Deer Browsing Effects on Temperate Forest Biogeochemistry, Plant Community Composition, and Plant Chemistry

by

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Dedication

Voor Thea, Jean, Sophie, en Geert, jullie geven mij moed en vertrouwen.

Voor Rhea en Mira, mijn grootste voorbeelden in het simpelweg gelukkig zijn.

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⁂

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Table of Contents

Dedication **ii**

Acknowledgements **iii**

List of Tables **viii**

List of Figures **xi**

Abstract **xvi**

Chapter 1 Introduction **1**

- Alteration of successional pathways: A case study in salt marshes 1
- Increased biomass and productivity with herbivory: A case study in the Serengeti 2
- Decreased productivity with herbivory: A case study in Minnesota grasslands 3
- Conflicting hypotheses 4
- Herbivore effects on nitrogen dynamics 5
- Temperate forests 6
- Soil components, processes, and feedbacks 6
- Deer impacts on temperate forests: what do we know? 7
- White-tailed deer 8
    - Range 8
    - Physical description 8
    - Diet 9
    - Implications of deer browsing 10
- Deer browsing in temperate forests: Dissertation outline 11
- Figures 14
- References 15

Chapter 2 Deer Browsing Effects on Temperate Forest Soil Nitrogen Cycling Shift from Positive to Negative Across Fertility Gradients **20**

Abstract 20
Introduction 21
Methods 23
  Study sites 23
  Soil sampling 24
  Soil processes 25
  Leaf litter 25
  Data analysis 26
Results 27
  N mineralization and CO_2 respiration rates across fertility gradients 27
  Browsing effects on N mineralization and CO_2 respiration rates across a fertility gradient 28
Discussion 29
Acknowledgments 33
Figures 34
Tables 38
References 41
Supplementary Figures 46

Chapter 3 Deer Browsing Decreases Understory Plant Abundance But Has No Detectable Effects on Plant Community Composition 51

Abstract 51
Introduction 52
Methods 56
  Study sites 56
  Plant community structure and composition 57
  Canopy litter inputs to soil 58
  Soil C:N analyses 59
  Data Analyses 59
Results 61
  Deer browsing effects on plant community structure 61
  Deer browsing effects on plant community composition 62

Discussion 64
    Deer browsing effects on plant community structure 65
    Deer browsing effects on plant community composition 68
Conclusions 69
Acknowledgements 70
Figures 71
Tables 73
References 76

Chapter 4 Deer Browsing Effects on Plant Chemical and Nutrient Concentrations 80
Abstract 80
Introduction 81
Methods 86
    Study sites 86
    Vegetation surveys 87
    Leaves 88
    Environmental variables 89
    Chemical analyses 89
    Data analyses 91
Results 94
    Deer browsing effects on foliar nitrogen and C:N 94
    Deer browsing effects on plant defense compounds 95
    Plant chemical richness and diversity 96
    Plant chemical composition 98
    Site level chemistry 98
Discussion 99
    Plant nitrogen and C:N 99
    Plant defense compounds 100
    Plant chemical richness and diversity 103
    Plant chemical composition 103
Conclusion 105
Acknowledgements 106

Figures 107
Tables 119
References 122

Chapter 5 Conclusion **128**
Further questions and considerations 130
References 131

List of Tables

Table 2.1 Study sites and plots (n=8), with average litterfall (kg ha^{-1} yr^{-1}), C:N ratio in litter and soil, deer population density (deer km^{-2}), N mineralization rates (g N g^{-1} d^{-1}) in non-browsed control plots, and CO_2 respiration (µmol C m^{-2} s^{-1}) rates in non-browsed control plots. Mean rates of N mineralization and CO_2 respiration are shown with standard error range. We excluded the Lake Loop plot in the Stony Creek site from statistical analyses due to exceptionally low leaf litter C:N and litterfall values in this plot. 38

Table 2.2 Mixed effect results for deer browsing effects and soil C:N on N mineralization (g N g^{-1} d^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects. 39

Table 2.3 Mixed effect results for deer browsing effects and litter C:N on N mineralization (g N g^{-1} d^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects. 39

Table 2.4 Mixed effect results for deer browsing effects and soil C:N on CO_2 respiration (µmol C m^{-2} s^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects. 40

Table 2.5 Mixed effect results for deer browsing effects and litter C:N on CO_2 respiration (µmol C m^{-2} s^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects. 40

Table 3.1 Factor level criteria for each continuous environmental variable: N mineralization (g N g soil $^{-1}$ d^{-1}), CO_2 respiration (µmol C m^{-2} sec^{-1}), Litterfall (g m^{-2} yr^{-1}), C:N Soil, and C:N Litter. 73

Table 3.2 Linear mixed effects model summary for plant community structure. (****) indicates significance <0.0001, (***) indicates significance <0.001, (**) indicates significance <0.05, (*) indicates significance <0.1. Plant community structure data has coefficient estimates for significant factors. Deer browsing and other environmental factors have significant effects on plant community structure, and significant interactions effects between deer and environmental variables were found across structural measures. R^2 marginal provides variance explained only by fixed effects, and R^2 conditional provides the variance explained by the entire model, both fixed effects and random effects. 74

Table 3.3 Model summary for PERMANOVA analyses of plant community composition. (*) indicates significance p <0.1. The model R^2 is 0.4, explaining 40% of variance in plant community composition. P-values below 0.1 are considered ecologically significant. 74

Table 3.4 All plant genus/species found at study sites, with significance indicator of deer browsing effects (*p<0.1,**p<0.05, ***p<0.001). Significance is indicated in bold, and represents an overall deer browsing effect when considering all sites. Most species were present at only a few sites, indicated by “present at site”. Minimum and maximum percent cover, the average % cover of species at these sites, and associated plant growth forms are listed as well. Note that “trees” are only seedlings and saplings, not including overstory................................. 75

Table 4.1 Linear mixed effects model summary for plant tissue chemical content and composition. (****) indicates significance <0.0001, (***) indicates significance <0.001, (**) indicates significance <0.05, (*) indicates significance <0.1. Phenol and Richness results have coefficient estimates for significant factors, indicate strength and direction of effect. Quinone has a “+” to indicate direction of effect. Linear mixed models were used to analyze %N, C:N, richness, diversity, Phenol, terpene, glycoside, and quinone contents were analyzed using linear models. 119

Table 4.2 Model summary for PERMANOVA analyses of chemical community composition. Significance level p<0.1 is indicated with *, p<0.01***. The model R2 is 0.1. explaining 10% of variance in chemical community composition. P-values below 0.1 are considered ecologically significant. 120

Table 4.3 Plant species leaf %C and C:N, and the effect of deer on each species. Species marked with a “*” were significantly affected by browsing (p<0.05). Deer browsing decreased %C overall (p=0.006), but effects varied between species. %C increased in *U. americana* (p=0.05), and decreased in *E. umbellata* (p=0.01). C:N increased in *U. americana* (p=0.001) and *P. quinquefolia* (p=0.03), and decreased in *E. umbellata* (p=0.03). 121

Table 4.4 Plant species leaf %N and the effect of deer on each species’ percent cover. %N values marked with a “*” were retrieved from the TRY plant database (Kattge and Knoll 2019). 121

List of Figures

Figure 1.1 Herbivores can have accelerating and decelerating effects on ecosystem nutrient cycling. Accelerating effects are often associated with high nutrient environments, and decelerating effects in low nutrient environments. 14

Figure 1.2 Microorganisms consume inorganic compounds to construct cells, enzymes and other organic compounds needed to grow, they are said to be “immobilizing” nutrients. When organisms excrete inorganic waste compounds they are said to be “mineralizing” nutrients. These excreted inorganic waste compounds are essential elements for plant growth and are assimilated by plant roots to sustain growth 14

Figure 2.1 Map of study sites located in southeastern Michigan. Kensington, Stony Creek, and Oakwoods, each have three experimental deer exclosures (n=9) 34

Figure 2.2 Net N mineralization (g N g soil $^{-1}$ day^{-1}) with and without deer browsing at each site. Filled circles are rates of net N mineralization outside deer exclosures (browsed), and empty circles are inside deer exclosures (non-browsed). 35

Figure 2.3 CO_2 respiration (µmol C m^{-2} sec^{-1}) rates with and without deer browsing at each site. Filled circles are rates of net N mineralization outside deer exclosures (browsed), and empty circles are inside deer exclosures (non-browsed). 36

Figure 2.4 The effect of deer browsing on (a,b) net N mineralization (g N g soil $^{-1}$ day^{-1}, browsed minus non-browsed) and (c,d) CO_2 respiration (µmol C m^{-2} sec^{-1}, browsed minus non-browsed) across a (a,c) litter C:N gradient, (b,d) soil C:N gradient. The dotted line indicates no difference between browsed and control. Values above *y*=0 indicate that rates increased with browsing, while values below *y*=0 indicate that rates decreased. N Mineralization increased with deer browsing at sites with low soil and litter C:N, and decreased with deer browsing at sites with high soil C:N(a,b). C respiration decreased with deer browsing at sites with low litter C:N and increased with deer browsing at sites with high litter C:N (c). 37

Figure 3.1 Plant community structure responses to deer browsing: A) Richness, B) % Cover, C) Diversity, D) Biomass, E) Sapling density, F) %seedlings. Responses to deer browsing are not uniform across sites. Overall deer significantly decreased percent cover, sapling density, and tree seedlings. Parks are abbreviated as follows: Kensington (K), Oakwoods (OW), and Stony Creek (SC). Sites are abbreviated as follows: Spring Hill (SH), Tamarack (TR), Wild Wing (WW), Big Tree (BT), Long Bark (LB), Railroad (RR), Lake Loop (LL), Return Trail (RT), and West Branch (WB). 71

Figure 3.2 Plant community composition NMDS averages with standard error overlaid on original NMDS points for C:N litter and C:N soil. Significance is indicated by non-overlapping error bars. Continuous data on C:N were binned in three categories: High (black), Medium (yellow), and Low (green) for NMDS visualization purposes. 72

Figure 3.3 Percent cover of plants grouped by growth form, with and without deer browsing. Linear mixed effect model showed that deer browsing significantly decreases cover of forbs (p=0.005 and vines (p=0.05), and significantly increases cover of graminoids (p=0.01). 72

Figure 4.1 %N in plant leaf tissues with and without deer browsing in the twelve most common plant species across all sites. Significance level α=0.05, overall effect of browsing on %N was analyzed using a mixed effect model, with sites as random effects. No overall effect was found. Browsing effects on individual species were analyzed using a linear model (anova), significance was found in two species and is indicated with a *: *U. americana* decreased %N with browsing (p<0.01) and *E. umbellata* increased %N with browsing (p<0.05). %N also varied between species, species with different letters were significantly different. 107

Figure 4.2 Total phenol (μg mg^{-1}) in plant leaves with and without deer browsing, in the twelve most common species. Significance level α=0.05, overall effect of browsing on phenol was analyzed using a mixed effect model, with sites as random effects. No overall effect of browsing was found, but species differed. Browsing effects within individual species were analyzed using a linear model, significance was found in four species: *A. saccharum, E. umbellata, P. quinquefolia,* and *U. americana*........ 108

Figure 4.3 Total phenol and percent cover per species. Twelve taxa were found overall, five taxa were found only in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Phenol concentration varied among species, and not all species respond similarly to deer browsing. 109

Figure 4.4 Relative terpene amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative terpene amount varies among species, and not all species respond similarly to deer browsing. 110

Figure 4.5 Relative glycoside amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative glycoside amount varies among species, and not all species respond similarly to deer browsing. 111

Figure 4.6 Relative quinone amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative quinone amount varies among species, and not all species respond similarly to deer browsing. 112

Figure 4.7 Chemical compound richness and diversity with and without deer browsing, across 9 study sites. Chemical richness varied at the site level (p<0.0001), and browsing effects varied (p=0.04); Chemical compound richness at LB (53) and RR (53), was higher than at SH (38). Diversity of chemical compounds varied between sites (p<0.0001), but browsing had no effect. 113

Figure 4.8 Chemical compound richness and diversity with and without deer browsing in 12 different plant species that were found inside and outside exclosures. Asterix (*) indicates significant browsing effect. Richness significantly increased with browsing in *Fraxinus sp.* (p=0.005). *P. peltatum* and *Fraxinus* had highest foliar chemical richness in their leaves (59 and 58 respectively), and were significantly different from *U. americana* (38), *graminoids* (37), and *E. umbellata* (35). diversity increased with browsing in *E. umbellata* (p=0.003). *Fraxinus* and *C. lutetiana* had higher diversity (13.4 and 12.0) than the other 10 species. 114

Figure 4.9 Chemical community composition NMDS averages with standard error overlaid on original NMDS points for deer browsing treatment, C:N soil and C:N litter. Deer browsing treatment is “Non-browsed” and “Browsed. Continuous values for C:N were grouped into three levels: low (green), medium (yellow) and high (black). 115

Figure 4.10 Average NMDS points for community composition of chemical compounds with standard error bars. PERMANOVA models show differences among twelve most common plant species (p=0.001). 116

Figure 4.11 Average NMDS points for community composition of chemical compounds with standard error bars. Deer browsing effects on plant chemical communities differ among species (p=0.001). 117

Figure 4.12 Total phenol load per site. Total phenol load represents a weighted sum of phenolic concentration per species based on percent cover at each site. A linear model did not detect significant browsing effects, but sites varied significantly (p=0.003). SH and LB were significantly higher in total phenolic levels than the other 6 sites. 118

Supplementary Figures Chapter 2

Supplementary Figure 2-1 Soil and litter C:N ratios are not correlated. 46

Supplementary Figure 2-2 Rates of N mineralization (g N g^{-1} d^{-1}) correlate positively with litter C:N ratios. 46

Supplementary Figure 2-3 Rates of N mineralization (g N g^{-1} d^{-1}) correlate positively with soil C:N ratios. 46

Supplementary Figure 2-4 Rates of N mineralization (g N g^{-1} d^{-1}) in browsed do not correlate with litter C:N ratios. 47

Supplementary Figure 2-5 Rates of N mineralization (g N g^{-1} d^{-1}) in non-browsed correlate positively with litter C:N ratios. 47

Supplementary Figure 2-6 Rates of N mineralization (g N g^{-1} d^{-1}) in browsed plots correlate positively with soil C:N ratios. 47

Supplementary Figure 2-7 Rates of N mineralization (g N g^{-1} d^{-1}) in non-browsed plots correlate positively with soil C:N ratios. 48

Supplementary Figure 2-8 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) do not correlate with litter C:N ratios. 48

Supplementary Figure 2-9 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) do not correlate with soil C:N ratios. 48

Supplementary Figure 2-10 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) in browsed plots do not correlate with litter C:N ratios. 49

Supplementary Figure 2-11 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) in non-browsed plots do not correlate with litter C:N ratios. 49

Supplementary Figure 2-12 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) in browsed plots do not correlate with soil C:N ratios. 49

Supplementary Figure 2-13 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) in non-browsed plots do not correlate with soil C:N ratios. 50

Abstract

Herbivores influence ecosystem nutrient dynamics in many ways. Impacts on soil biogeochemical processes often include increasing nutrient cycling rates under high nutrient availability and decreasing nutrient cycling rates under low nutrient availability. Herbivores also alter plant communities as well as concentrations of nutrients and secondary defense chemicals in plant tissues, which further impact decomposition and nutrient cycling. These patterns are far from universal, and interactions between soil fertility and herbivory are under continuing investigation. This dissertation focusses on the interactions between herbivores and primary producers, more specifically the white-tailed deer (*Odocoileus virginianus*) and temperate forest nutrient cycles. Increasing deer populations have been posing threats to their habitats for decades. Deer overabundance affects forest regeneration and understory vegetation cover and composition, with consequences across trophic levels such as insects and birds. Research thus far, however, has found inconsistent effects of deer overabundance on soil biogeochemical processes as well as on plant community structure. The net effects of deer browsing on soils remains difficult to predict, which is reflected in inconsistencies within and across studies.

By sampling inside and outside deer exclosures across a network of forest sites in southeast Michigan, I investigated deer browsing effects on temperate forest soil nitrogen (N) and carbon (C) cycling along gradients of soil and litter C:N and canopy litterfall. Deer browsing increased net N mineralization rates in high nutrient environments and decreased N mineralization rates in low nutrient environments, whereas browsing decreased soil CO_2 respiration in high nutrient environments and increased soil CO_2 respiration in low nutrient environments. My work shows that deer browsing can have significant effects on net N mineralization and C respiration in temperate forest soils and that the direction and magnitude of deer browsing effects on soil N and C cycling vary across fertility gradients.

Differences in deer browsing effects on soil processes could be mediated by plant responses to herbivory across gradients of resource availability. Deer effects on plant communities were significant but did not vary with environmental factors. Browsing significantly decreased several plant structural measures, including plant percent cover, tree seedling cover, and sapling density, but had no detectable effects on plant community composition overall. Other important browsing effects on plant community composition, however, are reflected in significant changes in percent cover of nearly 25% of plant species and 3 plant functional types with deer browsing.

