weight of the initial oven-dried plant sample.

Phosphorus

I determined phosphorus content (phosphate), using a protocol compiled by Dr. Yvonne Vadeboncoeur, Wright State University. I weighed 4 – 8 mg of dried, ground plant samples into Pyrex vials. I placed these samples into a muffle furnace at 500°C for 1 hour. I included a spinach standard (NIST #1570a) for reference. I added 15 ml of ultrapure water and 3 ml of 1 N HCl to the ashed samples, tightly capped the samples to prevent water loss and placed them in a drying oven at 105°C for 2 hours.

After this digestion step, I added 250 µl of the samples to a microplate along with 50 µl of a color reagent composed of ascorbic acid and an acid molybdate reagent. After allowing the color to develop for 15 min, I measured the absorbance at 880 nm in a spectrophotometer (Biotek Synergy HT). I created a standard curve from the digested spinach standard. I measured phosphorus content of *A. saccharum, M. canadense* and *D. intermedia* with 50% dilutions of the original digested sample as these values were initially too high for the standard curve. I multiplied the resulting measurements by two

to account for this dilution. I calculated phosphorus content as % phosphorus (w/w) by dividing the calculated amount of phosphorus in the samples over the sample weight.

Silica

I determined silica (SiO_2) content in *C. pensylvanica* using a method modified from that of Allen (1989). I dried samples of *C. pensylvanica* in an oven 70°C for 24 hours and ground them using an electric coffee grinder and mortar and pestle. I added 1 g of each dried, ground sample to a weighed porcelain crucible and heated in a muffle furnace at 550°C, following the same ashing procedure listed above. Following ashing, I added 10 ml 50% HCl to the crucibles. I covered the crucibles, and allowed them to simmer gently over medium heat on a hot plate. After 10 minutes of simmering, I removed the covers, allowed the liquid to evaporate, and baked the residue for 15 minutes. I repeated the addition of 10 ml 50% HCl, covered the crucibles and simmered for another 10 minutes. Using a Büchner funnel, I vacuum filtered the contents of each crucible through Whatman 541 filter paper (4.25 cm diameter). I washed the filter paper and residue thoroughly with hot water while filtering. I carefully transferred the filter paper and residue to weighed porcelain crucibles and heated at 550°C for 2 hours. After cooling in a desiccator, I weighed the material remaining in the crucibles. I calculated % silica by dividing the weight of the remaining residue after ashing over the initial weight of the dried, ground plant sample.

Fiber

Because of limited plant material, I pooled replicates for fiber analysis from each species/treatment for a total of eight samples. I sent these samples to be analyzed for fiber content by the Dr. Tamara Johnstone – Yellin lab at Bridgewater College (Bridgewater, VA). They analyzed the samples for acid detergent fiber (ADF) and neutral detergent fiber (NDF).

Saponins

I initially confirmed the presence of saponins in *M. canadense* using a qualitative foaming assay (Edeoga, Okwu, Mbaebie 2005). I extracted 0.5 g of *M. canadense* in 20 ml boiling distilled water for 30 minutes. I centrifuged this solution at 2,000 g for 5 minutes. I decanted the supernatant from the plant material and placed in a clean tube. I shook the tube containing the extract vigorously. The presence of a thick foam persisting for longer than 15 minutes indicates that saponins are present in this sample.

To quantify the saponins present in the sample, I first extracted saponins from *M. canadense* using a method modified from Motz et al. (2015). I removed 5 g of frozen *M. canadense* from storage at -20°C and extracted it in 40 ml 75% methanol at room temperature for 1 week. I then agitated the plant/methanol mixture at room temperature for 24 hours on a rocking platform. I removed the plant debris from the extract by filtering twice through Whatman grade 1 filter paper. I transferred the filtered extract to a rotary evaporator (Buchi Rotavapor RE) and evaporated the methanol at 60°C under

reduced pressure. I washed the remaining residue with 20 ml of distilled water into a 125 ml separation funnel. I purified this extract by adding 40 ml of ethyl acetate to the funnel, preserving the aqueous (bottom) layer. I repeated this process twice, with additions of 20 ml ethyl acetate. I separated the resulting aqueous layer with 20 ml of 1-butanol three times, preserving the 1-butanol (top) layer each time. I combined the 1-butanol layers and air-dried them in a pre-weighed beaker at room temperature for 24 – 48 hours. I increased the speed of evaporation by directing a stream of compressed air over the surface of the solution. I added water equivalent to the weight of the initial fresh weight of the *M. canadense* minus the weight of the dried extract to create a plant strength saponin solution (1:1 ratio of aqueous extract to plant material).

I quantified the saponin content in this solution using a vanillin colorimetric method (Hiai, Oura, Nakajima 1976). I performed this assay in a microplate. I diluted the saponin samples in a 1:3 ratio with pure water. I mixed 20 µl of the saponin extract with 20 µl of an 8 % (w/v) vanillin/ethanol solution and 200 µl of 72% (v/v) sulfuric acid. I kept the plate on ice while the reagents were added. After mixing, I warmed the plate in a 60°C water bath for 10 minutes, and then cooled it on ice for five minutes. I immediately measured the absorbance of the samples in a spectrophotometer (Molecular Devices SpectraMAX 190) at 535 nm. I used commercial saponins extracted from *Quillaja* bark (Sigma, 20-35% sapogenin content) as a standard with concentrations ranging from 0.047 – 6 mg/ml. I prepared blank samples using distilled water. I calculated saponin content as

µg/g *Quillaja* saponin equivalents, dividing saponin content over the fresh weight of the plant samples. I multiplied by a factor of four to account for the dilution.

Maianthemum canadense growth experiment

In order to study induced responses to herbivory in *M. canadense*, I attempted to grow *M. canadense* from seed. The seeds ultimately did not germinate and thus this experiment does not factor into my conclusions. Since the literature on the growth of *M. canadense* is very limited, I list my methods as information for future attempts to grow this plant.