Furthermore, my detailed analyses of plant chemical composition in temperate forest understory plant communities showed that deer browsing alters plant chemistry and that plant species vary in their response to browsing. Together with inherent differences among plant species in chemical and nutrient concentrations, this work highlights the importance of both selective browsing and browsing-induced defenses for ecosystem nutrient dynamics. Foliar chemistry did not vary with environmental variables, indicating that among-species differences are more important than within-species responses to browsing in driving plant community chemical responses to browsing.

Understanding the factors that contribute to changes in forest C and N cycling continues to be critically important, especially considering predicted scenarios of climate change. Overall, my dissertation provides support and insights into how interactions between above- and belowground processes are important drivers of ecosystem functioning.

Chapter 1 Introduction

The world consists of a complex web of trophic interactions, many of which influence ecosystem functioning and energy cycling (Wardle 2002). Numerous interactions between organisms within and across trophic levels, including primary producers, herbivores, and top predators, influence plant chemistry and ecosystem nutrient cycling through a variety of pathways (Hunter 2001, Sterner and Elser 2002, Wardle 2002, Hunter et al. 2012). This dissertation focuses on interactions between herbivores and primary producers, specifically the role of white-tailed deer (*Odocoileus virginianus*) in temperate forest plant community composition, plant chemistry, as well as nutrient cycling. Herbivores drive important changes in various ecosystems around the world in different ways, which I discuss below through a series of case study examples.

Alteration of successional pathways: A case study in salt marshes

Succession can be highly impacted by herbivory in a wide range of ecosystems including plankton turnover in lakes and the successional trajectories of boreal forests (Leibold 1989, Hidding et al. 2013). Succession is largely driven by a trade-off between competitive and dispersal ability, with fast growing and high dispersing plants colonizing disturbed habitat first, but over time being replaced by slow growing more competitive species (Tilman 1988). Consumers can alter rates of species replacement, and thereby participate in long-term ecosystem dynamics and autotroph chemistry on a large scale. In particular, herbivore disturbance can increase species diversity by suppressing dominant, highly competitive species (Pierce et al. 2007).

For example, in Argentinian salt marshes, crabs and wild guinea pigs selectively consume cordgrass, *Spartina densiflora*, which is a secondary successional species, and in the absence of herbivory is a dominant competitor. This selective consumption of cordgrass allows early successional species like pickleweed, *Sacrocornia perennis*, and alkaliweed, *Cressa truxillensis*, to successfully colonize and dominate disturbed patches (Daleo et al. 2014). Consequently, the presence of herbivores slows down the rate of succession. This is an example of how selective browsing pressure on late-successional species, slows species replacement and the ecosystem remains in a state of early succession.

Increased biomass and productivity with herbivory: A case study in the Serengeti

Herbivory *increases* productivity in systems in which plants respond to the stressor by compensatory growth to enable fast recovery. In turn, increased productivity can result in rapid decomposition of litter, and increased mineralization rates due to nitrogen (N) rich waste products of herbivores (McNaughton 1976). Herbivory can thus increase nutrient availability, stimulating aboveground productivity and resulting in a positive feedback that increases production.

The Serengeti grassland ecosystem, located in Tanzania and Kenya, is a key example of how herbivores can increase productivity (McNaughton 1976, Mcnaughton 1985). The Serengeti grasslands are heavily populated by large herds of ungulate browsers and grazers such as zebra, wildebeest, gazelle, buffalo, and many others. These large herds of migratory grazers have the capacity to consume vast swaths of grassland areas, substantially impacting grassland productivity. The wildebeest population was estimated to be around 1.4 million in 1974, contributing roughly 50-70% to the total migratory population. When passing through grassland areas, which takes the herds about 4 days, green biomass was reduced up to 85% and plant height by 56%. After the herd moved through, the areas that were grazed sustained net primary productivity of 2.6 g m^{-2}, whereas areas protected from grazing showed a biomass decline of 4.9 g m^{-2}. This increase in productivity in grazed areas demonstrates that the wildebeest transformed a previously senescent plant community into a productive one and provides a clear example of how ungulate grazing can change ecosystem dynamics.

Decreased productivity with herbivory: A case study in Minnesota grasslands

Alternatively, herbivores can *decrease* productivity and rates of nutrient cycling via selective browsing on nutrient rich species. When herbivores selectively browse on nutrient rich species, more browse-resistant and nutrient poor plant species can increase in dominance. Leaf litter from these resistant species will decompose more slowly than leaf litter from nutrient rich species, thereby reducing nutrient availability in soils (Hobbie 1992, Hobbs 1996, Ritchie et al. 1998). Reduced nutrient availability favors competition by more nutrient-poor species with low nutrient requirements, leading to a positive feedback loop that reduces productivity and nutrient availability. For example, exclusion of herbivores (deer, *Homoptera* and *Orthoptera* spp.) greatly increased plant cover and biomass in a nitrogen-limited savanna in Minnesota (Ritchie et al. 1998). Decreased belowground biomass was observed associated with increased aboveground biomass. Aboveground biomass, leaf litter, and belowground tissue also showed increases in N concentration after exclosure from herbivory. Increases in biomass and nutrient concentration resulted in higher soil nitrate availability and total available N; total soil and plant N were not altered. These results suggest that herbivory can decrease nutrient cycling rates, by removing plant species with high N content. Furthermore, the effects may be particularly strong when browsers forage selectively on a few important species that control resource abundance, such as nitrogen-fixing legumes (Ritchie and Tilman 1995).

Conflicting hypotheses

These two conflicting hypotheses (Figure 1.1) indicate that the effect herbivory has on nutrient cycling and productivity can vary, and might depend on the resource by which plants are most limited (Ritchie and Tilman 1995). If the dominant plant community is primarily N limited, and thus has lower N concentration in tissues, herbivores are likely to slow down nutrient cycling (Tilman 1988). However, if the plant community is high in N content, and is limited more by other factors (e.g. light, water), or resists browsing in a way that does not involve tissue chemistry, herbivores might accelerate nutrient cycling (Hobbie 1992). The research described above on grasslands and savannas has shown both acceleration and deceleration of nutrient cycling by herbivores, yet questions remain on how herbivores impact temperate forest nutrient cycling. Although there has been progress in understanding biogeochemical responses to grazing in grassland ecosystems (McNaughton et al. 1997), boreal forests (Pastor and Cohen 1997), and managed lands (Bardgett et al. 2001), it remains hard to predict the effects herbivores will have on nutrient cycling (Sitters and Olde Venterink 2015, Forbes et al. 2019). Studies report mixed results, sometimes nitrogen (N) mineralization rates increase (McNaughton et al. 1997), decrease (Persson et al. 2005), or are not affected due to browsing (Barthelemy et al. 2015). Most conceptual models predict increased nutrient cycling rates under high nutrient availability and decreased in nutrient cycling rates under low nutrient availability (Cherif and Loreau 2013). High nutrient environments allow for plants to have high nutrient content, and herbivores increase nutrient cycling by excreting highly labile N and phosphorous (hereafter, P) products, thus stimulating compensatory plant growth. When nutrients are scarce, some plant species increase their chemical defenses instead of tolerating browsing with compensatory growth. Damaged plants often become tougher and higher in polyphenol concentrations in response to tissue loss due to herbivory (Hunter and Schultz 1993), which consequently decreases decomposition and nutrient cycling (Findlay et al. 1996). To make better predictions about how herbivores affect nutrient cycling, more long-term experimental studies on the relative strength of the different mechanisms driving these effects are needed.

Herbivore effects on nitrogen dynamics

Nutrient cycling studies focus mainly on N, because it is one of the most limiting factors of productivity in boreal, temperate, and several types of tropical forests (Barnes et al. 1998). Spatial patterns in N availability often determine plant biomass, competitive relationships, and biodiversity (Gilliam 2007). Ungulate herbivores can influence nutrient cycling directly and indirectly; therefore, it is important to determine the magnitudes of direct and indirect effects in order to better understand community structure (Abrams 1995). Ungulate herbivores can directly increase nutrient cycling rates in soils through excretion of fecal pellets on the forest floor (Murray et al. 2013). Alternatively, ungulates can indirectly effect plant communities by selectively browsing on palatable species. As consumption reduces relative abundance of palatable plants, which are usually high in nutrients and easily decomposed, less favorable species increase in relative abundance and decompose more slowly, and nutrient cycling rates can decrease (Pastor et al. 1993, Pastor and Cohen 1997). Results of the effects of large ungulates (i.e. moose and deer) on nutrient cycling are mixed; sometimes N cycling increases (McNaughton et al. 1997, Frank and Groffman 1998, Stark et al. 2000), and sometimes N cycling decreases (Pastor et al. 1993, Ritchie et al. 1998, Côté et al. 2004) with ungulate herbivory. The relative magnitude of direct and indirect effects at play can vary and cause variation in browsing effects on nutrient cycling.

Temperate forests

Temperate forests represent 25% of forests worldwide, and regulate important ecosystem functions including energy cycling, hydrologic cycling, and atmospheric composition (Barnes et al. 1998). Globally, forests store an estimated 45% of terrestrial C in biomass and soils, and are becoming increaslingly important in a changing global climate (Landsberg and Waring 2014). Temperate forests occur in both the Northern and Southern hemisphere, and can be classified into three main biogeographic regions based on vegetation characteristics: Evergreen coniferous temperate fotests, evergeen deciduous temperate forests, and mixed temperate forests (Currie and Bergen 2008, Landsberg and Waring 2014).

Northern US forests are good systems to explore herbivore effects on ecosystem processes because deer densities have been increasing and range from almost 0 deer per km^2 to more than 20 deer per km^2 across this region (Mudrak et al. 2009). Moreover, most forests within the Northern US region are mixed temperate forests and thus comparable to one another (Barnes et al. 1998).

Soil components, processes, and feedbacks

Soil is central to forest functioning, providing physical support, recycling of nutrients, and water retention. Soils are diverse and rich with organisms including macrofauna (millipedes, worms, centipedes), mesofauna (arthropods), microbiota (protists, rotifers, nematodes), prokaryotes (bacteria, archaea), and eukaryotic microorganisms (fungi) (Thomas and Packham 2007). The energy and nutrient inputs to these organisms and the food webs they populate comes from litter produced by aboveground vegetation. Carbon and nutrients in litterfall are recycled through decomposition and mineralization. Nitrogen (N) is an important nutrient to make proteins in any living organism, including soil organisms and plants. Plant growth (and thus C sequestration) is limited by N, and relies on N mineralization by soil microbes (Vitousek et al.

2002). Soil nutrients generally occur in two forms: inorganic compounds dissolved in soil solution including nitrate and ammonium (NO_3^-, NH_4^+) or attached to minerals and organic compounds of living organisms and dead organic matter (Figure 1.2). Microbial communities, bacteria and fungi are always transforming nutrients between these two forms, mediated by environmental conditions (Balser and Firestone 2005, Högberg et al. 2013). When microorganisms consume inorganic compounds to construct cells, enzymes and other organic compounds needed to grow, they are said to be “immobilizing” nutrients. When organisms excrete inorganic waste compounds they are said to be “mineralizing” nutrients. These excreted inorganic waste compounds are essential elements for plant growth and are assimilated by plant roots to sustain growth. As a result, any changes in these biological processes will have consequences throughout trophic levels below- and above-ground (Wardle 2002).

Overall, soil is an essential part of terrestrial ecosystems, including forests. To fully understand herbivore effects on ecosystem functioning, one must consider effects on soils and the feedbacks between above- and belowground organisms and processes.

Deer impacts on temperate forests: what do we know?

Populations of large ungulates such as deer, moose, and elk, have increased to various degrees in the Midwest and Northeastern regions of the United States since the 19th century. Historically, deer abundance was estimated to be 2-4 deer per km^2, but since the 20th century, deer are the most abundant large mammal in the region with densities reaching >20 deer per km^2 (Alverson et al. 1988). The increase in deer abundance is partly due to milder winters, favorable habitat conditions, and winter provisioning. Deer habitat has also greatly improved because of game management policies focused on sustaining deer populations across much of the US (deCalesta and Stout 1997, Waller and Alverson 1997, Waller et al. 2009). Increasing deer populations have been posing an increasing threat to their natural environments by browsing heavily on the vegetation (Hobbs 1996). These large deer densities are capable of radically changing patterns of tree regeneration and forest floor plant community structure (Waller et al. 2009). Management has focused on monitoring the effects of deer browsing on understory plant species diversity and abundance (Frerker et al. 2013) and on tree regeneration (White 2012). Although research on this topic has been ongoing for some decades now, questions regarding effects of deer herbivory on ecosystem processes remain.

White-tailed deer

Range

The white-tailed deer (*Odocoileus virginianus*) is native to North, Central-, and South America, and can be found in 47 states in the US, as well as in Canada and Mexico. Its range spans from southern Yukon and the Northwest Territories, throughout the US, down to Central America ("NatureServe Explorer: An Online Encyclopedia of Life." 2013). The species also has been introduced in Serbia, Finland, New Zealand, and the Caribbean Islands (Vercauteren and Hygnstrom 2011). In the US, they are most abundant in the Great Lakes region and the Northeast, where mostly hardwood and coniferous temperate forests are common. During winter, conifer forests mixed with hardwood forests close to lakes and rivers are preferred habitat for white-tailed deer (Vercauteren and Hygnstrom 2011).

Physical description

The largest white-tailed deer individuals are found in the Northeast, where adult bucks weigh about 100 kg. Smaller subspecies are found near the equator, and at low elevations, where adult bucks weigh about 36 kg (Smith 1991). An average sized doe weighs about 60-75% of what adult bucks weigh (Feldhamer et al. 2003). Body size varies among regions, and is dependent on soil fertility (Turner 2004). Deer have particularly high body mass in areas with intense agriculture, such as in the Midwest, where deer have access to and forage on fertilized lands containing high nutrient crops.

Reproduction

In northern regions white-tailed deer breed in November, and the breeding season lasts about a month. Breeding seasons in more southern regions might not start until January, and near the equator deer breed year round (Turner 2004). Pregnancy lasts between 187 and 213 days, and fawning periods in the North are restricted to only a few months in the summer. Does typically move to specific fawning areas during pregnancy and remain there for 8 to 10 weeks (Feldhamer et al. 2003). Most white-tailed deer reach sexual maturity after one year and the average litter size is 2, but adults can have as many as 5 fawns per litter. Female reproductive success and fawn survival is highly dependent on nutritional status, with low nutrition resulting in lower fawn birth mass, longer pregnancies, and increased fawn mortality (Verme 1965, 1969).

Diet

White-tailed deer are browsing herbivores that consume a wide variety of plant species and plant parts, including stems, leaves, flowers, and seeds (Fulbright and Ortega-Santos 2013). Although deer may sample a large diversity of plant species, most of their diet consists of only a few key plant species. While diverse habitats throughout the white-tailed deer range may harbor many different plant species, deer diets typically consist of only 10% of the diversity in their habitat (Chamrad and Box 1968). This indicates that deer may selectively forage on the plant community. Average diets consist of 46% browse (green twigs and leaves), 24% forbs (net-veined, broad-leaved plants with non-woody stems), 11% mast , 8% grass, 4% agricultural crops and some other items, but diets vary substantially among regions and seasons (Turner 2004).

Implications of deer browsing

It is clear that large populations of native ungulates are threatening the diversity and integrity of the forests that sustain them (Rooney 2001, Côté et al. 2004, Waller et al. 2009). Management has focused on sustaining large deer populations because of their social and economic value mainly driven by the hunting industry (deCalesta and Stout 1997, Waller and Alverson 1997, Waller et al. 2009). In addition to management activities, the absence of large predators has contributed to the growth of deer populations (Alverson et al. 1988, Côté et al. 2004). As a result, large deer populations exist throughout the northeastern US, threatening the persistence of palatable plant species, reducing understory plant diversity, and slowing or diminishing regeneration of late successional tree species. It is therefore important to study interactions between deer and their environments and their effects on the ecosystem function (Waller et al. 2009).

White-tailed deer can influence plant species composition and diversity by selectively consuming palatable species and leaving the unpalatable species to increase in dominance in plant communities (Côté et al. 2004). This process changes plant community dynamics, which consequently alters patterns of forest succession (Waller and Alverson 1997; Rooney 2001; Russell et al. 2001; Rooney and Waller 2003; Côté et al. 2004) and could have downstream effects on ecosystem processes. However, it remains unclear specifically how deer browsing impacts nutrient cycles in temperate forests. A recent decomposition experiment on a forested island in Canada shows litter decomposition is slower with deer present (Sitka black-tailed deer, *Odocoileus hemionus sitkensis*), due to more recalcitrant litter (Chollet et al. 2019). Other studies have found contradicting effects of deer browsing on nutrient cycles; with slower decomposition in browsed Birch (*Betula pubescens*) in the Scottish Highlands (Harrison and Bardgett 2003), and increased decomposition rates in grazed tundra (Olofsson and Oksanen 2002). Clearly, direction of deer browsing effects (acceleration vs. deceleration) on nutrient cycling rates varies. But this variation thus far, has not been addressed in the deer browsing literature. In this dissertation I address the acceleration vs. deceleration hypotheses by investigating the contrasting effects of deer browsing on nutrient cycling in temperate forests and exploring potential underlying mechanisms.

Deer browsing in temperate forests: Dissertation outline

Deer interact with their environment in many ways, and research thus far has found inconsistent effects on soil biogeochemical processes as well as on plant community structure. Therefore, the effects of deer browsing on soils are difficult to predict, which is reflected in inconsistencies within and across studies. My goal for this dissertation is to explain the variation in browsing effects on ecosystem functioning and to explore underlying mechanisms. This dissertation includes three research chapters, each exploring the effects of deer browsing on different aspects of temperate forest ecosystems across an environmental gradient of soil fertility. By incorporating an environmental gradient, I aim to address some of the inconsistent, and sometimes confusing, variability in studies of deer impacts on forest ecosystems.

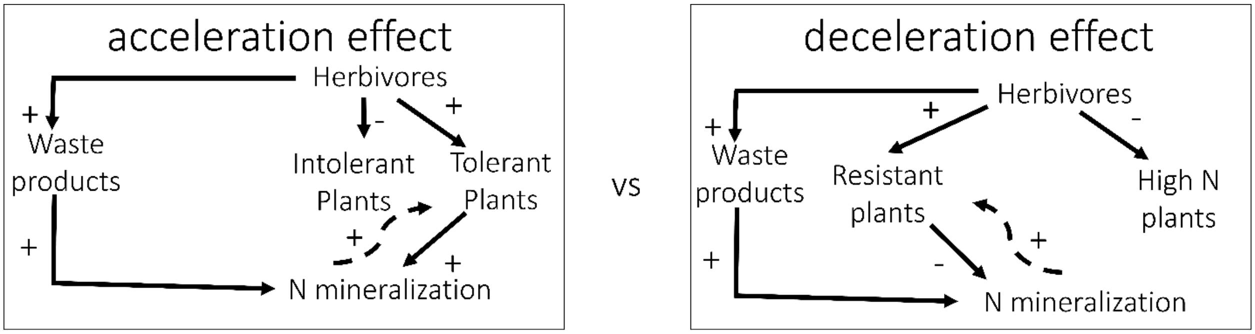
In **Chapter 2, "Deer browsing effects on temperate forest soil nitrogen cycling shift from positive to negative across fertility gradients",** I focus on the belowground biogeochemical cycles. My objective was to determine whether and how deer browsing influences soil C and N cycling rates in temperate forests, and whether effects of browsing change across fertility gradients. I predicted that deer browsing impacts on temperate forest soils are correlated with soil nutrient availability because soil fertility influences plant responses after browsing. This Chapter exists in published form (Popma and Nadelhoffer 2020).

Chapter 3, "Deer browsing decreases understory plant abundance but has no detectable effects on plant community composition", addresses the visible changes aboveground and shows how deer browsing alters plant community structure and plant community composition I predicted that under high soil nutrient availability, deer browsing would have significant effects on structural components of plant communities and that plant community composition would remain unaffected. My reasoning was that when resource availability is high, plants are more likely to have been selected to tolerate browsing damage via compensatory growth responses, and communities might not change as a result. In contrast, I predicted that under low soil nutrient availability deer browsing would significantly shift plant community composition towards better defended plant communities, because in resource-poor environments plants are more likely to have been selected to resist herbivory and cannot grow fast enough to outgrow deer browsing; selective browsing would shift community composition to species with better plant defenses.

Chapter 4, "**Deer browsing effects on plant chemical and nutrient concentrations**" investigates plant chemical traits in browsed and non-browsed plant communities. In this chapter I explore two mechanisms by which plant community chemistry might change: 1) intraspecific variation in plant species: how browsing-induced chemical defenses alter plant leaf chemistry, and 2) interspecific variation among plant species: how selective browsing alters the quality and abundance of leaf litter in the plant community. I predicted that plant defense compounds would increase with deer browsing in low nutrient environments and that plant chemical communities would change as a result. In contrast, I predicted that plant defense compounds would remain similar under high nutrient environments. The underlying premise here is that environments with low resource availability favor plant species with higher levels of defense compounds that lack the ability to allocate resources to compensatory growth in response to browsing. Also, I predicted that plant leaf N will increase with deer browsing under high nutrient environments, but will decrease under low nutrient environments, because resource rich environments select for fast-growing plants with low levels of costly defenses that outcompete plants producing costly defense compounds that diminish potentials for compensatory growth.

Finally, **Chapter 5, “Conclusion”** briefly summarizes and synthesizes the important findings from each research chapter. In this chapter I highlight the insights and support that my work provides into how interactions between above- and belowground processes are important drivers of ecosystem functioning. I also discuss further questions and considerations.

Figures



Modified from Ritchie et al.
Ecology (1998)

Figure 1.1 Herbivores can have accelerating and decelerating effects on ecosystem nutrient cycling. Accelerating effects are often associated with high nutrient environments, and decelerating effects in low nutrient environments.

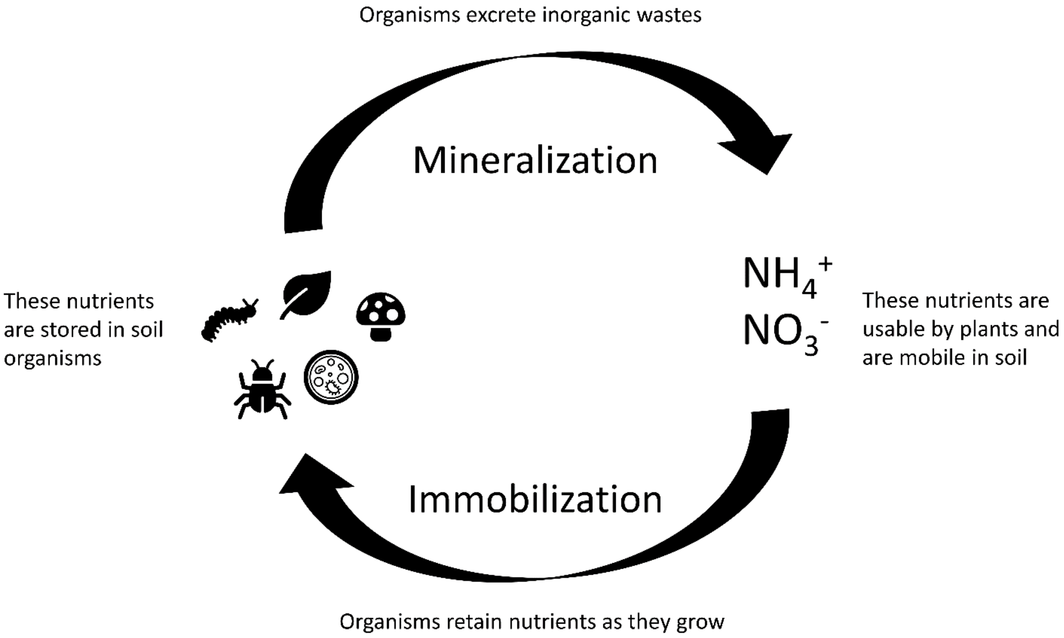


Figure 1.2 Microorganisms consume inorganic compounds to construct cells, enzymes and other organic compounds needed to grow, they are said to be “immobilizing” nutrients. When organisms excrete inorganic waste compounds they are said to be “mineralizing” nutrients. These excreted inorganic waste compounds are essential elements for plant growth and are assimilated by plant roots to sustain growth

References

Abrams, P. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. Am. Nat. **146**(1): 112–134. doi:10.2307/2678832.

Alverson, W.S., Waller, D.M., and Solheim, S.L. 1988. Forests Too Deer : Edge Effects Forests Northern Wisconsin. Conserv. Biol. **2**(4): 348–358. doi:10.1111/j.1523-1739.1988.tb00199.x.

Balser, T.C., and Firestone, M.K. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry **73**: 395–415. doi:10.1007/s10533-004-0372-y.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., and Hobbs, P.J. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub montane ecosystems. **33**: 1653–1664. doi:10.1016/S0038-0717(01)00086-4.

Barnes, B. V., Zak, D.R.,.H Denton, S.R Spurr, S. 1998. Forest ecology. John Wiley & Sons, Inc. Available from https://books.google.com/books/about/Forest_ecology.html?id=bKoPAQAAMAAJ&pgis=1.

Barthelemy, H., Stark, S., and Olofsson, J. 2015. Strong Responses of Subarctic Plant Communities to Long-Term Reindeer Feces Manipulation. Ecosystems **18**: 740–751. doi:10.1007/s10021-015-9856-y.

Chamrad, A.D., and Box, T.W. 1968. Food Habits of White-Tailed Deer in South Texas'. J. Range Manag. **21**(3): 158–164.

Cherif, M., and Loreau, M. 2013. Plant-herbivore-decomposer stoichiometric mismatches and nutrient cycling in ecosystems. Proc. R. Soc. B **280**: 20122453. doi:10.1098/rspb.2012.2453.

Chollet, S., Maillard, M., Grayston, S., and Martin, J. 2019. Deer slow down litter decomposition by reducing litter quality in a temperate forest. Peer Community Ecol.: 1–32.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C., and Waller, D.M. 2004. Ecological Impacts of Deer Overabundance. Annu. Rev. Ecol. Evol. Syst. **35**(2004): 113–147. doi:10.1146/annurev.ecolsys.35.021103.105725.

Currie, W.S., and Bergen, K.M. 2008. Temperate Forest. *In* Encyclopedia of Ecology. *Edited by* S.E. Jørgensen and B.D. Fath. Academic Press, Oxford. pp. 3494–3503. doi.org/10.1016/B978-008045405-4.00704-7.

Daleo, P., Alberti, J., Pascual, J., Canepuccia, A., and Iribarne, O. 2014. Herbivory affects salt marsh succession dynamics by suppressing the recovery of dominant species. Oecologia **175**: 335–343. doi:10.1007/s00442-014-2903-0.

deCalesta, D.S., and Stout, S.L. 1997. Relative deer density and sustainability : a conceptual framework for integrating deer management with ecosystem management. Wildl. Soc. Bull. **25**(2): 252–258.

Feldhamer, G., Thompson, B., and Chapman, J. (*Editors*). 2003. Wild Mammals of North America: Biology, Management, and Conservation. JHU Press. Available from https://books.google.com/books?id=-xQalfqP7BcC&pgis=1.

Findlay, S., Carreiro, M., Krischik, V., and Jones, C.G. 1996. Effects of Damage to Living Plants on Leaf Litter Quality. Ecol. Appl. **6**(1): 269–275. doi:10.2307/2269570.

Forbes, E. S., J. H. Cushman, D. E. Burkepile, T. P. Young, M. Klope, and H. S. Young. 2019. Synthesizing the effects of large, wild herbivore exclusion on ecosystem function. Functional Ecology 33:1597–1610.

Frank, D.A., and Groffman, P.M. 1998. Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone National Park. Ecology **79**(7): 2229–2241. doi:10.1890/0012-9658(1998)079[2229:UVLCOS]2.0.CO;2.

Frerker, K., Sonnier, G., and Waller, D.M. 2013. Browsing rates and ratios provide reliable indices of ungulate impacts on forest plant communities. For. Ecol. Manage. **291**: 55–64. doi:10.1016/j.foreco.2012.11.041.

Fulbright, T.E., and Ortega-Santos, J.A. 2013. White-Tailed Deer Habitat: Ecology and Management on Rangelands. Texas A&M University Press. Available from https://books.google.com/books?hl=en&lr=&id=t9BGvuVx6eAC&pgis=1.

Gilliam, F.S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. Bioscience **57**(10): 845. doi:10.1641/B571007.

Green, M. L., A. C. Kelly, D. Satterthwaite-Phillips, M. B. Manjerovic, P. Shelton, J. Novakofski, and N. Mateus-Pinilla. 2017. Reproductive characteristics of female white-tailed deer (Odocoileus virginianus) in the Midwestern USA. Theriogenology 94:71–78.

Harrison, K.A., and Bardgett, R.D. 2003. How browsing by red deer impacts on litter decomposition in a native regenerating woodland in the Highlands of Scotland. Biol. Fertil. Soils **38**: 393–399. doi:10.1007/s00374-003-0667-5.

Hidding, B., Tremblay, J.P., and Cǒté, S.D. 2013. A large herbivore triggers alternative successional trajectories in the boreal forest. Ecology **94**(12): 2852–2860. doi:10.1890/12-2015.1.

Hobbie, S.E. 1992. Effects of plant species on nutrient cycling. Trends Ecol. Evol. **7**(1975): 336–

9. doi:10.1016/0169-5347(92)90126-V.

Hobbs, N.T. 1996. Modifications of ecosystems by ungulates. J. Wildl. Manage. **60**(4): 695–713.

Högberg, M.N., Högbom, L., and Kleja, D.B. 2013. Soil microbial community indices as predictors of soil solution chemistry and N leaching in Picea abies (L .) Karst . forests in S . Sweden. Plant Soil **372**(1–2): 507–522. doi:10.1007/s11104-013-1742-9.

Hunter, M.D. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agric. For. Entomol. **3**: 77–84. doi:10.1046/j.1461-9563.2001.00100.x.

Hunter, M.D., Reynolds, B.C., Hall, M.C., and Frost, C.J. 2012. Trait-Mediated Indirect Interactions. *Edited By*T. Ohgushi, O. Schmitz, and R.D. Holt. Cambridge University Press, Cambridge. doi:10.1017/CBO9780511736551.

Hunter, M.D., and Schultz, J.C. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. Oecologia **94**(2): 195–203. Springer.

Landsberg, J., and Waring, R. 2014. Forests in Our Changing World. Island Press/Center for Resource Economics. doi:10.5822/978-1-61091-497-0.

Leibold, M. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. Am. Nat. **134**(6): 922–949. doi:10.2307/2678832.

Mcnaughton, S.J. 1985. Ecology of a Grazing Ecosystem : The Serengeti. Ecol. Monogr. **55**(3): 259–294.

McNaughton, S.J. 1976. Serengeti migratory wildebeest: facilitation of energy flow by grazing. Science **191**(4222): 92–94. doi:10.1126/science.191.4222.92.

McNaughton, S.J., Banyikwa, F.F., and McNaughton, M.M. 1997. Promotion of the Cycling of Diet-Enhancing Nutrients by African Grazers. Science (80-.). **278**(December): 1798–1801. doi:10.1126/science.278.5344.1798.

Mudrak, E.L., Johnson, S.E., and Waller, D.M. 2009. Forty-Seven Year Changes in Vegetation at the Apostle Islands: Effects of Deer on the Forest Understory. Nat. Areas J. **29**(2): 167–176.

Murray, B.D., Webster, C.R., and Bump, J.K. 2013. Broadening the ecological context of ungulate-ecosystem interactions: The importance of space, seasonality, and nitrogen. Ecology **94**(6): 1317–1326. doi:10.1890/12-1582.1.

NatureServe Explorer: An Online Encyclopedia of Life. 2013. Available from http://explorer.natureserve.org/index.htm.

Olofsson, J., and Oksanen, L. 2002. Role of Litter Decomposition for the Increased Primary

Production in Areas Heavily Grazed by Reindeer : A Litterbag Experiment. Oikos **96**(3): 507–515.

Pastor, J., and Cohen, Y. 1997. Herbivores, the functional diversity of plants species, and the cycling of nutrients in ecosystems. Theor. Popul. Biol. **51**: 165–179. doi:10.1006/tpbi.1997.1327.

Pastor, J., Dewey, B., Naiman, R.J., Mcinnes, P.F., Cohen, Y., and Mar, N. 1993. Moose Browsing and Soil Fertility in the Boreal Forests of Isle Royale National. Ecology **74**(2): 467–480. doi:10.1006/tpbi.1997.1327.

Persson, I.L., Pastor, J., Danell, K., and Bergström, R. 2005. Impact of moose population density on the production and composition of litter in boreal forests. Oikos **108**(2): 297–306. doi:10.1111/j.0030-1299.2005.13844.x.

Pierce, S., Luzzaro, A., Caccianiga, M., Ceriani, R.M., and Cerabolini, B. 2007. Disturbance is the principal alpha-scale filter determining niche differentiation, coexistence and biodiversity in an alpine community. J. Ecol. **95**: 698–706. doi:10.1111/j.1365-2745.2007.01242.x.