I purchased 169 seeds of *M. canadense* from a small online seed vendor. I stratified these seeds by rolling them in a moist paper towel and storing the paper towel within a zip-top plastic bag. I kept the bagged seeds in a refrigerator (4°C) for 100 days (February 5, 2016 – May 15, 2016). After the stratification period, I planted them approximately 1.5 cm deep in the individual cells of a planting tray. I used a commercially available (Miracle-Gro Nature's Care) potting soil mix consisting primarily of sphagnum peat moss, coir, and perlite. I placed the trays in a greenhouse and checked water daily for 188 days (May 15, 2016 – November 19, 2016). The seeds did not germinate during this time, the majority of recovered seeds were not intact. Further experimentation with stratification and growing conditions are necessary for successful greenhouse growth of *M. canadense*.

Statistical analysis

I compared the results of the assays for energy, protein, ash, and phosphorus using a two factor ANOVA. I determined significance between groups using Tukey's HSD test. I compared the results of the assays for silica and saponin using Welch's t-test. I used an alpha value of 0.05 for these analyses. All analyses were performed in the R statistical computing environment (version 3.3.2).

RESULTS

Energy

I measured the energy content in each collected sample from all focal species for a total of 96 measurements. There was a significant difference between treatment plots, as exclosure plots (M = 19,215.96 J/g) had higher average energy content relative to control plots (M = 19,003.42 J/g), $F(1, 88) = 12.29$, $p < 0.001$. There was also a significant difference between species, $F(3, 88) = 65.30$, $p < 0.001$. There was a significant interaction between deer exclosure and plant species, $F(3, 88) = 4.66$, $p = 0.005$ (Figure 1). Treatment accounted for 3.96% of the variance, while species accounted for 63.16% of the variance.

Post hoc analysis with Tukey's HSD test indicated a significant difference in energy ($p = 0.010$) between control and exclosure communities of *M. canadense*. Exclosure communities were approximately 500 J/g higher in energy than control

communities. No other species significantly differed in energy between control and exclosure plots ($p > 0.05$). *D. intermedia* had the highest energy content ($M = 19{,}840.38$ J/g), significantly higher than the energy content of the other species ($p < 0.05$) (Figure 1).

Protein

I measured protein in each collected sample for the four focal species for a total of 96 measurements. Three measurements were unusable from *A. saccharum* control samples. To preserve equal sample groups, I randomly selected nine measurements from each species/treatment group for an updated total of 72 measurements. There was no significant difference in protein between treatments, $F(1, 0.21) = 0.36$, $p = 0.551$. There was a significant difference in protein between species, $F(3, 11.94) = 6.81$, $p < 0.001$. There was not a significant interaction between treatment and species, $F(3, 4.06) = 2.31$, $p = 0.084$ (Figure 2). Treatment accounted for 0.39% of the variance, while species accounted for 22.26% of the variance.

Post-hoc analysis with Tukey's HSD test indicated that there were no significant differences in protein between control and exclosure plots for each species ($p > 0.05$). Protein measurements between species generally overlapped, but *D. intermedia* ($M = 1.88$ mg/g) had on average over twice the protein content of *A. saccharum* ($M = 0.77$ mg/g) (Figure 2).

Ash

I measured ash content in samples collected from the four focal species, for a total of 96 measurements. Ash content significantly differed between treatment plots, with higher ash content on average in the exclosure plots (M = 6.54 %) relative to the control plots (M = 6.11 %), $F(1, 88) = 8.50$, $p = 0.004$. There was also a significant difference in ash content between species, $F(3, 88) = 158.97$, $p < 0.001$. The interaction between treatment and species was not significant, $F(3, 88) = 0.11$, $p = 0.955$ (Figure 3). Treatment accounted for 1.48% of the variance in ash content, while species accounted for 83.12% of the variance.

Post-hoc analysis by Tukey's HSD test indicated no significant differences between treatment plots for each species. *A. saccharum* had significantly lower ash content (M = 3.72 %) than the other species. The highest average ash content recorded was in *M. canadense* (M = 7.93 %) (Figure 3).

Phosphorus

I measured phosphorus content (phosphate), in samples collected from all four focal species, for a total of 96 measurements. There was no significant difference in phosphorus content between treatment plots, $F(1, 88) = 1.38$, $p = 0.243$. There was a significant difference in phosphorus content between species, $F(3, 88) = 173.59$, $p < 0.001$. The interaction between treatment and species was not significant, $F(3, 88) = 0.02$,

p = 0.055 (Figure 4). Treatment accounted for 0.22% of the variance in phosphorus content, while species accounted for 84.26% of the variance.

Post-hoc analysis with Tukey's HSD test indicated that there was no significant difference between treatment plots for each species ($p > 0.05$). There were no large differences in phosphorus content between *A. saccharum*, *M. canadense,* and *C. pensylvanica*. *D. intermedia* (M = 0.495 %) had approximately twice the average phosphorus content of the other three focal species (M = 0.258 %) (Figure 4).

Silica

Silica content of the 12 samples of *C. pensylvanica* collected from the control plots (M = 2.13 %, SD = 0.83) was not significantly different from the silica content of the 12 samples of *C. pensylvanica* collected from the exclosure plots (M = 2.21 %, SD = 0.92), $t(21.79) = -0.22$, p = 0.832 (Figure 5).

Fiber

Fiber content (NDF and ADF) was measured for the pooled samples collected from the control and exclosure plots for all four focal species. *A. saccharum* from the control plots had lower ADF (24.7%) than that measured in the exclosure plots (30.5%). Conversely, ADF of *D. intermedia* was much higher in the control plots (41.0%) compared to the exclosure plots (32.2%). Both *M. canadense* (control = 27.8%, exclosure

= 26.2%) and *C. pensylvanica* (control = 31.9%, exclosure = 31.5%) had ADF contents that were very similar between treatment plots (Figure 6).