Ritchie, M.E., and Tilman, D. 1995. Responses of legumes to herbivores and nutrients during succession on a nitrogen-poor soil. Ecology **76**(8): 2648–2655. doi:10.2307/2265835.

Ritchie, M.E., Tilman, D., and Knops, J.M.H. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. Ecology **79**(1): 165–177. doi:10.1890/0012-9658(1998)079[0165:HEOPAN]2.0.CO;2.

Rooney, T.P. 2001. Deer impacts on forest ecosystems: a North American perspective. Forestry **74**(3): 201–208. doi:10.1093/forestry/74.3.201.

Rooney, T.P., and Waller, D.M. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. For. Ecol. Manage. **181**: 165–176. doi:10.1016/S0378-1127(03)00130-0.

Russell, F., Zippin, D., and Fowler, N. 2001. A Review of Effects of White-tailed Deer (Odocoileus virginianus) on Plants, Plant Populations and Communities. Am. Midl. Nat. **146**(1): 1–26. doi:10.1674/0003-0031(2001)146[0001:EOWTDO]2.0.CO;2.

Sitters, J., and H. Olde Venterink. 2015. The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling. Plant and Soil 396:421–426.

Smith, W. 1991. Odocoileus virginianus. Am. Soc. Mammal.: 1–13.

Stark, S., Wardle, D.A., Ohtonen, R., Helle, T., and Yeates, G.W. 2000. The effect of reindeer grazing on decomposition, mineralisation and soil biota in a dry oligotrophic Scot pine forest. Oikos **90**(2): 301–309.

Sterner, R.W., and Elser, J.J. 2002. Ecological Stoichiometry: The Biology of Elements from

Molecules to the Biosphere. Princeton University Press. Available from https://books.google.com/books?id=53NTDvppdYUC&pgis=1.

Thomas, P., and Packham, J. 2007. Ecology of Woodlands and Forests : Description, Dynamics and Diversity. Cambridge University Press, Cambridge, UNITED KINGDOM. Available from http://ebookcentral.proquest.com/lib/umichigan/detail.action?docID=803013.

Tilman, D. 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press. Available from https://books.google.com/books?hl=en&lr=&id=HYnSkKTKYegC&pgis=1.

Vercauteren, K., and Hygnstrom, S.E. 2011. Managing White-tailed Deer : Midwest North America. Pap. Nat. Resour.

Verme, L.J. 1965. Reproduction Studies on Penned White-Tailed Reproduction. J. Wildl. Manage. **29**(1): 74–79.

Verme, L.J. 1969. Reproductive patterns of white-tailed deer related to nutritional plane. J. Wildl. Manage. **33**(4): 881–887. doi:10.2307/3799320.

Vitousek, P.M., Hättenschwiler, S., Olander, L., Allison, S., Ambio, S., and Nitrogen, O. 2002. Nitrogen and Nature. Ambio **31**(2): 97–101.

Waller, D.M., and Alverson, W.S. 1997. Ecological Aspects of Management: The White-tailed deer. Wildl. Soc. Bull. **25**(2): 217–226. Available from http://www.jstor.org/stable/3783435.

Waller, D.M., Johnson, S., Collins, R., and Williams, E. 2009. Threats posed by ungulate herbivory to forest structure and plant diversity in the Upper Great Lakes Region-with a review of methods to assess those threats. *In* Natural Resource Report NPS/GLKN/NRR–2009/102. Fort Collins, Colorado. Available from http://scholar.google.com/scholar?q=related:lAqlEY3oju8J:scholar.google.com/&hl=en&num=30&as_sdt=0,5%5Cnpapers2://publication/uuid/155B5688-0373-4904-872E-A94DABA444BC.

Wardle, D.A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press. Available from https://books.google.com/books?hl=en&lr=&id=b35IAaamCDcC&pgis=1.

White, M.A. 2012. Long-term effects of deer browsing: Composition, structure and productivity in a northeastern Minnesota old-growth forest. For. Ecol. Manage. **269**: 222–228. Elsevier B.V. doi:10.1016/j.foreco.2011.12.043.

Chapter 2 Deer Browsing Effects on Temperate Forest Soil Nitrogen Cycling Shift from Positive to Negative Across Fertility Gradients

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Abstract

Herbivores impact soil biogeochemical processes, often increasing nutrient cycling rates under high nutrient availability and decreasing nutrient cycling rates under low nutrient availability. These patterns are far from universal, and interactions between habitat fertility and herbivore effects are under continuing investigation. By sampling inside and outside a network of deer exclosures, we determined deer browsing effects on temperate forest soil N and C cycling along a gradient of soil and litter C:N ratios across our network of sites. Deer browsing increased net N mineralization rates in high nutrient environments and decreased N mineralization rates in low nutrient environments, whereas browsing decreased CO_2 respiration rates in high nutrient environments and increased CO_2 respiration rates in low nutrient environments. Differences in deer browsing effects on soil processes could be explained by plant responses to herbivory across gradients of resource availability. To our knowledge, our study is one of the first to show that deer browsing can have significant effects on net N mineralization and C respiration in temperate forest soils and that the direction and magnitude of deer browsing effects on soil N and C cycling vary across fertility gradients.

Introduction

Herbivores are known to have strong effects on ecosystem processes such as primary production (Milchunas and Lauenroth 1993), fire regimes (Van Langevelde et al. 2003, Holdo et al. 2009), plant community composition (Gornall et al. 2009, Frerker et al. 2014), and ecosystem type shifts (Zimov et al. 1995). Although there has been progress in understanding biogeochemical responses to grazing in grassland ecosystems (McNaughton et al. 1997), boreal forests (Pastor and Cohen 1997), and managed lands (Bardgett et al. 2001), it remains difficult to predict how herbivores influence soil biogeochemical processes. Conceptual models often predict increased nutrient cycling rates under high nutrient availability and decreased nutrient cycling rates under low nutrient availability (Pastor et al. 2006, Cherif and Loreau 2013). Nutrient rich environments favor plants with high nutrient contents, and herbivores increase nutrient cycling by excreting highly labile N and P products, thus stimulating compensatory plant growth in grazed or browsed plants. For example, herbivory in nutrient rich grasslands can result in enhanced nutrient concentrations in leaves (Holland and Detling 1990, Hamilton and Frank 2001) and similar results have been found in boreal forest ecosystems (Kielland et al. 1997). In contrast, damaged plants can become tougher and increase polyphenol concentrations in response to tissue loss due to herbivory (Hunter and Schultz 1993), which consequently decreases decomposition and nutrient cycling rates (Findlay et al. 1996).

These studies highlight the importance of studying effects of herbivores along environmental gradients. Studies that address mammalian herbivore effects on soil processes have mostly focused on grasslands, while forest ecosystems are less well studied. While grasslands cover a substantial area globally, forests are widespread as well and are strongly linked to global carbon, nutrient, water, and energy cycles. Studies show mammalian herbivores drive changes in forest plant communities and successional pathways, but a research gap remains in predicting their effects on soil biogeochemical processes.

In order to make better general predictions about mammalian herbivore effects on soil biological processes, we investigated C and N dynamics in temperate forest ecosystems in the U.S. Midwest. Forested regions of the midwestern and northeastern US have experienced increases in white-tailed deer (*Odocoileus virginianus* an ungulate browser, hereafter referred to as “deer”) populations since the 1970s, with densities of up to 20 deer per km^2 in some areas (Alverson et al. 1988, DNR 2010). Deer browsing has well known effects on forest vegetation, such as altering plant community composition and forest successional pathways (Waller and Alverson 1997, Rooney 2001, Russell et al. 2001, Rooney and Waller 2003, Côté et al. 2004, Turner 2004, Frerker et al. 2014). It is not known, however, how and to what extent deer influence forest soil carbon and nutrient cycling. Research thus far indicates three general mechanisms through which ungulates, including deer, can affect soil nutrient cycling. The first is by changing the *quantity* of plant inputs to soils. Ungulates affect net primary production, and therefore litter production (Pastor et al. 1993, Harrison and Bardgett 2003). The second is by shifting plant community composition; thereby affecting the chemical *quality* of litter inputs to soil. Selective and intensive herbivory in plant communities can favor less palatable plant tissues and produce more recalcitrant litter which decomposes slowly (Grime et al. 1996, Cornelissen et al. 2004), thereby slowing nutrient cycles and soil respiration. The third involves introducing *excretion products* (feces & urine) to soils that can be high in labile N and other nutrients and that decompose rapidly (Bump et al. 2009, Murray et al. 2013). However, effects of excretion products are not always positive, and can vary across forage quality gradients due to shifts in herbivore excretion pathways (Pastor et al. 2006).

Few studies have identified the effects of mammalian herbivory on forest soil nutrient cycling and most predictions are based on theoretical conceptual models (Seagle et al. 1992, Hobbs 1996, Côté et al. 2004, Harrison and Bardgett 2004, Abbas et al. 2012, Murray et al. 2013). Although direct effects of deer excretion and cadavers are known to alter biogeochemistry on smaller spatial scales (Bump et al. 2009), it is unknown to what extent indirect effects such as selective browsing alter nutrient cycling rates at larger spatial scales. In this paper we present results of our study of deer browsing effects on N and C cycling rates in north temperate deciduous forests located in southeastern Michigan, USA. Our objective was to determine whether and how deer browsing influences soil C and N cycling rates in temperate forests, and whether effects of browsing change across fertility gradients. We predicted that deer browsing impacts on temperate forest soils are influenced by soil nutrient availability because soil fertility influences plant responses after browsing.

Methods

Study sites

We selected nine study sites in Oakland, Macomb, and Wayne counties in the greater Detroit Metro area of Michigan, USA. Temperate forests in the US Midwest and Northeast such as those we selected have experienced increases in deer populations since the 1970s, with densities reaching up to 20 deer per km^2 in some areas (Alverson et al. 1988, DNR 2010). The study areas lie within three Huron Clinton Metroparks: Kensington (42°32'28.1"N 83°38'16.2"W), Stony Creek (42°42'36.2"N 83°04'16.8"W), and Oakwoods (42°06'57.8"N 83°21'28.1"W) (Figure 2.1). We sampled within and outside of nine 10m x 10m experimental deer exclosures constructed in 1999, three in each of the three Metroparks. These 20-yr-old exclosures are in dry-mesic and mesic forest types. Dry-mesic forests are oak or oak-hickory forest types in southern Lower Michigan and typically grow on sandy loam or loamy soils. Mesic forests in southern Michigan are typically dominated by American beech and sugar maple, growing on loamy upland soils. The exclosures are 2.5m tall and exclude ungulates (deer) while allowing small herbivores to move in and out. Mean annual temperature in southeastern Michigan is 9°C, mean annual rainfall is 81cm, mean annual snowfall is 130cm (U.S. Climate Data), and mean annual litterfall is 3422 kg dry mass ha^{-1} y^{-1} (See Table 2.1). The main ungulates are white-tailed deer (*Odocoileus virginianus*), with densities of 8, 8, and 16 deer per square kilometer at the Stony Creek, Oakwoods and Kensington parks, respectively. Deer populations are estimated and actively managed by Huron Clinton Metroparks (Ryan Colliton, personal communication 2018). Helicopter fly-over estimates for Oakwoods were more difficult to obtain due to proximity to the Detroit Metropolitan Airport airspace and could potentially be over- or under-estimating deer population densities.

Soil sampling

We established a 10 x 10m plot outside each exclosure with matching slope and aspect and collected one composite soil sample inside and outside of each deer exclosure (n=9) in June 2017. Each composite sample consisted of five 2.5cm diameter soil cores sampled to a depth of 10cm. Cores were collected randomly from within each fenced and nearby unfenced plot. Composite soil samples were stored in a cooler in the field until refrigerated upon return to our laboratory at the University of Michigan in Ann Arbor. Soil samples were sieved using a 2-mm sieve and roots were removed manually. Composite soil samples were used for determining moisture, pH, %C, %N, C:N, and rates of N mineralization, nitrification, and CO_2 respiration. Soil pH was determined using an Accumet® pH meter, on 15g fresh, sieved soil duplicate subsamples diluted to a 1:2 ratio of soil to deionized water. C:N, %C, %N soil were determined using a CN Analyzer (University of Michigan Biological Station Analytical Lab, Pellston, MI, USA).

Soil processes

Fresh soils were stored at 4°C and prepared within 5 days of collection for an 8-week laboratory incubation experiment to determine the effects of large herbivores on rates of net N mineralization, net nitrification, and microbial respiration. Samples were brought to field capacity prior to laboratory incubations, and we dried a subsample of each composite for 48h at 80°C to determine moisture content. We incubated 5 composite soil sample replicates (10g fresh soil each) at 20°C in 500 ml Thermo Scientific® clear glass jars equipped with septa for headspace gas sampling(Robertson et al. 1999). Microbial respiration was estimated by sampling CO_2 accumulation every other week in the headspace during an 8wk incubation. CO_2 concentrations were measured using a Thermo Fisher Trace GC 2000 with HS2000 autosampler. After each CO_2 sampling event, incubation jars were aerated and soils within each jar were brought back up to field capacity. CO_2 respiration rate (µmol C m^{-2} sec^{-1}) calculations were based on the sum of CO_2 respired over 8 weeks. Net N mineralization rate (g N g $soil^{-1}$ d^{-1}) and % nitrification were calculated by extracting initial and final NH_4^+ and NO_3^- from soil using a 1N KCl solution (Robertson et al. 1999). We measured NH_4^+ and NO_3^- using a Smartchem Westco Scientific Instruments Inc. analyzer, then used solution volumes, ion concentrations, and soil dry mass equivalents to calculate masses of NH_4^+-N and NO_3^--N in each sample prior to and following incubation. C and N cycling rates were scaled to a per area basis using the surface area of our soil cores and bulk density data. All rates are expressed per m^{-2}, from the soil surface to 10 cm depth.

Leaf litter

Leaf litter fall was collected inside each exclosure (n=9) between October and December 2018. Collection baskets were 66cm x 46cm, modified from commercial laundry baskets by drilling drainage holes in the bottoms. We placed five leaf litter baskets inside each exclosure in

October and collected leaf litter in December. Leaf litter was dried for 48h at 80°C to estimate biomass productivity. Leaf litter input C:N was analyzed for each individual species. Leaves were ground for 2 minutes to a fine powder for C:N, %C, and %N analyses. Site C:N ratios were calculated by first multiplying the mass of each represented species by the %C and %N in the litter of that species, resulting in grams of C and N in each species' litter for that site. Site level C:N ratios were then calculated by adding up mass of C and N of all the species at each site, and dividing total grams C by total grams N.

Data analysis

Data analyses were conducted using R Studio (version 3.5.1) and significance was accepted at $\alpha=0.05$. We used linear mixed effects model analyses from the lme4 R-package to test whether deer browsing, our treatment variable, altered rates of N mineralization (n=80) and CO_2 respiration (n=80). We also included soil and litter C:N ratios (measures of fertility) in our models as predictors because fertility strongly affects nitrogen and carbon dynamics and varied among our parks and sites. We considered two-way interactions between deer browsing treatment and fertility. Park (n=3) and site (n=8) were used as random nested effects because we expected rates to be influenced by geographic location. We excluded data from the Lake Loop site in Stony Creek because the leaf litter C:N average was more than 2 standard deviations outside the distribution of other plots, likely due to the presence of large amounts of *Tilia americana* (American basswood) leaf litter at this site. Because there was no correlation between C:N ratios in soil and litter we chose to compare their individual effects on the magnitude and direction of deer browsing effects on net rates of N mineralization and CO_2 respiration in two separate models. N mineralization and CO_2 respiration rates were calculated based on incubations of soil samples collected from outside and inside of deer exclosures at each site.

Results

The nine exclosure locations represented gradients in litter and soil C:N ratios (Table 2.1). Litter C:N ratios ranged from 59.2 at the Spring Hill (SH) plot to 62.4 at the West Branch (WB) plot. Soil C:N ratios ranged from 11.5 to 18.5, at Wild Wing (WW) and Rail Road (RR) respectively (Table 2.1). Soil C:N ratios were not correlated with litter C:N ratios (Supplementary figure 2.1) across sites, therefore soil C:N and litter C:N ratios at our study sites were treated as independent factors. Litterfall measurements ranged from 273 g m^{-2} yr^{-1} at WW to 392 g m^{-2} yr^{-1} at Big Tree (BT) (Table 2.1). Rough deer density estimates showed that our study sites represented a range from medium (8 deer per km^2) to high (16 deer per km^2) population densities (Table 2.1). Deer densities are generally considered high at levels > 10-15, medium at 7-12, low at <7 deer per km^2 (Russell et al. 2001).