The same general patterns were observed in the more inclusive NDF. *A. saccharum* from control plots had lower NDF (33.2%) than exclosure plots (40.1%). NDF of control *D. intermedia* (51.4%) was much higher than that found in exclosure plots (42.4%). *M. canadense* (control = 35.8%, exclosure = 34.3%) and *C. pensylvanica* (control = 62.17%, exclosure = 60.9%) did not show strong shifts in NDF content between treatment plots (Figure 7).

Saponin

Saponin content of the 12 samples of *M. canadense* collected from the control plots (M = 29.33, SD = 6.01) was not significantly different from the saponin content of the 12 samples of *M. canadense* collected from the exclosure plots (M = 32.25, SD = 4.21), $t(19.69) = -1.38$, $p = 0.183$ (Figure 8).

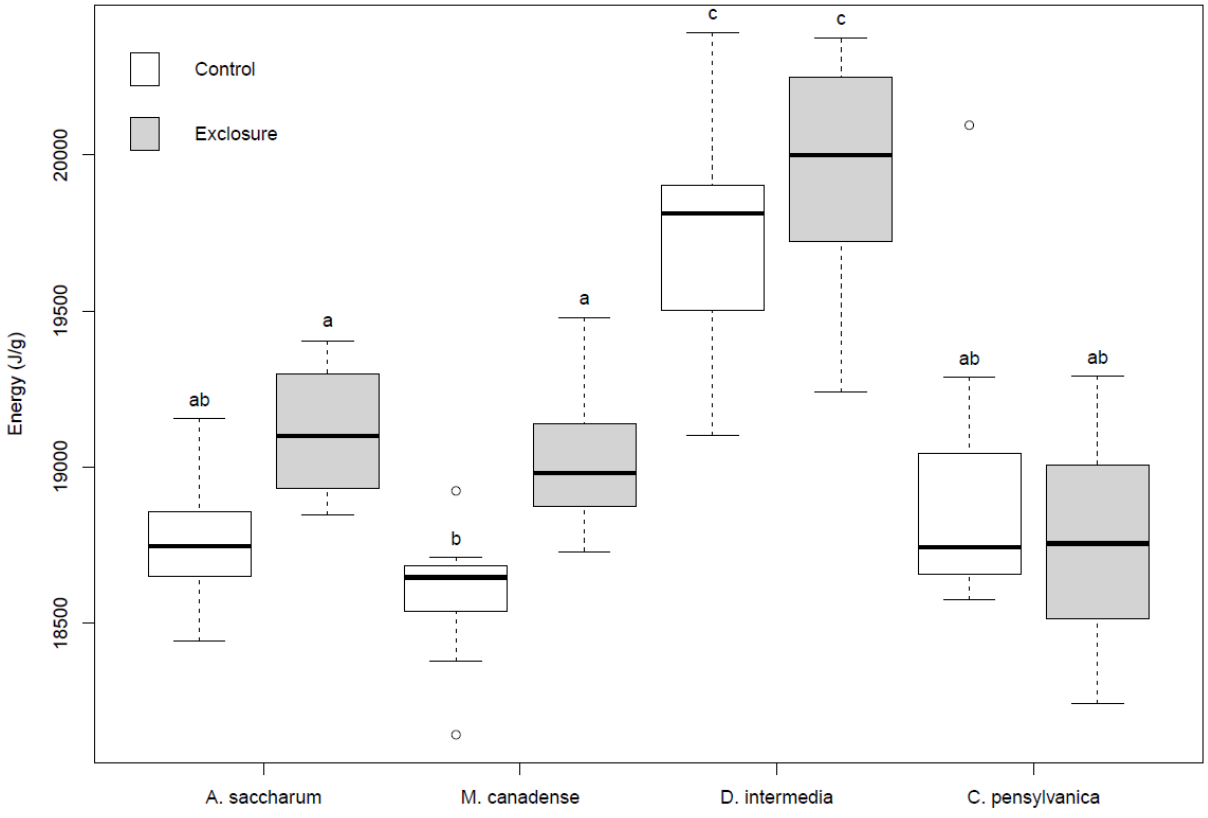

Figure 1. Energy (J/g) measured for all focal species. Letters above each box represent significance groups, calculated using Tukey's HSD test. Different letters represent groups that are significantly different from each other ($\alpha = 0.05$).

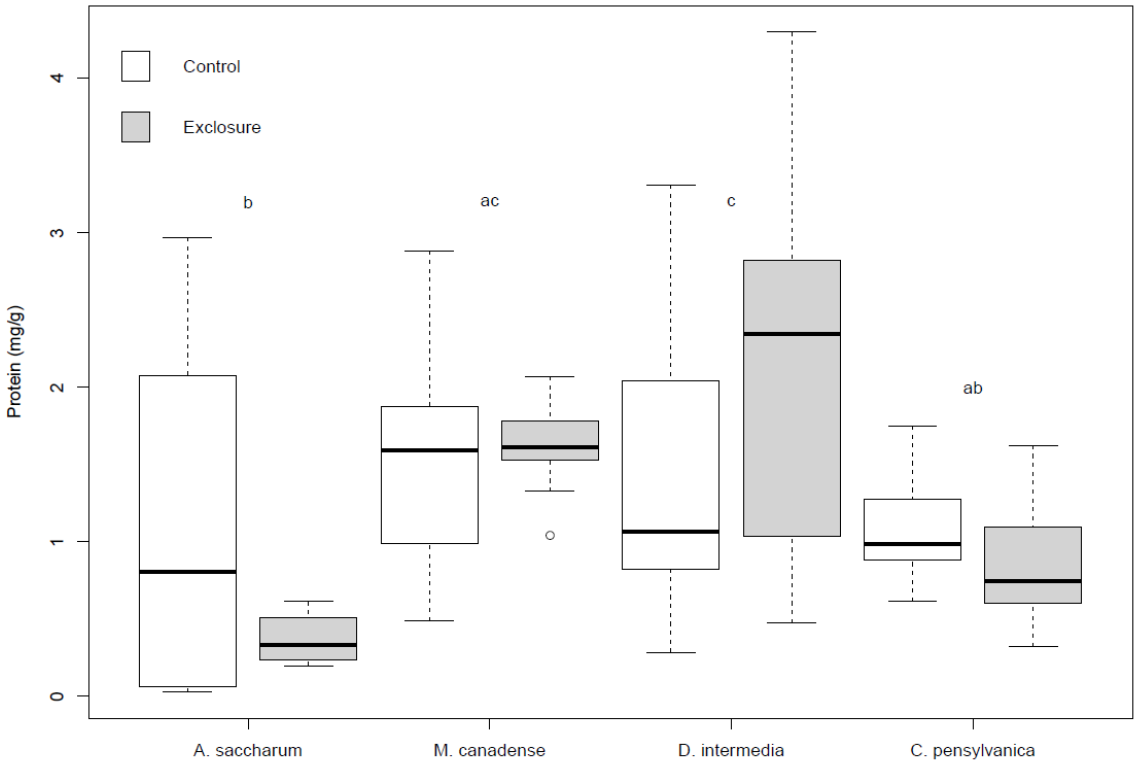

Figure 2. Protein (mg/g) measured for all focal species. Letters above the boxes represent significance groups, calculated using Tukey's HSD test. Different letters represent species that are significantly different from each other ($\alpha = 0.05$).

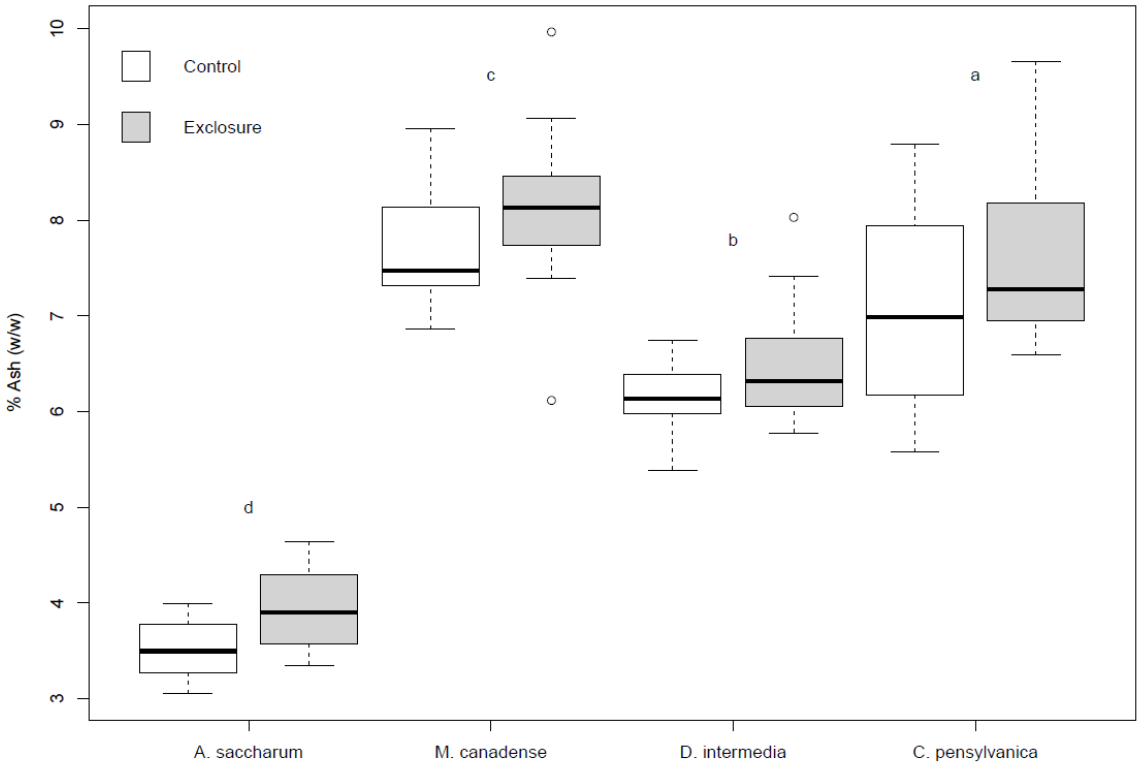

Figure 3. % Ash (w/w) measured for all focal species. Letters above the boxes represent significance groups, calculated using Tukey's HSD test. Different letters represent species that are significantly different from each other ($\alpha = 0.05$).