N mineralization and CO_2 respiration rates across fertility gradients

Overall net N mineralization rates were positively correlated with both litter and soil C:N (Supplementary figures. 2.2, 2.3), and this correlation was true for both browsed and non-browsed plots as well (Supplementary figures 2.5, 2.6, 2.7). Although we did not detect a significant trend between N mineralization rates in browsed plots and litter C:N ratios (Supplementary figure 2.4). Overall CO_2 respiration rates were not significantly correlated with either litter or soil C:N ratios (Supplementary figure 2.8, 2.9), and no significant trends were detected when browsed and non-browsed plots were analyzed separately either. (Supplementary figures 2.10, 2.11, 2.12, 2.13). N mineralization rates and CO_2 respiration rates both showed significant grouping based on exclosure location (site) and some grouping was detected based on park location (Tables 2,3,4,5).

Browsing effects on N mineralization and CO_2 respiration rates across a fertility gradient

Rates of N mineralization and CO2 respiration differed among sites, and the effect of deer browsing varied from positive, to neutral, to negative (Figures 2.2, 2.3). As predicted, variation in deer browsing effects on N mineralization and CO_2 respiration was significantly correlated with fertility gradients, however we found no effects on nitrification. Mixed effects models show that soil and litter C:N ratios influence the strength and direction of deer browsing effects on N mineralization ($p<0.0001$, Tables 2,3), and litter C:N ratios influence the strength and direction of deer browsing effects on CO_2 respiration ($p<0.01$, Table 2.5).

Net N mineralization rates in incubated soils from non-browsed plots ranged from 0.15 to 3.33 g N g soil $^{-1}$ d^{-1}, at BT and WB respectively (Table 2.1) and deer browsing effects on net N mineralization differed across soil and litter C:N ratios (Figure 2.4 a,b). Browsing decreased net N mineralization rates at sites with high soil and litter C:N ratios and increased rates at sites with low soil and litter C:N ratios (Figure 2.4 a,b).

Soil CO_2 respiration in incubations from non-browsed plots ranged from 1.51 to 5.00 µmol C m^{-2} sec^{-1} (Table 2.1) and the effects of deer browsing on CO_2 respiration differed significantly across litter C:N ratios, but not across soil C:N ratios (Tables 4,5). The direction of deer browsing effect on CO_2 respiration versus litter C:N was opposite of the effects of browsing on N mineralization. Deer decreased CO_2 respiration at sites with low litter C:N ratio, and increased CO_2 respiration at sites with high litter C:N ratios (Figure 2.4).

Model R^2 values were much higher when including the explanatory power of random effects (conditional R^2) compared to model R^2 values including only the explanatory power of fixed effects (marginal R^2), with values ranging between 0.68-0.95 and 0.02-0.23 respectively (Tables 2,3,4,5). Our models greatly improved when including nested random effects of park and site.

Discussion

Our results show that deer browsing effects on soil N mineralization switch from positive to negative, and that effects on soil respiration switch from negative to positive across gradients of increasing litter and soil C:N ratios. Results are consistent with the acceleration vs. deceleration framework, in which resource availability determines the direction of indirect herbivore effects on ecosystem processes (Côté et al. 2004, Schmitz et al. 2015). Deer browsing research in temperate forests has historically focused on changes in plant communities and forest succession. To our knowledge, our study is one of the first to show that deer browsing effects on soil nutrient cycling in temperate forests are significant and variable across habitat fertility gradients.

Deer browsing increased N mineralization in high nutrient conditions and decreased N mineralization in low nutrient conditions in our forest sites (Figure 2.4 a,b). This shift in browsing effects across fertility gradients could be explained by multiple, not mutually exclusive, mechanisms. Firstly, shifts in the chemical landscape of plant communities due to browsing can lead to changes in soil biogeochemical cycling. When resource availability is low, plants are likely to invest in becoming more resistant when browsed on, resulting in tougher leaves that have higher defense and more recalcitrant secondary compound concentrations in the understory (Bardgett and Wardle 2003). This defense against herbivory also likely confers resistance against microbial breakdown, thereby down-regulating soil nutrient cycling (Findlay et al. 1996). The opposite effects are found in high nutrient environments, where plants can tolerate browsing by investing in compensatory growth, thereby stimulating microbial community N turnover with inputs of high quality litter (Bardgett and Wardle 2003).

Secondly, the shift in browsing effects could be explained by a shift in plant communities towards species that are inherently better at deterring herbivores (in low nutrient systems) or tolerating herbivores (in high nutrient systems) (Ritchie et al. 1998, Bardgett and Wardle 2003, Wardle David A. et al. 2004).

Finally, browser excretion decomposability can shift across gradients of foliar nutrient quality (Pastor et al. 2006). Using conceptual models, Pastor et al. showed that when browsers are starved for N due to low %N in diet, N retention is high, and excretory products mostly consist of recalcitrant fecal material. Whereas when diet %N is high, browsers excrete N-rich urine that is readily available to plants. Their model suggests this shift in excretory pathway happens around 1.5% N in forage. When forage is higher than 1.5% N, N rich urea can promote microbial activity in soil. When forage is lower than 1.5% N browser excretion is N- poor and is slow to mineralize and become available for plant uptake. Although we did not directly measure %N of foliage, our observed tipping point in deer effects on soil biogeochemical cycling is at $\log_{10}$ C:N 1.783 (Figure 2.4a,c). Assuming C is approximately 50% of dried litter mass, and 50% of N is reabsorbed before litter senesces, a $\log_{10}$ C:N 1.783 translates roughly to 1.6%N of green leaves, which is close to the modeled 1.5% N. To test these mechanisms, more studies are needed that investigate plant community responses, browser excretory pathways, and soil nutrient cycling across fertility gradients in temperate forests.

Effects of deer browsing on soil CO_2 respiration were opposite those on net N mineralization. Deer browsing decreased CO_2 respiration in high nutrient environments and increased CO_2 respiration in low nutrient environments (Figure 2.4d). The contrasting patterns of deer browsing effects on CO_2 respiration and net N mineralization rates could be explained by microbial community responses to amendments in N inputs. As mentioned before, at high nutrient sites, plants can tolerate browsing by increasing productivity. This increased productivity likely leads to more N-rich plant detritus (low C:N) entering the soil decomposition cycle, which can reduce microbial C respiration (Frey et al. 2014). Frey et. al. show that chronic N additions consistently suppressed C respiration and enzyme activity in N addition plots in a temperate forest. Their responses in the microbial community were highest in the organic horizon of the hardwood forest soil, which correlates to our study where we sampled organic and mineral soils. Furthermore, molecular analyses of the soil communities at the Harvard forest sites in Frey et. al.'s study showed that N additions also resulted in a shift in bacterial and fungal communities (Turlapati et al. 2013). The opposite seems to be true at low nutrient sites, where N is less available. At low nutrient sites plants often respond to browsing by increasing resistance. Increased resistance leads to more recalcitrant, N poor plant material (high C:N) entering the soil decomposition cycle, increasing microbial CO_2 respiration in order to access N.

Changes in aboveground plant community structure and chemical composition caused by deer browsing likely affect the belowground phytochemistry environment as well (Hunter 2008). Aboveground herbivory can signal roots to produce chemical defense compounds and can alter root nutrient concentration (Seastedt et al. 1988, Baldwin et al. 1994). Plants can reallocate newly acquired resources away from areas under attack (Tao and Hunter 2013). This functions not only to protect resources from being eaten, but also to reduce nutritional value and attractiveness to herbivores (Tao and Hunter 2011). Although we did not directly measure root properties, differences in reallocation strategies could influence the direction and strength of deer browsing effects on soil N and C cycling. Depending on plant nutrient availability, reallocation strategies of plants could possibly shift across fertility gradients. For example, moose (*Alces americanus*) browsing affects belowground processes in Alaskan taiga ecosystems, by significantly decreasing root production (Kielland et al. 1997). Deer are likely to suppress fine root biomass production as was demonstrated for moose browsing (Kielland et al. 1997), which could consequently affect mycorrhizal fungi associations, nematodes and soil insects (Rooney and Waller 2003). Moose and deer are similar in that both are ungulates that feed on forest ground vegetation. However, given that deer predominantly browse in temperate forest ecosystems and moose in more boreal regions, moose and deer browsing should not be generalized. Therefore, future research on the effects of deer browsing on forest ecosystems should focus more on belowground processes linked to aboveground alterations due to deer. Deer density could also have affected the direction of browsing effects on CO_2 respiration and net N mineralization rates. Because our deer density population estimates were limited and broad, we could not address this factor appropriately with our model. Importantly, however, all sites had high deer densities, ranging from 8 to 16 deer km^{-2}, and no sites had low deer densities. Additionally, population estimates at the Oakwoods park have a larger margin of error due to flying restrictions during helicopter surveys. It would be interesting to study the effects of deer population density in more detail, specifically to test whether intermediate levels of deer browsing can have positive effects on plant diversity and nutrient cycling, but effects diminish as deer densities pass a certain threshold. Consistent with the hypothesis historically known as the grazing optimization hypothesis, plant productivity can sometimes increase under moderate levels of herbivory (Owen and Wiegert 1976). Following the grazing optimization hypothesis, herbivory can result in premature leaf abscission, stimulating decomposition and nutrient cycling. To fully understand the effects of deer density on the direction and strength of browsing effects on N mineralization, studies are needed that include sites with more accurate estimates of deer population densities and include sites with low deer density populations.

In conclusion, our results provide evidence that deer browsing effects on C and N cycling in temperate forests are significant and can shift completely in strength and direction depending on site fertility. We also show that deer browsing effects on C cycling are opposite to those on N cycling, but both show a shift in direction across fertility gradients. These results highlight the importance of studying herbivore effects along environmental gradients. Understanding the underlying mechanisms of deer on their environment across gradients is an important future challenge.

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Figures

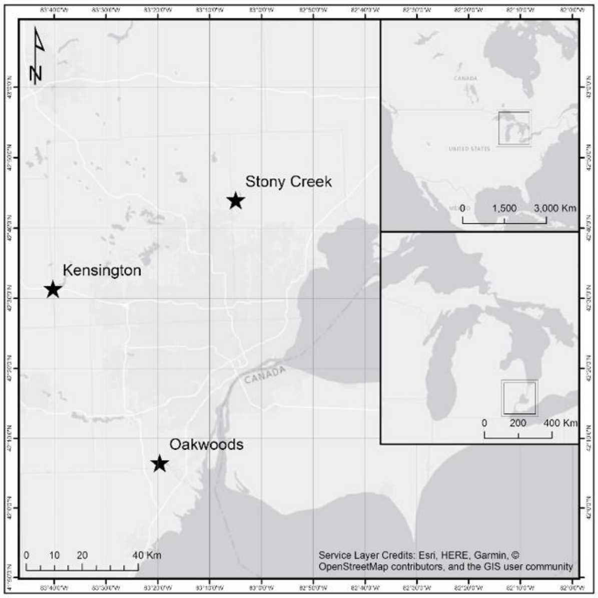


Figure 2.1 Map of study sites located in southeastern Michigan. Kensington, Stony Creek, and Oakwoods, each have three experimental deer exclosures (n=9)

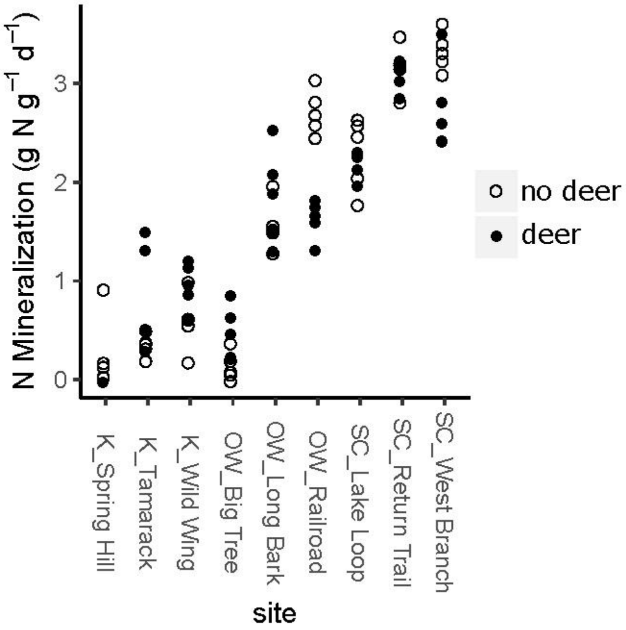


Figure 2.2 Net N mineralization (g N g soil $^{-1}$ day^{-1}) with and without deer browsing at each site. Filled circles are rates of net N mineralization outside deer exclosures (browsed), and empty circles are inside deer exclosures (non-browsed).

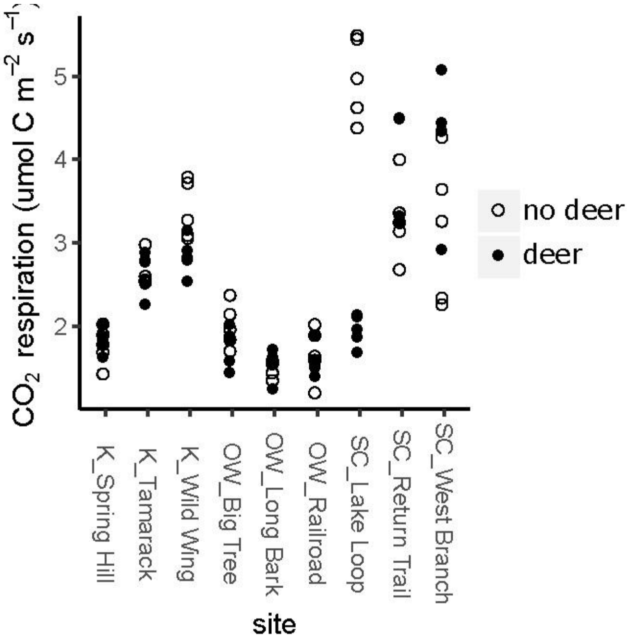


Figure 2.3 CO_2 respiration (µmol C m^{-2} sec^{-1}) rates with and without deer browsing at each site. Filled circles are rates of net N mineralization outside deer exclosures (browsed), and empty circles are inside deer exclosures (non-browsed).

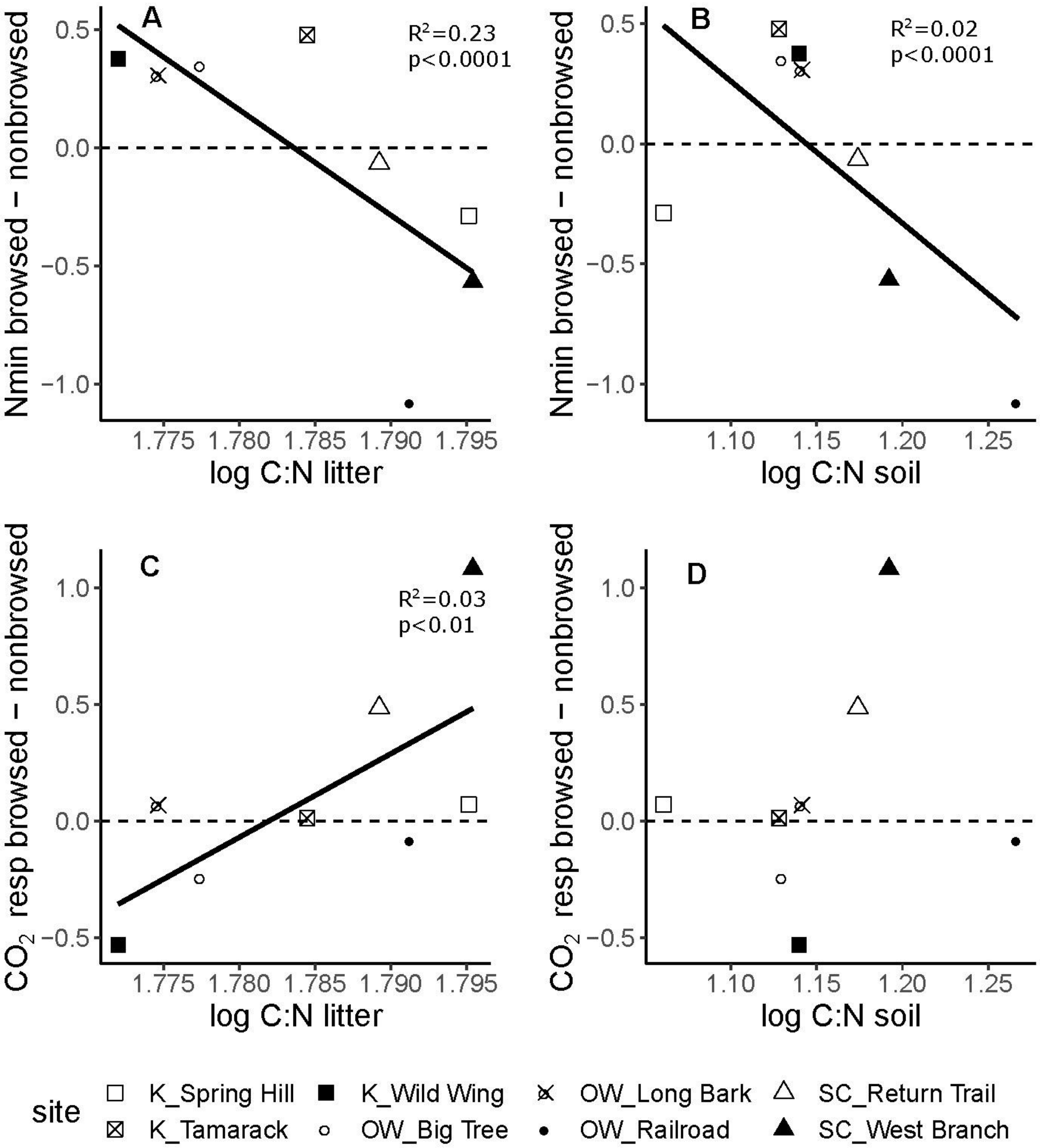


Figure 2.4 The effect of deer browsing on (a,b) net N mineralization (g N g soil $^{-1}$ day^{-1}, browsed minus non-browsed) and (c,d) CO_2 respiration (µmol C m^{-2} sec^{-1}, browsed minus non-browsed) across a (a,c) litter C:N gradient, (b,d) soil C:N gradient. The dotted line indicates no difference between browsed and control. Values above *y*=0 indicate that rates increased with browsing, while values below *y*=0 indicate that rates decreased. N Mineralization increased with deer browsing at sites with low soil and litter C:N, and decreased with deer browsing at sites with high soil C:N(a,b). C respiration decreased with deer browsing at sites with low litter C:N and increased with deer browsing at sites with high litter C:N (c).