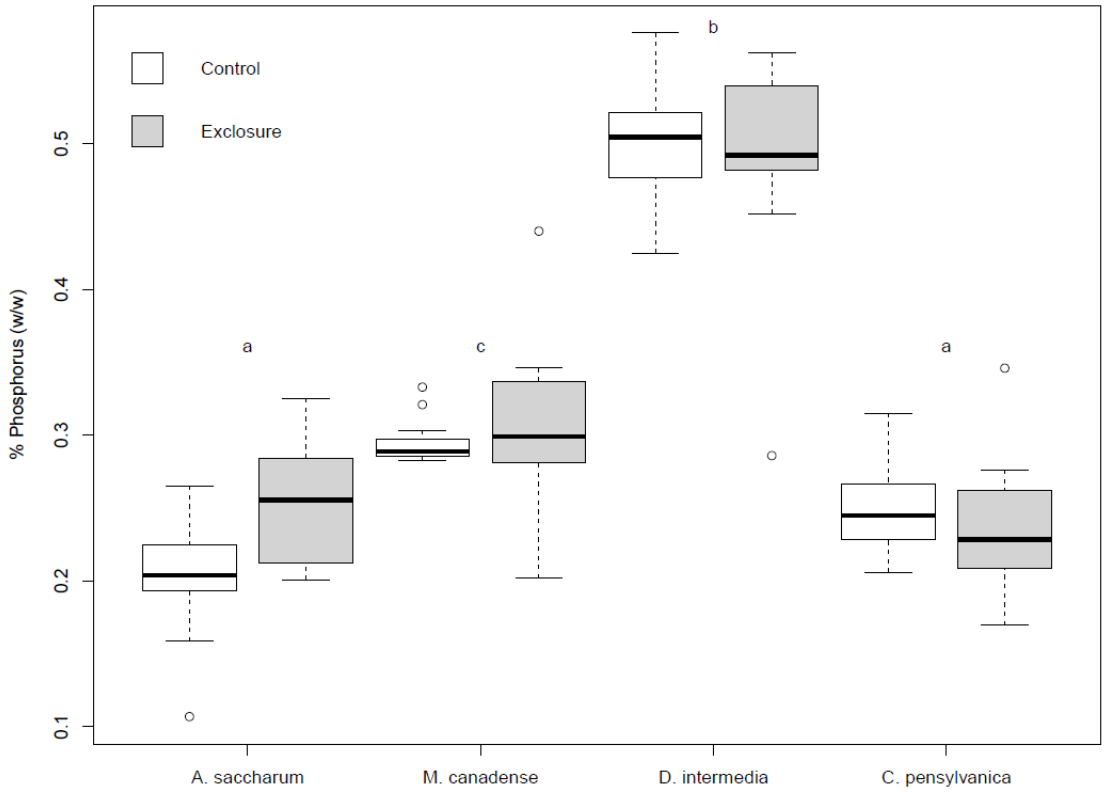

Figure 4. % Phosphorus (w/w) measured for all focal species. Letters above the boxes represent significance groups, calculated using Tukey's HSD test. Different letters represent species that are significantly different from each other ($\alpha = 0.05$).

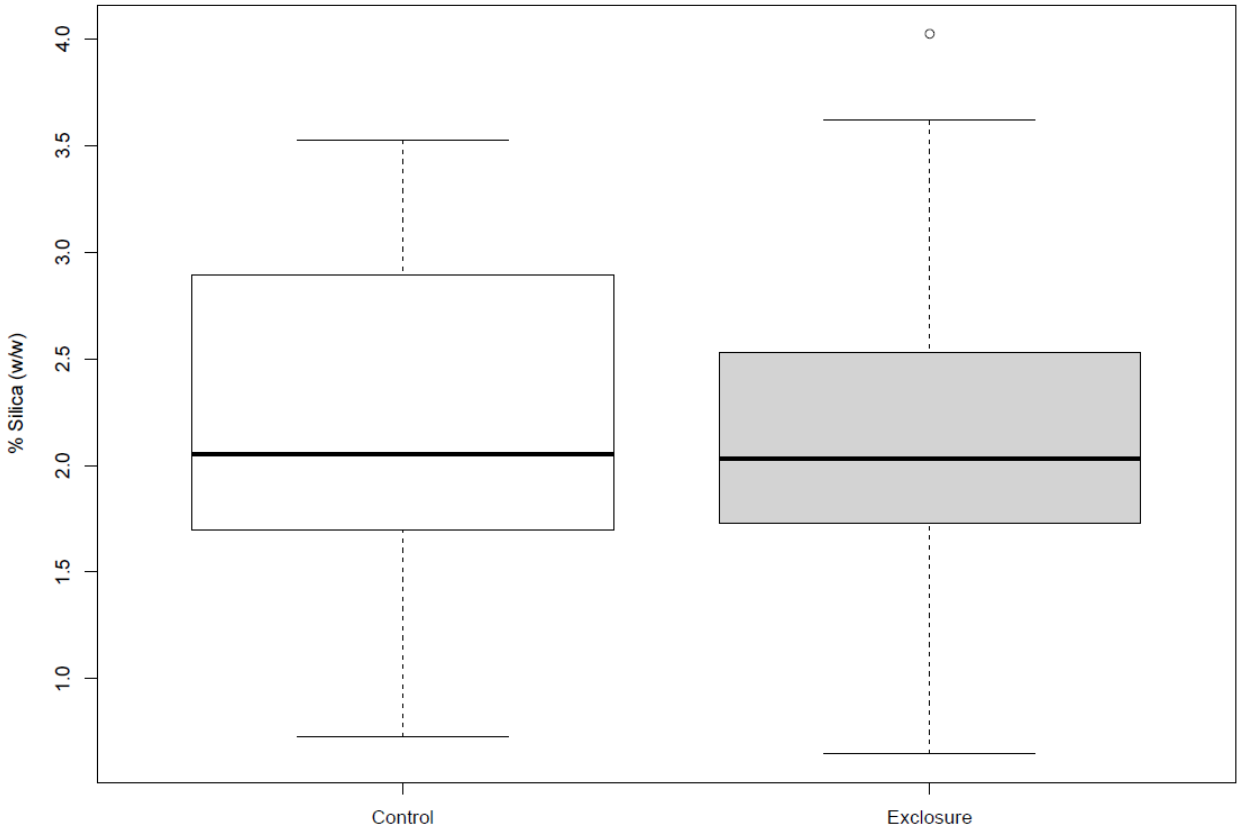

Figure 5. % Silica (w/w) measured in *Carex pensylvanica.*

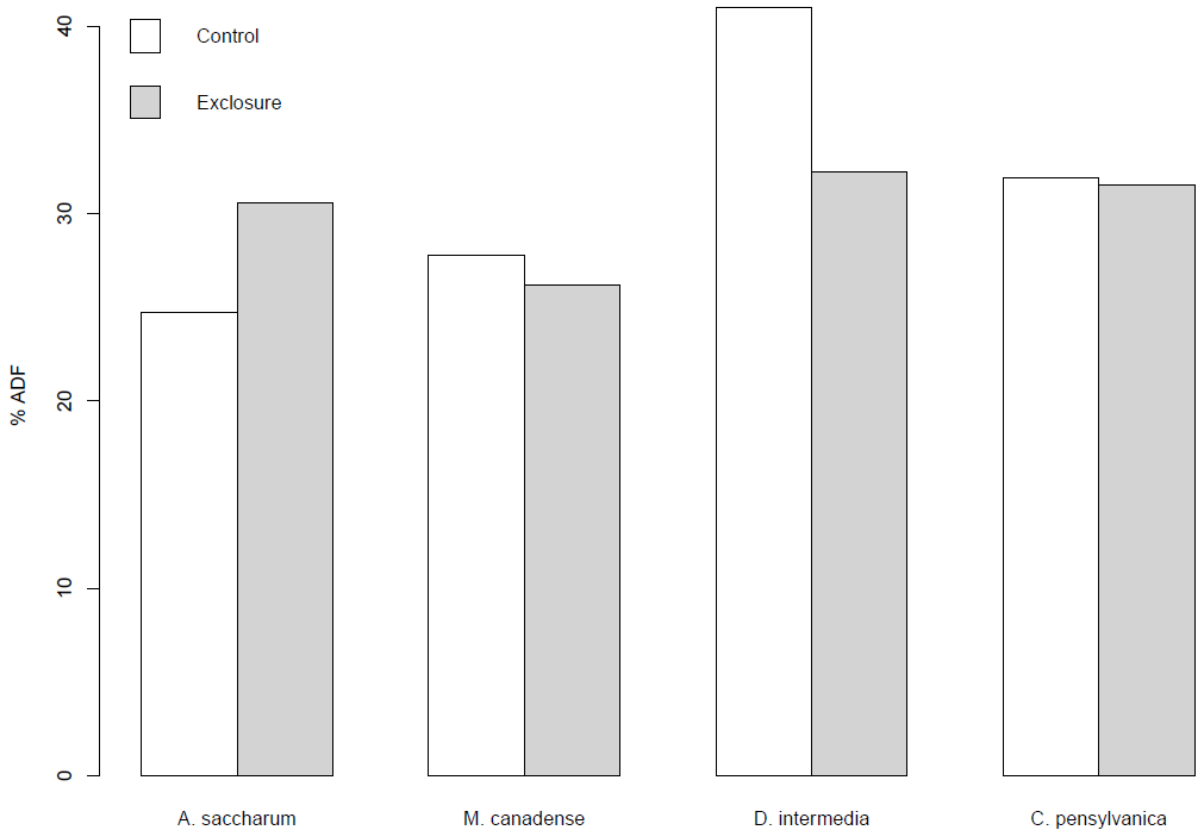

Figure 6. % ADF measured for all focal species.

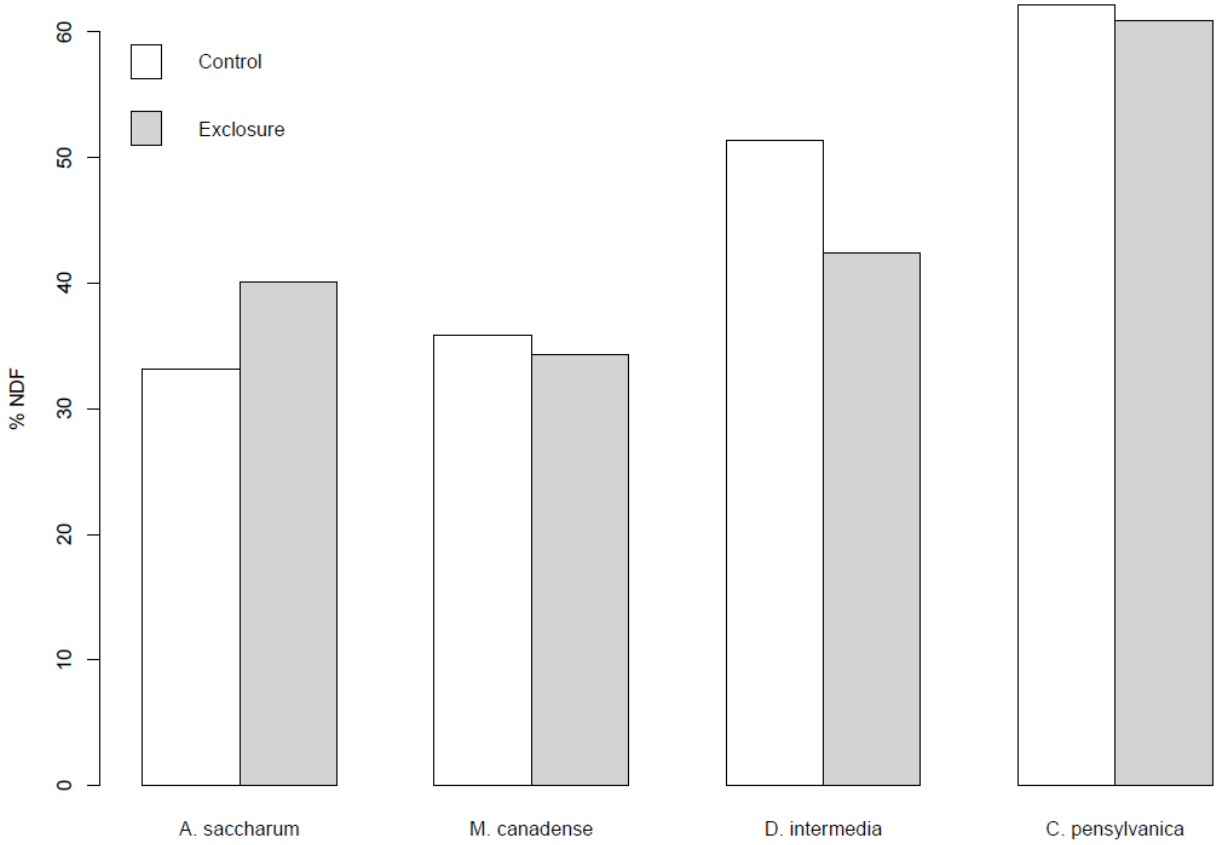

Figure 7. % NDF measured for all focal species.