Tables

Table 2.1 Study sites and plots (n=8), with average litterfall (kg ha^{-1} yr^{-1}), C:N ratio in litter and soil, deer population density (deer km^{-2}), N mineralization rates (g N g^{-1} d^{-1}) in non-browsed control plots, and CO_2 respiration (µmol C m^{-2} s^{-1}) rates in non-browsed control plots. Mean rates of N mineralization and CO_2 respiration are shown with standard error range. We excluded the Lake Loop plot in the Stony Creek site from statistical analyses due to exceptionally low leaf litter C:N and litterfall values in this plot.

Site	Plot	Litterfall (g m^{-2} yr^{-1})	C:N Litter	C:N Soil	Deer km^{-2}	Net N mineralization non-browsed g N g^{-1} d^{-1}	CO_2 respiration non-browsed µmol C m^{-2} s^{-1}
Stony Creek	Return Trail (RT)	387 ± 17.88	61.6	14.9	8.1	3.17 ± 0.11	3.30 ± 0.21
Stony Creek	Lake Loop (LL)	241 ± 11.27	34.1	14.5	8.1	2.30 ± 0.17	5.00 ± 0.22
Stony Creek	West Branch (WB)	364 ± 22.33	62.4	15.6	8.1	3.33 ± 0.09	3.17 ± 0.38
Oakwoods	Railroad (RR)	310 ± 19.71	61.9	18.5	8.7	2.72 ± 0.10	1.69 ± 0.14
Oakwoods	Long Bark (LB)	361 ± 11.98	59.5	13.9	8.7	1.57 ± 0.11	1.51 ± 0.04
Oakwoods	Big Tree (BT)	392 ± 26.78	59.9	13.5	8.7	0.15 ± 0.07	2.02 ± 0.12
Kensington	Wild Wing (WW)	273 ± 27.90	62.4	11.5	16.0	0.60 ± 0.13	3.40 ± 0.16
Kensington	Spring Hill (SH)	385 ± 28.56	59.2	13.8	16.0	0.29 ± 0.16	1.78 ± 0.10
Kensington	Tamarack (TR)	367 ± 20.86	60.9	13.4	16.0	0.36 ± 0.05	2.66 ± 0.09

Table 2.2 Mixed effect results for deer browsing effects and soil C:N on N mineralization (g N g^{-1} d^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects.

	N Mineralization				
Fixed Effect	Estimate	Std. Error	df	t-value	Pr(>|t|)
(Intercept)	1.61	0.52	2	3.08	0.09
Browsing	-0.06	0.07	79	-0.90	0.37
C:N Soil	5.70	1.65	6	3.45	**0.01**
Browsing * Soil C:N	-2.57	0.59	79	-4.33	**<0.0001**
Random Effect					Pr(>Chisq)
(1|Exclosure:Park)					**<0.0001**
(1|Park)					**0.04**
Marginal R^2	0.23				
Conditional R^2	0.92				

Table 2.3 Mixed effect results for deer browsing effects and litter C:N on N mineralization (g N g^{-1} d^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects.

	N Mineralization				
Fixed Effect	Estimate	Std. Error	df	t-value	Pr(>|t|)
(Intercept)	1.66	0.72	1.83	2.31	0.16
Browsing	-0.06	0.07	70.00	-0.81	0.42
C:N Litter	15.61	14.71	4.46	1.06	0.34
Browsing * Litter C:N	-19.41	3.74	70.00	-5.20	**<0.0001**
Random Effect					Pr(>Chisq)
(1|Exclosure:Park)					**<0.0001**
(1|Park)					0.14
Marginal R^2	0.02				
Conditional R^2	0.95				

Table 2.4 Mixed effect results for deer browsing effects and soil C:N on CO_2 respiration (µmol C m^{-2} s^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects.

	CO$_2$ respiration				
Fixed Effect	Estimate	Std. Error	df	t-value	Pr(>|t|)
(Intercept)	2.73	0.56	2.07	4.89	**0.04**
Browsing	-0.24	0.15	79.00	-1.64	0.11
C:N Soil	1.10	1.50	7.62	0.74	0.48
Browsing * Soil C:N	0.48	1.20	79.00	0.40	0.69
Random Effect					Pr(>Chisq)
(1|Exclosure:Park)					**0.02**
(1|Park)					**0.01**
Marginal R^2	0.03				
Conditional R^2	0.68				

Table 2.5 Mixed effect results for deer browsing effects and litter C:N on CO_2 respiration (µmol C m^{-2} s^{-1}). Marginal R squared values are those associated with the fixed effects, the conditional ones are those of the fixed effects plus the random effects.

	CO$_2$ respiration				
Fixed Effect	Estimate	Std. Error	df	t-value	Pr(>|t|)
(Intercept)	2.59	0.66	1.95	3.91	0.06
Browsing	-0.11	0.09	70.00	1.13	0.26
C:N Litter	-21.12	7.47	5.07	-2.83	**0.04**
Browsing * Litter C:N	15.56	4.82	70.00	3.23	**<0.01**
Random Effect					Pr(>Chisq)
(1|Exclosure:Park)					**<0.0001**
(1|Park)					0.14
Marginal R^2	0.03				
Conditional R^2	0.95				

References

Abbas, F., Merlet, J., Morellet, N., Verheyden, H., Hewison, A.J.M., Cargnelutti, B., Angibault, J.M., Picot, D., Rames, J.L., Lourtet, B., Aulagnier, S., and Daufresne, T. 2012. Roe deer may markedly alter forest nitrogen and phosphorus budgets across Europe. Oikos **121**(October 2011): 1271–1278. doi:10.1111/j.1600-0706.2011.20103.x.

Alverson, W.S., Waller, D.M., and Solheim, S.L. 1988. Forests Too Deer : Edge Effects Forests Northern Wisconsin. Conserv. Biol. **2**(4): 348–358. doi:10.1111/j.1523-1739.1988.tb00199.x.

Baldwin, I.T., Schmelz, E. a., and Ohnmeiss, T.E. 1994. Wound-induced changes in root and shoot jasmonic acid pools correlate with induced nicotine synthesis inNicotiana sylvestris spegazzini and comes. J. Chem. Ecol. **20**(8): 2139–2157. doi:10.1007/BF02066250.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., and Hobbs, P.J. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub montane ecosystems. **33**: 1653–1664. doi:10.1016/S0038-0717(01)00086-4.

Bardgett, R.D., and Wardle, D. a. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology **84**(9): 2258–2268. doi:10.1890/02-0274.

Bump, J.K., Webster, C.R., Vucetich, J.A., Peterson, R.O., Shields, J.M., and Powers, M.D. 2009. Ungulate carcasses perforate ecological filters and create biogeochemical hotspots in forest herbaceous layers allowing trees a competitive advantage. Ecosystems **12**(6): 996–1007. doi:10.1007/s10021-009-9274-0.

Burghardt, K.T., Bradford, M.A., and Schmitz, O.J. 2018. Acceleration or deceleration of litter decomposition by herbivory depends on nutrient availability through intraspecific differences in induced plant resistance traits. J. Ecol. **106**(6): 2380–2394. doi:10.1111/1365-2745.13002.

Cherif, M., and Loreau, M. 2013. Plant-herbivore-decomposer stoichiometric mismatches and nutrient cycling in ecosystems. Proc. R. Soc. B **280**: 20122453. doi:10.1098/rspb.2012.2453.

Cornelissen, J.H.C., Quested, H.M., Gwynn-Jones, D., Van Logtestijn, R.S.P., De Beus, M. a H., Kondratchuk, a., Callaghan, T. V., and Aerts, R. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. Funct. Ecol. **18**: 779–786. doi:10.1111/j.0269-8463.2004.00900.x.

Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C., and Waller, D.M. 2004. Ecological Impacts of Deer Overabundance. Annu. Rev. Ecol. Evol. Syst. **35**(2004): 113–147. doi:10.1146/annurev.ecolsys.35.021103.105725.

DNR. 2010. Michigan deer management plan. Available from http://www.michigan.gov/documents/dnre/WLD_Deer_Management_Plan_FINAL_5.8_320639_7.pdf?20150917085823.

Findlay, S., Carreiro, M., Krischik, V., and Jones, C.G. 1996. Effects of Damage to Living Plants on Leaf Litter Quality. Ecol. Appl. **6**(1): 269–275. doi:10.2307/2269570.

Frerker, K., Sabo, A., and Waller, D. 2014. Long-Term Regional Shifts in Plant Community Composition Are Largely Explained by Local Deer Impact Experiments. PLoS One **9**(12): 1–17. doi:10.1371/journal.pone.0115843.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., and Wickings, K. 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry **121**(2): 305–316. doi:10.1007/s10533-014-0004-0.

Gornall, J.L., Woodin, S.J., Jónsdóttir, I.S., and van der Wal, R. 2009. Herbivore impacts to the moss layer determine tundra ecosystem response to grazing and warming. Oecologia **161**(4): 747–758. doi:10.1007/s00442-009-1427-5.

Grime, J.P., Cornelissen, J.H.H.C., Thompson, K., and Hodgson, J.G. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. Oikos **77**(3): 489–494. Available from https://www.jstor.org/stable/3545938.

Hamilton, E.W., and Frank, D.A. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology **82**(9): 2397–2402. doi:10.1890/0012-9658(2001)082[2397:CPSSMA]2.0.CO;2.

Harrison, K.A., and Bardgett, R.D. 2003. How browsing by red deer impacts on litter decomposition in a native regenerating woodland in the Highlands of Scotland. Biol. Fertil. Soils **38**: 393–399. doi:10.1007/s00374-003-0667-5.

Harrison, K.A., and Bardgett, R.D. 2004. Browsing by red deer negatively impacts on soil nitrogen availability in regenerating native forest. Soil Biol. Biochem. **36**: 115–126. doi:10.1016/j.soilbio.2003.08.022.

Hobbs, N.T. 1996. Modifications of ecosystems by ungulates. J. Wildl. Manage. **60**(4): 695–713.

Holdo, R.M., Sinclair, A.R.E., Dobson, A.P., Metzger, K.L., Bolker, B.M., Ritchie, M.E., and Holt, R.D. 2009. A disease-mediated trophic cascade in the Serengeti and its implications for ecosystem C. PLoS Biol. **7**(9): e1000210. doi:10.1371/journal.pbio.1000210.

Holland, E.A.., and Detling, J.K.. 1990. Plant Response to Herbivory and Belowground Nitrogen Cycling. Ecology **71**(3): 1040–1049. Available from http://www.pnas.org/lookup/doi/10.1073/pnas.1502549112.

Hunter, M.D. 2008. Root herbivory in forest ecosystems. *In* Root Feeders. An Ecosystem Perspective. *Edited by* P.J.M. Scott N. Johnson. CAB International. pp. 68–95. Available from https://books.google.com/books?hl=en&lr=&id=Y7GVUAa1U5YC&pgis=1.

Hunter, M.D., and Schultz, J.C. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. Oecologia **94**(2): 195–203. Springer.

Kielland, K., Bryant, J.P., and Ruess, R.W. 1997. Moose herbivory and carbon turnover of early successional stands in interior Alaska. Oikos **80**(1): 25–30. doi:10.2307/3546512.

Van Langevelde, F., Van De Vijver, C.A.D.M., Kumar, L., Van De Koppel, J., De Ridder, N., Van Andel, J., Skidmore, A.K., Hearne, J.W., Stroosnijder, L., Bond, W.J., Prins, H.H.T., and Rietkerk, M. 2003. Effects of fire and herbivory on the stability of savanna ecosystems. Ecology **84**(2): 337–350. doi:10.1890/0012-9658(2003)084[0337:EOFAHO]2.0.CO;2.

McNaughton, S.J., Banyikwa, F.F., and McNaughton, M.M. 1997. Promotion of the Cycling of Diet-Enhancing Nutrients by African Grazers. Science (80-.). **278**(December): 1798–1801. doi:10.1126/science.278.5344.1798.

Milchunas, D., and Lauenroth, W. 1993. Quantitative Effects of Grazing on Vegetation and Soils Over a Global Range of Environments. Ecol. Monogr. **63**(4): 327–366. doi:10.2307/2937150.

Murray, B.D., Webster, C.R., and Bump, J.K. 2013. Broadening the ecological context of ungulate-ecosystem interactions: The importance of space, seasonality, and nitrogen. Ecology **94**(6): 1317–1326. doi:10.1890/12-1582.1.

Owen, A.D.F., and Wiegert, R.G. 1976. Do Consumers Maximize Plant Fitness? Oikos **27**(3): 488–492. Available from http://www.jstor.org/stable/3543467.

Pastor, J., and Cohen, Y. 1997. Herbivores, the functional diversity of plants species, and the cycling of nutrients in ecosystems. Theor. Popul. Biol. **51**: 165–179. doi:10.1006/tpbi.1997.1327.

Pastor, J., Cohen, Y., and Hobbs, N.T. 2006. The roles of large herbivores in ecosystem nutrient cycles. *In* Large Herbivore Ecology, Ecosystem Dynamics and Conservation. *Edited By*K. Danell, P. Duncan, R. Bergstrom, and J. Pastor. Cambridge University Press. doi:10.1017/CBO9780511617461.012.

Pastor, J., Dewey, B., Naiman, R.J., Mcinnes, P.F., Cohen, Y., and Mar, N. 1993. Moose Browsing and Soil Fertility in the Boreal Forests of Isle Royale National. Ecology **74**(2): 467–480. doi:10.1006/tpbi.1997.1327.

Ritchie, M.E., Tilman, D., and Knops, J.M.H. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. Ecology **79**(1): 165–177. doi:10.1890/0012-9658(1998)079[0165:HEOPAN]2.0.CO;2.

Robertson, G.P., Coleman, D.C., Bledsoe, C.S., and Sollins, P. 1999. Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, Cary, UNITED STATES. Available from http://ebookcentral.proquest.com/lib/umichigan/detail.action?docID=241674.

Rooney, T.P. 2001. Deer impacts on forest ecosystems: a North American perspective. Forestry **74**(3): 201–208. doi:10.1093/forestry/74.3.201.

Rooney, T.P., and Waller, D.M. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. For. Ecol. Manage. **181**: 165–176. doi:10.1016/S0378-1127(03)00130-0.

Russell, F., Zippin, D., and Fowler, N. 2001. A Review of Effects of White-tailed Deer (Odocoileus virginianus) on Plants, Plant Populations and Communities. Am. Midl. Nat. **146**(1): 1–26. doi:10.1674/0003-0031(2001)146[0001:EOWTDO]2.0.CO;2.

Schmitz, O.J., Buchkowski, R.W., Burghardt, K.T., and Donihue, C.M. 2015. Functional Traits and Trait-Mediated Interactions: Connecting Community-Level Interactions with Ecosystem Functioning. *In* Advances in Ecological Research, 1st edition. Elsevier Ltd. doi:10.1016/bs.aecr.2015.01.003.

Seagle, S.W., McNaughton, S.J., and Ruess, R.W. 1992. Simulated effects of grazing on soil nitrogen and mineralization in contrasting Serengeti grasslands. Ecology **73**(3): 1105–1123. doi:10.2307/1940184.