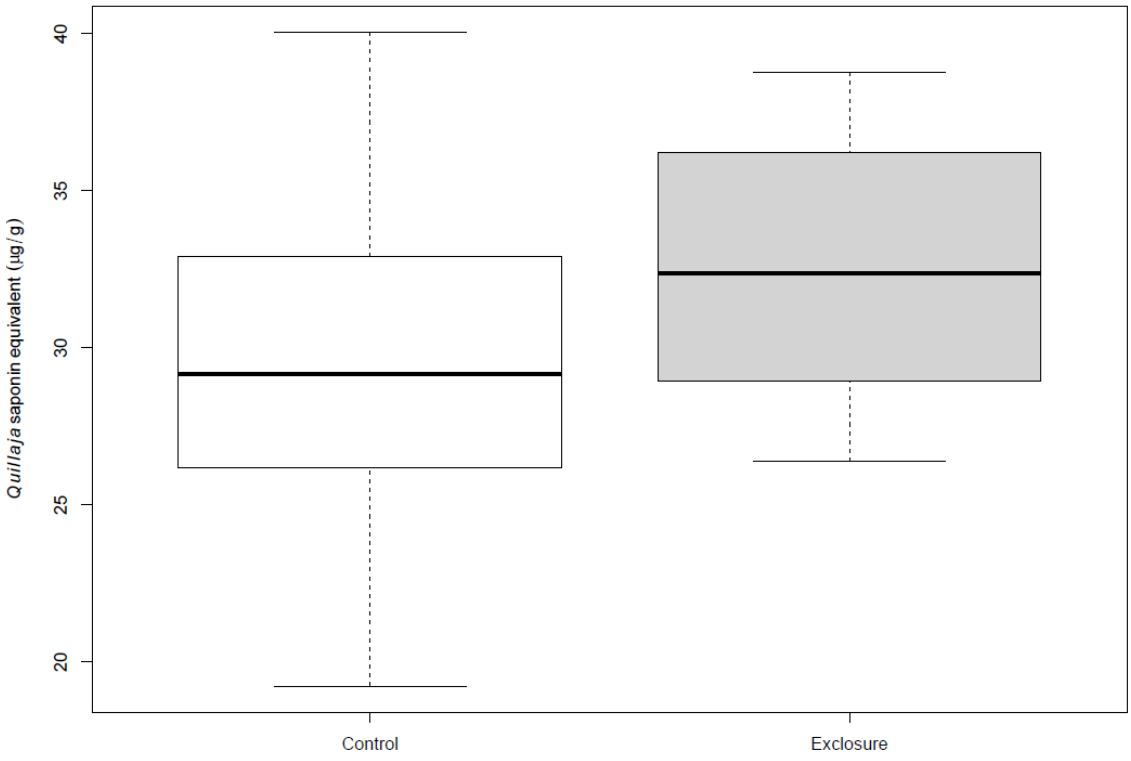

Figure 8. Saponin content (*Quillaja* saponin equivalent µg/g) measured in *Maianthemum canadense*.

DISCUSSION

Deer browsing impacts on forage quality

To identify impacts of deer browsing on the forage quality of a particular species, I focused on the pairwise comparisons between treatment plots within a species produced by Tukey's HSD test. Although the omnibus ANOVA found significant differences between treatment plots for energy and ash, treatment only accounts for a small amount of the overall variance in the data.

The significant difference in energy content of *M. canadense* stands out as the only forage quality parameter that was statistically different between treatment plots within a single species. The *M. canadense* protected from deer herbivory had significantly higher energy content than the *M. canadense* contained in the control plots ($p = 0.011$). Lipid content of plants is often associated with differences in energy content due to the high chemical energy contained in lipid molecules. Caloric content tends to increase in herbs as they flower and fruit (Bliss 1962). The difference in energy content of *M. canadense* could be explained by more mature plants on average in the exclosures. Measuring the lipid content of plants and counting flowering plants in the treatment plots are logical next steps to characterize the nature of this difference. Interestingly, *A. saccharum*, the other focal species sensitive to deer herbivory, shows a similar but non-significant trend. Mean energy content in the exclosure plots ($M = 19{,}114.42$ J/g) is

higher than that in the control plot (M = 18,764.17 J/g), but the difference is not significant (p = 0.087).

There was no difference in protein, ash, or phosphorus content between treatment plots for any of the focal species. Analysis of silica content of *C. pensylvanica* and saponin content of *M. canadense* produced results in which the mean values of these factors were essentially identical between treatment plots. Individually, there are several influences that have contributed to the lack of significant difference between these factors. First, an inclusive protein measurement does not communicate the relevance of these proteins to the diet of an organism. Utility of dietary protein to wildlife can be dependent on the amino acid composition of forages (Parrish and Martin 1977). Second, mineral content of plants is largely determined by soil mineral content (Van Soest 1994). The control and exclosure plots are located adjacent to each other on the same soil type, which can explain the low measured variation in plant mineral content. Third, the measured saponin content in *M. canadense* likely represents the baseline, constitutive levels of saponins present in this species. There was little evidence of herbivory on samples of *M. canadense* that we collected. Saponins have been demonstrated to be involved in induced defense mechanisms of plants (Szakiel, Paczkowski, Henry 2011). It is possible that deer herbivory could induce production of saponins in *M. canadense*, but that remains to be conclusively demonstrated.

The results for fiber show a different pattern. Both *M. canadense* and *C. pensylvanica* have similar ADF and NDF in both treatment plots. However, *A. saccharum* has higher fiber content in the exclosure plots. This may be explained by more mature individuals on average for this species in the exclosure plots. The majority of the *A. saccharum* in the exclosure plots were 1-2 meters tall, while the individuals found in the control plots were all seedlings around 10 cm tall. Fiber content generally increases as plants mature (Buxton and Redfearn 1997). An opposite trend is observed in *D. intermedia*, as higher fiber content on average was observed in individuals located in the control plots. Although ferns are not preferred forage items for deer, field observations have confirmed that ferns are occasionally browsed by deer (Crawford 1982). It is possible that this difference in fiber content may reflect a filtering effect caused by deer herbivory. However, the small sample size of these fiber measurements does not support definitive conclusions. Further collection and study of fiber content at this study site is necessary.