Seastedt, T.R., Ramundo, R. a, and Hayes, D.C. 1988. Maximization of Densities of Soil Animals by Foliage Herbivory: Empirical Evidence, Graphical and Conceptual Models. Oikos **51**(2): 243–248. doi:10.2307/3565649.

Tao, L., and Hunter, M.D. 2011. Effects of Insect Herbivores on the Nitrogen Economy of Plants. *In* Ecological Aspects of Nitrogen Metabolism in Plants. *Edited by* J.C. Polacco and C.D. Todd. John Wiley & Sons, Inc. pp. 255–279. doi:10.1002/9780470959404.ch12.

Tao, L., and Hunter, M.D. 2013. Allocation of resources away from sites of herbivory under simultaneous attack by aboveground and belowground herbivores in the common milkweed, Asclepias syriaca. Arthropod. Plant. Interact. **7**: 217–224. doi:10.1007/s11829-012-9235-y.

Turlapati, S.A., Minocha, R., Bhiravarasa, P.S., Tisa, L.S., Thomas, W.K., and Minocha, S.C. 2013. Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. FEMS Microbiol. Ecol. **83**(2): 478–493. doi:10.1111/1574-6941.12009.

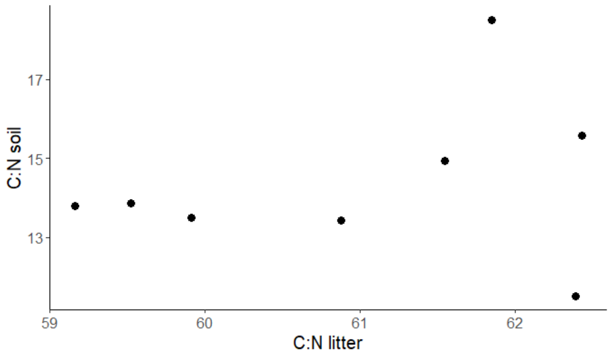
Turner, J.A.Y.C.E.E.L. 2004. Biology and management of. *Edited By*D. Hewitt. CRC Press, Boca Raton, FL. doi:10.1093/neuonc/now016.

Waller, D.M., and Alverson, W.S. 1997. Ecological Aspects of Management: The White-tailed deer. Wildl. Soc. Bull. **25**(2): 217–226. Available from http://www.jstor.org/stable/3783435.

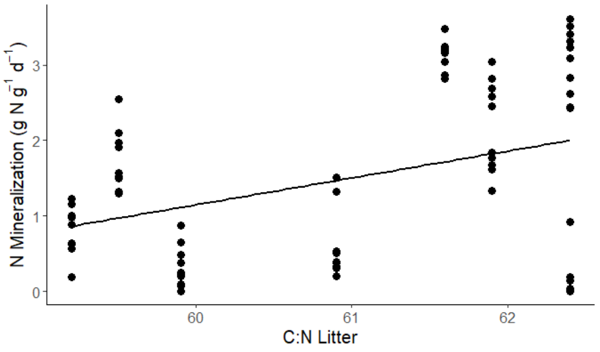
Wardle David A., Bardgett Richard D., Klironomos John N., Setälä Heikki, van der Putten Wim H., and Wall Diana H. 2004. Ecological Linkages Between Aboveground and Belowground Biota. Science (80-.). **304**(June): 1629–1633. doi:10.1126/science.1094875.

Zimov, S.A., Chuprynin, V.I., Oreshko, A.P., Iii, F.S.C., Reynolds, J.F., and Chapin, M.C. 1995. Steppe-Tundra Transition: A Herbivore-Driven Biome Shift at the End of the Pleistocene. Am. Nat. **146**(5): 765. doi:10.1086/285824.

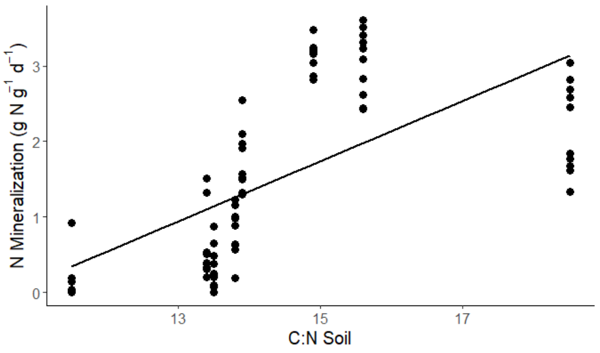
Supplementary Figures



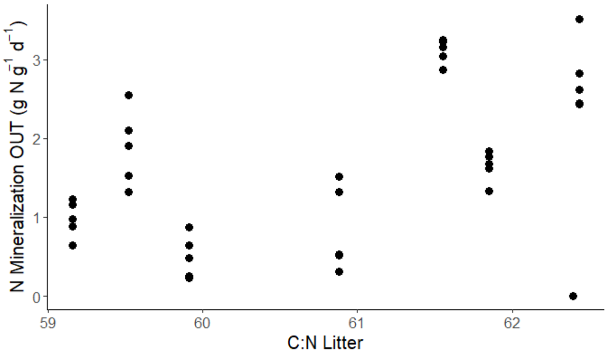
Supplementary Figure 2-1 Soil and litter C:N ratios are not correlated.



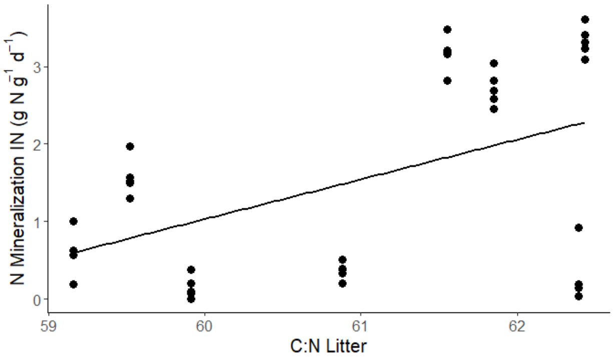
Supplementary Figure 2-2 Rates of N mineralization (g N g^{-1} d^{-1}) correlate positively with litter C:N ratios.



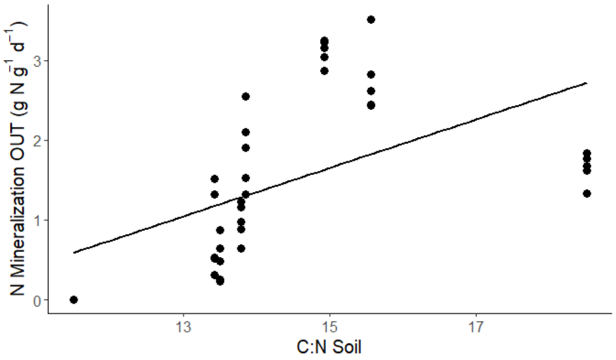
Supplementary Figure 2-3 Rates of N mineralization (g N g^{-1} d^{-1}) correlate positively with soil C:N ratios.



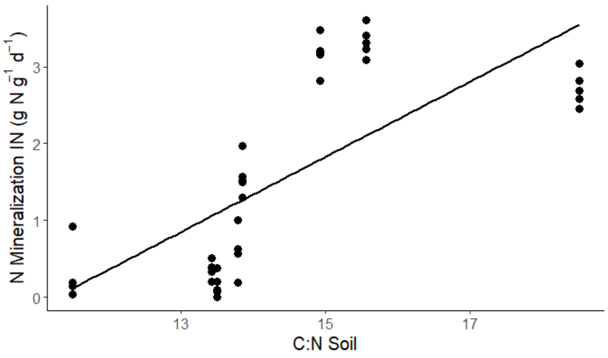
Supplementary Figure 2-4 Rates of N mineralization (g N g^{-1} d^{-1}) in browsed do not correlate with litter C:N ratios.



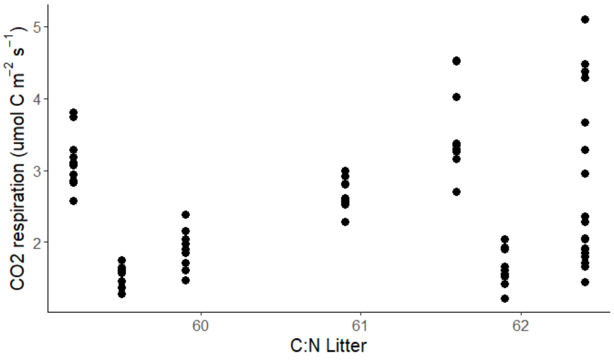
Supplementary Figure 2-5 Rates of N mineralization (g N g^{-1} d^{-1}) in non-browsed correlate positively with litter C:N ratios.



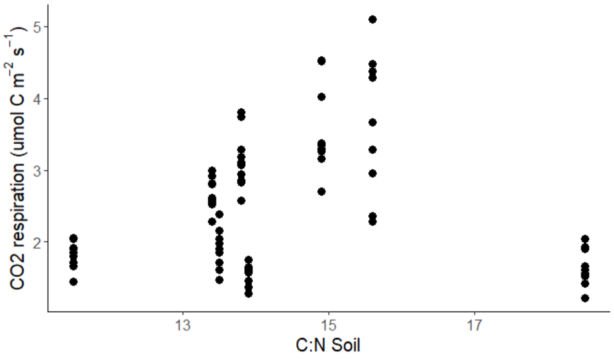
Supplementary Figure 2-6 Rates of N mineralization (g N g^{-1} d^{-1}) in browsed plots correlate positively with soil C:N ratios.



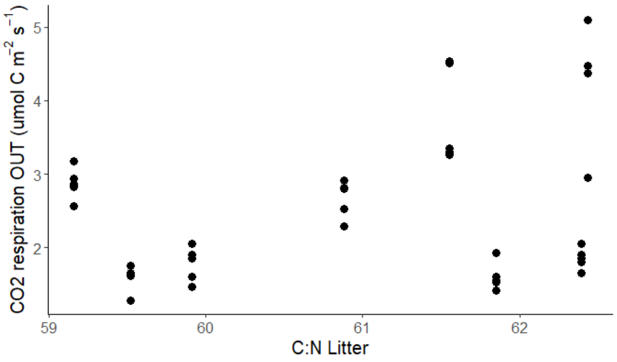
Supplementary Figure 2-7 Rates of N mineralization (g N g^{-1} d^{-1}) in non-browsed plots correlate positively with soil C:N ratios.



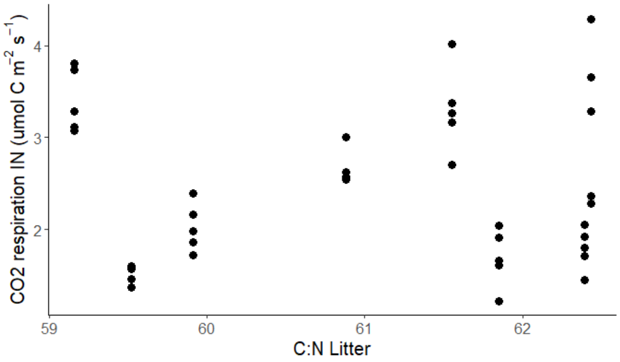
Supplementary Figure 2-8 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) do not correlate with litter C:N ratios.



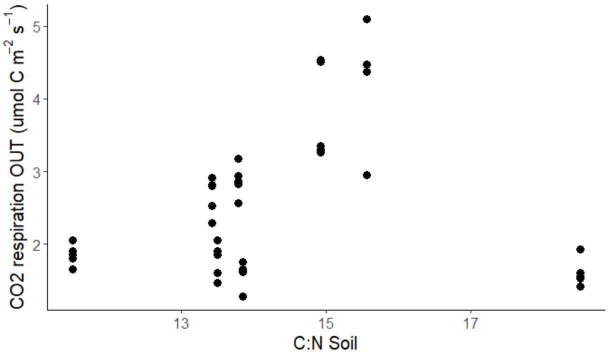
Supplementary Figure 2-9 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) do not correlate with soil C:N ratios.



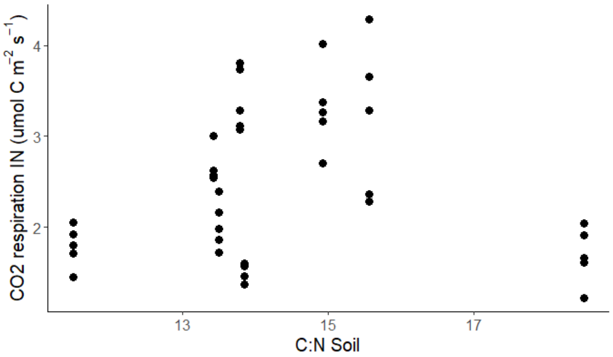
Supplementary Figure 2-10 Rates of CO_2 respiration (μmol C m^{-2} sec^{-1}) in browsed plots do not correlate with litter C:N ratios.



Supplementary Figure 2-11 Rates of CO_2 respiration (μmol C m^{-2} sec^{-1}) in non-browsed plots do not correlate with litter C:N ratios.



Supplementary Figure 2-12 Rates of CO_2 respiration (μmol C m^{-2} sec^{-1}) in browsed plots do not correlate with soil C:N ratios.



Supplementary Figure 2-13 Rates of CO_2 respiration (µmol C m^{-2} sec^{-1}) in non-browsed plots do not correlate with soil C:N ratios.

Chapter 3 Deer Browsing Decreases Understory Plant Abundance But Has No Detectable Effects on Plant Community Composition

Abstract

White-tailed deer populations in the northeastern US have increased dramatically in recent decades. As a result, understory plant community composition and diversity are shifting, but patterns are far from universal, and interactions between plant resource availability and deer browsing are under continuing investigation. By sampling inside and outside deer (*Odocoileus virginianus*) exclosures across a network of sites in southeast Michigan (USA), I tested the non-mutually exclusive hypotheses about how resource availability levels can sometimes favor plant species or taxa that can initiate a compensatory growth response to outgrow herbivory, or that are inherently better defended against herbivores. I predicted that under high soil nutrient availability, deer browsing will have significant effects on plant community structural components and that plant community composition will remain unaffected, because in resource-rich environments plants selected for rapid compensatory growth in response to browsing are dominant. My reasoning was that when resource availability is high, selection likely favors plants that tolerate browsing damage via growth responses, and communities might not change as a result. My results are consistent with this prediction; Deer browsing effects on plant communities were significant but did not vary strongly with environmental factors. Specifically, browsing significantly decreased several plant community structural measures, including plant percent cover, tree seedling cover, and sapling density, but had no detectable effects on plant community composition overall. Other important browsing effects on plant community composition, however, are reflected in significant changes of nearly 25% of plant species and 3

plant functional types with deer browsing. Whether these patterns shift with resource availability as indicated by litter and soil C:N ratios and by total litterfall inputs to soil remains inconclusive, given the narrow ranges of C:N in soil and litter across my sites. Further research expanding this environmental gradient is needed to fully test hypotheses on plant community responses to herbivory under differing resource availability.

Introduction

Nutrient availability and herbivore activity can have large impacts on plant community composition and key ecosystem processes. Nutrient limitation has important effects on plant diversity (Bobbink et al. 2010), productivity and chemistry (LeBauer and Treseder 2008) across a range of ecosystem types. For example, nitrogen (N) enrichment is known to alter many ecosystem properties and processes including primary production, competition, plant-microbe interactions, soil acidification, and herbivory. These ecosystem properties and processes can mediate plant community responses to nutrient enrichment, with N functioning as a key nutrient affecting plant community characteristics such as species composition and diversity (Farrer and Suding 2016). Herbivores can also influence primary producers and habitats in many ways; For example, in terrestrial ecosystems, such as the Serengeti in East Africa, where wildebeest grazing has been shown to increase aboveground productivity in grasslands (McNaughton 1976, Mcnaughton 1985), to Minnesotan oak savannas where deer browsing decreases plant cover and biomass (Ritchie et al. 1998), and in aquatic systems such as the Galapagos archipelago where giant tortoises engineer freshwater wetland ecosystems (Froyd et al. 2014), to Michigan lakes where bluegill sunfish alter successional pathways of zooplankton and phytoplankton (Leibold 1989).

Herbivore effects on plant communities and ecosystem processes can vary widely in strength and direction, partly because plant community responses have been found to both respond and feed back to nutrient availability. One hypothesis is that when resource availability is high, plants can initiate compensatory growth to recover from herbivore damage quickly and thereby increase productivity. In turn, increased productivity often means N-rich compensatory tissue growth that decomposes quickly and releases N and other nutrients for plant uptake (McNaughton, 1976). Furthermore, N-rich waste products of herbivores can increase mineralization rates (McNaughton, 1976). Herbivory stimulating aboveground productivity can thus result in increased nutrient availability, creating a positive feedback that increases production.

Another, non-mutually exclusive, hypothesis is that herbivores selectively browse palatable and nutrient rich species, and thus increase the dominance of species that are browse-resistant and low in nutrient content. Leaf litter from these resistant species decomposes more slowly than leaf litter from nutrient rich species, thereby reducing nutrient availability to plants (Hobbie, 1992; Mattson, 1992, Hobbs, 1996; Ritchie et al., 1998). Reduced nutrient availability can in turn select for more nutrient-poor species with low nutrient requirements, resulting in a feedback that, up to certain thresholds, reduces productivity and nutrient availability.