Collectively, these data do not provide support for the concept of deer herbivory driving a depression in forage quality in these species, through the mechanism of biotic filtering or release from herbivory. In addition to the individual factors mentioned above, several broader principles may explain the similarity of the measurements.

First, deer abundance has declined on the property since the regular feeding of deer stopped in the year 2000 (Rooney 2006). The fact that the plant communities in

control and exclosure plots remain distinct even with reduced deer herbivory pressure can be explained by the concept of alternate stable states. There is strong evidence that disturbance by heavy deer herbivory can alter the composition of a forest such that normal forest regeneration is halted (Stromayer and Warren 1997). These alternate stable states can be maintained by mechanisms such as heavy fern cover which can significantly reduce tree regeneration (Krueger and Peterson 2009). Given this idea of alternate stable states, the drastic plant community differences between exclosure and control sites at my study area can persist even without continued heavy deer browsing pressure. The combination of these factors may indicate that there is not a persistent strong filtering effect driven by deer at this study site.

Second, there is evidence that supplemental feeding of unbalanced, nutrient-rich diets can disrupt the digestion of a ruminant. This has been observed in moose populations. Moose that were provided with supplemental feed exhibited a compensatory feeding response, and increased their consumption of browse items (Felton et al. 2016). This scenario is applicable to the deer population on the Dairymen's property. The long history of supplemental feeding of deer likely increased deer impacts in vegetation in addition to increasing the local abundance of deer. Current deer populations do not need to consume additional browse to compensate for the unbalanced macronutrient profile of supplemental feeding.

Third, an important distinction to make with any exclosure study is that the comparison between treatment plots is not strictly a comparison between undisturbed and disturbed vegetation. Rather, the exclosures represent plant communities once subject to the same disturbances as the control communities. The exclosure communities differ in that they have been allowed to recover for the last several decades.

Fourth, I want to be careful to recognize the scope of my study and the forage quality measurements. I focused on only four species from an ecosystem recovering from heavy deer impacts. There are other forage quality metrics that I have not explored in this study. In particular, expanding focus to a broader secondary metabolite profile would be a particularly interesting aspect for future research. For example, tannins are a category of secondary metabolites associated with defense against large mammal herbivory (Belovsky 1981). Tannins have been identified as present in *A. saccharum* (Baldwin, Schultz, Ward 1987). It would be interesting to study the fluctuations of tannins in response to deer herbivory.

Characteristics of preferred forages

To investigate the forage characteristics of preferred deer browse in this study system, I divided my focal species into preferred and non-preferred categories. *A. saccharum* and *M. canadense* formed the preferred category, while *D. intermedia* and *C. pensylvanica* formed the non-preferred category. These distinctions were made after reference to a 2006 survey of the plants in the treatment plots at my study site. I

considered a large decline in relative abundance of a species between exclosure and control to be indicative of the sensitivity of the species to deer herbivory. The relative abundance of *A. saccharum* in exclosure plots (22.02%), was drastically higher than the control plots (0.22%). The same pattern is observed in *M. canadense*, as exclosure relative abundance (23.95%) is much higher than control (0.74%). *D. intermedia* does not show such a drastic decline, but exclosure relative abundance (6.57%) is still higher than control (1.04%). *C. pensylvanica* shows an opposite trend, with lower relative abundance in exclosure plots (3.00%) compared to control plots (25.39%) (Rooney 2009).

These categories are supported by observations by other researchers. Field studies confirm that deer feed on both *A. saccharum* and *M. canadense* (Horsley, Stout, DeCalesta 2003; Rooney 1997). As discussed previously, heavy deer herbivory is often associated with increases in the abundance of ferns and graminoids (Frerker, Sabo, Waller 2014; Rooney 2009). These species increase in the presence of deer herbivory stress as they are well equipped for herbivory tolerance and defense. Graminoids such as *C. pensylvanica* tolerate herbivory pressure structurally by their basal meristem, allowing them to regenerate lost tissue. Graminoids are typically not characterized by extensive chemical defenses, although silica and lignin content can reduce availability of nutrients (Coughenour 1985). Ferns are typically defended by a wide range of defensive secondary metabolites including various terpenes, cyanogenic glycosides, flavonoids, phenolics, and tannins (Cooper-Driver 1990).

The forage quality profiles between the preferred and non-preferred categories differ in a number of factors. A major difference between these two categories is fiber content. The preferred species have lower fiber content (ADF and NDF) than the non-preferred species. This corresponds with the observation that high fiber content reduces the digestible energy available to an herbivore. Strikingly, *D. intermedia* has significantly higher energy and phosphorus content relative to the other species. This underscores that gross energy and nutrient measurements must be considered in light of their availability for digestion. Fluctuations in the gross energy of *D. intermedia* are likely related to seasonal and life stage fluctuations in lipid levels (Rozentsvet et al. 2002). Chemical defenses likely play a role in the low preference deer show for *D. intermedia*. Study of baseline levels of defensive secondary metabolites in *D. intermedia* similar to my study of saponins in *M. canadense* would allow for more firm conclusions in this area. Silica content is likely another important difference between preferred and non-preferred forage categories. Although I focused only on the silica content of *C. pensylvanica*, other researchers have identified high silica content as an herbivory deterrent in ferns (Cooper-Driver 1990; Mazumdar 2011). Silicon content of *Carex* spp. and *Dryopteris* spp. reported in an exhaustive review are on average higher than those reported for *A. saccharum* and *Maianthemum japonicum,* a close relative to *M. canadense* (Hodson et al. 2005).

The shift in the plant community composition observed at this site combined with these forage quality measurements support the predicted increasing abundance of

unpalatable plant species in response to deer herbivory pressure. This represents a direct impact on the vegetation of this study site, catalyzing further indirect impacts to the ecosystem. Continued monitoring of the plant community and forage quality at this site is necessary to track changes over time. An expansion of the forage quality parameters measured will further contribute to a more complete understanding of deer forage preferences. These data can be used to predict the impacts, both direct and indirect, of deer on ecosystems.

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