Developing a better understanding of the inconsistencies of plant community responses to herbivores requires attention to above- and belowground processes across productivity gradients (Bardgett and Wardle 2003). I test here these non-mutually exclusive hypotheses that resource availability levels can favor plants that can initiate a compensatory growth response to outgrow herbivory and can favor plants that are better defended against herbivores, with a case study of plant community responses to white-tailed deer browsing (*Odocoileus virginianus*) across gradients of environmental fertility in temperate forests.

Temperate forest plant community structure and function are influenced by many factors, including browsing by white-tailed deer, particularly over the past several decades. Large populations of deer and other native ungulates in the United States increasingly threaten the diversity and integrity of the forests that sustain them (Rooney, 2001; Côté et al., 2004; Waller et al., 2009). Management has focused on sustaining large deer populations because of their social and economic value (deCalesta & Stout, 1997; Waller & Alverson, 1997; Waller et al., 2009). In addition to management activities, the absence of large predators has contributed to the growth of deer populations (Alverson et al., 1988; Côté et al., 2004). As a result, large deer populations throughout the northeastern US are reducing the abundances of palatable species, decreasing understory plant diversity, and slowing or preventing regeneration of dominant tree species (Frerker et al. 2014, Bradshaw and Waller 2016). Historically, deer browsing studies focused mainly on tree species and less on understory species, even though understory vegetation is important for other organisms, succession, and ecosystem processes (Hobbs 1996, Côté et al. 2004, Sakata and Yamasaki 2015, Landsman and Bowman 2017). It is therefore important to better understand the nature and extent of the interactions between deer and the ecosystems in which they live (Waller et al., 2009). Because white-tailed deer forage selectively, they can influence plant species composition and diversity (Waller & Alverson, 1997; Rooney, 2001; Russell et al., 2001; Rooney & Waller, 2003; Côté et al., 2004; Hewitt, 2011). However, the effects of deer browsing on plant communities are neither uniform nor predictable. Variations exist in strength and direction of browsing on diversity, richness, and community composition

(Begley-Miller et al. 2019, Schäfer et al. 2019). It is not known whether plant community responses to deer browsing vary in relation to soil nutrient availability.

In this study, I compare deer browsing effects on understory plant communities across soil nutrient availability and litterfall gradients in temperate deciduous forests located in southeastern Michigan, USA. I used fenced exclosures along gradients of soil fertility, as indicated by soil and litter C:N ratios and total litterfall inputs to soil, to test for direct deer browsing effects on vegetation communities and associations between browsing and environmental factors. Based on the two non-mutually exclusive hypotheses on how resource availability levels might favor plants that can initiate a compensatory growth response to outgrow herbivory, and sometimes favor plants that are better defended against herbivores, I hypothesized that browsing effects on plant community structure and composition vary with nutrient availability, both in magnitude and direction. My foci here are on 1) plant community structure as described by species richness, diversity, abundance (percent cover), tree regeneration (seedlings and saplings), and biomass, and 2) plant community composition as described by visual estimation of the proportion of area covered (or percent cover) by each species within sampling areas in browsed and non-browsed experimental plots (protected from deer browse for 20 years). I predicted that under high soil nutrient availability, deer browsing would have significant effects on structural components of plant communities and that plant community composition would remain unaffected, because in resource-rich environments plants selected for rapid compensatory growth in response to browsing are dominant. My reasoning was that when resource availability is high, plants are more likely to have been selected to tolerate browsing damage via growth responses, and communities might not change as a result. In contrast, I predicted that under low soil nutrient availability deer browsing would significantly shift plant community composition towards better defended plant communities, because in resource-poor environments plants are more likely to have been selected to resist herbivory and cannot grow fast enough to outgrow deer browsing; selective browsing would shift community composition to species with better plant defenses.

Methods

Study sites

I selected nine study sites in Oakland, Macomb, and Wayne counties in the greater Detroit Metro area of Michigan, USA (Popma and Nadelhoffer 2020). Temperate forests in the US Midwest and Northeast such as those I selected have experienced increases in deer populations since the 1970s, with densities reaching up to 20 deer per km^2 in some areas (Alverson et al. 1988, DNR 2010). The study areas lie within three Huron Clinton Metroparks: Kensington (K) (42°32'28.1"N 83°38'16.2"W), Stony Creek (SC) (42°42'36.2"N 83°04'16.8"W), and Oakwoods (OW) (42°06'57.8"N 83°21'28.1"W) (Fig. 1). Sites are abbreviated as follows: Within Kensington there is Spring Hill (SH), Tamarack (TR), and Wild Wing (WW); within Oakwoods there is Big Tree (BT), Long Bark (LB), and Railroad (RR); Within Stony Creek there is Lake Loop (LL), Return Trail (RT), and West Branch (WB). In June and July of 2017, I sampled within (non-browsed plot) and outside (browsed plot) of nine 10 m x 10 m experimental deer exclosures constructed in 1999, three in each of the three Metroparks. These 20-yr-old exclosures are in dry-mesic and mesic forest types. Dry-mesic forests are oak or oak-hickory forest types in southern Lower Michigan and typically grow on sandy loam or loamy soils. Mesic forests in southern Michigan are typically dominated by American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*), growing on loamy upland soils. The exclosures are 2.5m tall and exclude ungulates (deer) while allowing small herbivores to move in and out. Mean annual temperature in southeastern Michigan is 9°C, mean annual rainfall is 81cm, mean annual snowfall is 130 cm (US Climate Data), and mean annual litterfall is 3422 kg dry mass ha^{-1} y^{-1}. The main ungulates are white-tailed deer (*Odocoileus virginianus*), with densities of 8, 8, and 16 deer per square kilometer at the Stony Creek, Oakwoods and Kensington parks, respectively. Deer populations are estimated and actively managed by Huron Clinton Metroparks (Ryan Colliton, *personal communication* 2018). Helicopter fly-over estimates for Oakwoods were more difficult to obtain due to proximity to the Detroit Metropolitan Airport airspace and could potentially be over- or under-estimating deer population densities.

Plant community structure and composition

Vegetation surveys were performed inside and outside deer exclosures to test for the effects of deer browsing on plant communities. Plots outside deer exclosures were established within 10 meters of each exclosure to create a control plot similar in size, slope, aspect, and habitat. I randomly selected locations for three 1 m x 1 m frame quadrats within each browsed and non-browsed (deer exclosure) plot for replication of ground layer and understory plant species measurements. I focused on ground layer vegetation, including woody and non-woody species, and understory vegetation because these layers are accessible to browsing deer. Tree seedlings and saplings were included in the vegetation surveys; tree stems of 1 m in height or higher were considered saplings and those under 1 m were considered seedlings. Tree stems with a diameter at breast height of > 9cm were considered adult tree and excluded from vegetation surveys.

Plant species richness was calculated by summing the number of unique species present in a quadrat. Total plant percent cover was calculated by summing the percent cover values of all plant species in a quadrat. Plant species diversity (inverse Simpson) was calculated using percent cover data for each species, with the vegan package in R (R Core Team 2019). Ground cover biomass, including tree seedlings and saplings, was estimated by randomly placing a 20 cm x 50 cm frame on the ground and clipping all plant biomass inside the frame. I collected ground cover biomass from three frames in each plot inside and outside deer exclosures. Plant biomass was stored in paper bags until further processing at the University of Michigan laboratory. Plant biomass from each frame was oven dried at 60˚C for 48 hours and weighed.

Percent cover was visually estimated for each species or taxa (or growth form in the case of graminoids), and bare ground if there was no vegetation. Percent cover of all species was then summed per quadrat, sometimes resulting in values above 100% if vegetation was overlapping.

Sapling density (shrub layer) was recorded by counting all saplings above 1 m and a diameter smaller than 9cm at breast height. Sapling density was recorded at the plot level (100 m^2), inside and outside exclosures. Tree seedling cover was estimated by summing percent cover of all tree seedlings in a quadrat. Tree seedling percent cover was included in overall percent cover, as well as estimated separately.

See *Data Analyses* for methods used to quantify plant community composition.

Canopy litter inputs to soil

Canopy leaf litterfall (kg ha^{-1} yr^{-1}) was estimated using five, 0.31 m^2 litter baskets (Popma and Nadelhoffer 2020) within each browsed (n = 9) and non-browsed plot (n = 9). Leaves were collected once in October and once in December (2018), separated by species, and dried for 48h at 80˚C in our University of Michigan laboratory. Collection baskets were 66 cm x 46 cm, modified from commercial laundry baskets by drilling drainage holes in the bottoms.

Canopy leaf litter was sorted by species and dried for 48h at 80°C to analyze leaf C:N for each individual species. Leaves were ground for 2 minutes to a fine powder prior to C:N, %C, and %N analyses. Site-level C:N ratios were calculated by first multiplying the mass of each represented species by the %C and %N in the litter of that species, resulting in grams of C and N in litter of each species at each site. Site level litter C:N ratios were then calculated by summing the masses of C and N of all the species at each site, and dividing total grams C by total grams N.

Soil C:N analyses

I collected one composite soil sample inside and outside of each deer exclosure (n=9) in June 2017. Each composite sample consisted of five 2.5cm diameter soil cores sampled to a depth of 10cm. Composite soil samples were used for determining soil C:N. Soil C:N was determined using a CN Analyzer (University of Michigan Biological Station Analytical Lab, Pellston, MI, USA). Fresh soils were stored at 4°C and prepared within 5 days of collection for an 8-week laboratory incubation experiment to determine the effects of large herbivores on rates of net N mineralization, net nitrification, and microbial respiration. For more details see Popma & Nadelhoffer 2020.

Data Analyses

Data analyses were conducted using R Studio (version 3.5.1) and significance was accepted at $\alpha<0.05$, and marginally significant results accepted at $\alpha<0.1$. I used linear mixed effects model analyses from the lme4 R-package to test whether deer browsing altered indices of plant community structure: plant species richness, diversity (inverse simpson), plant percent cover, understory biomass, sapling density, and seedling percent cover. In addition to deer browsing as a fixed effect, I also included C:N litter, C:N soil, and litterfall in my models, as well as the interactions of each with deer browsing. Park (n=3) and site (n=8) were used as random nested effects because I expected rates to be influenced by geographic location. Linear mixed effects models were performed on n=8 sites (excluding the “lake loop” site).

I analyzed deer browsing effects on all plant species and on broad groups of plant growth forms including forbs, graminoids, shrubs, trees, and vines. I used linear mixed effects model analyses from the lme4 R-package to test whether deer browsing altered the average percent cover of each species and growth form at all sites (n=8). Park (n=3) and site (n=8) were used as random nested effects because I expected rates to be influenced by geographic location.

I analyzed main effects of deer browsing and three environmental factors on plant community composition, and the interactions between deer browsing and each environmental factor, using PERMANOVA models. The environmental factors included C:N soil, C:N canopy litter, and canopy litterfall. PERMANOVA models were executed with the adonis2() function in the vegan package in R (R Core Team 2019). The response variable was a community dissimilarity index, calculated by subjecting plant species abundance data to a Hellinger transformation, followed by the calculation of Euclidian distances (Legendre and Gallagher 2001). I chose the Hellinger transformation because it is particularly suited for species abundance data with many zeros. Sites (n=8, excluding the lake loop site) were accounted for as random effects because I was mainly interested in environmental effects. I also ran a model focused specifically on site effects, to test for changes in deer effects across sites. PERMANOVA analyses were performed on n=8 sites, excluding n=1 site “lake loop”. I excluded data from the Lake Loop site in the Stony Creek forest because the leaf litter C:N average here was more than 2 standard deviations outside the distribution of other plots, likely due to the presence of large amounts of *Tilia americana* (American basswood) leaf litter at this site.

Data were plotted using non-metric-dimensional scaling (NMDS) scores of plant community composition of two deer browsing treatments and along environmental gradients.

NMDS scores were calculated using the metaMDS function in the vegan package in R (R Core Team 2019). In order to visualize effects of continuous environmental variables on plant community composition, I transformed each environmental variable (C:N soil, C:N litter, litterfall) from continuous into a three-level factor variable: High, Medium, Low. Sites were assigned to High, Medium, or Low bins based on histogram distributions of site level environmental averages for each variable: Litterfall, C:N Soil, and C:N Litter. Plotting a histogram of site averages for each variable allowed me to bin sites into High, Medium, or Low, using natural breaks in distributions (Table 3.1).

Results

The nine exclosure locations represented gradients in litter and soil C:N ratios (Chapter 2, Table 2.1). Soil C:N ratios were not correlated with litter C:N ratios across sites, therefore I treated soil C:N and litter C:N ratios as independent factors across my study sites. As such, these factors represent two independent proxies for soil nitrogen (N) availability, with annual litterfall representing a third fertility proxy. Deer density estimates showed that the study sites represented a range from medium (8 deer per km^2) to high (16 deer per km^2) population densities (Chapter 2, Table 2.1). All sites within the Kensington Metropark (Wild Wing, Spring Hill, and Tamarack) had high deer densities, whereas all sites within the Oakwoods and Stony Creek Metroparks had medium deer densities. Deer densities are generally considered high at levels > 10-15, medium at 7-12, low at <7 deer per km^2 (Russell et al. 2001).

Deer browsing effects on plant community structure

Effects of deer browsing on plant community structure (measures of percent cover, tree seedlings and saplings, diversity, richness, biomass) varied among sites (Figure 3.1), ranging from positive to negative and from weak to strong (Table 3.2, Figure 3.1). Overall, linear mixed effects models showed that deer browsing had strong negative effects on overall percent cover of plants, tree sapling density, and tree seedling percent cover, as indicated by the large negative coefficient estimates (Table 3.2). Tree seedling percent cover was also correlated with environmental factors, showing a negative correlation with increased canopy litterfall, and a positive correlation with soil and litter C:N (Table 3.2). As predicted, these deer browsing effects on percent understory cover, sapling density, and tree seedling percent cover, covaried with litter C:N and deer effects on sapling density covaried with canopy litterfall (Table 3.2). Significant interactions between deer and environmental variables indicate that the effects of browsing were not uniform across sites. Specifically, browsing effects on percent cover and sapling density were positively associated with increasing litter C:N (coefficient estimate 23.2, Table 3.2), and effects on seedling cover were negatively associated with litter C:N (coefficient estimate -5.8, Table 3.2). Groundcover biomass was not affected by browsing, but showed negative associations with soil C:N and litterfall (Coefficient estimates -0.2 and -0.1 respectively, Table 3.2).

Variations in plant species richness, sapling density, and tree seedling cover could be explained by site locations, which were included as random effects in the models (Table 3.2). Contrary to my prediction however, deer browsing had no detectable effects on either plant species richness or diversity, and no variations in these two response variables were detected in relation to fixed environmental factors or their interactions with deer browsing (Table 3.2).

Deer browsing effects on plant community composition

Deer browsing had no detectable effects on overall plant community composition, but plant community composition was affected by both litter and soil C:N (Table 3.3, Figure 3.2). Overall, the PERMANOVA model explained nearly half the variation (R^2=0.40) in plant community composition. The strongest predictor of variation in plant community composition was the Residuals, explaining over half the variation (R^2=0.6). In contrast to my predictions, deer browsing had no detectable effects on plant community composition. However, plant community composition was associated with litter (R^2=0.09) and soil C:N (R^2=0.09), but associations were weak with only 18% of variation explained by these two environmental variables together (Table 3.2). Moreover, no significant interactions between deer browsing and any of the three environmental factors were found to influence overall plant community composition. To visualize plant community variation with litter and soil C:N, I plotted NMDS scores of plant communities (Figure 3.2).

I also tested the effects of deer browsing on individual plant taxa (either species or genera) and plant functional types (PFTs). In total, these included forbs, graminoids, shrubs, trees, and vines. Of the 41 different plant taxa and 2 PFTs in the forest understory, 10 were significantly affected by deer browsing (Table 3.4). Most of these were negatively impacted by deer browsing, except graminoids and two invasive species *Eleagnus umbellata* (Autumn olive) and *Celastrus orbiculatus* (Oriental bittersweet), which increased with deer browsing (Table 3.4). Note that results are average percent cover across all plots and therefore include many zeroes. Directions and magnitudes of deer browsing effects are variable among individual sites. For example, *Eleagnus umbellata* cover increased 70% with deer browsing on average at SH, but was not present at any other site except TR (Table 3.4).

Deer browsing significantly influenced three groups of plant growth forms: forbs, graminoids, and vines (Figure 3.3). Direction and magnitude of deer browsing effects varied within groups, depending on the site location, but overall deer browsing negatively influenced the percent cover of forbs and vines, and positively influenced cover of graminoids. Forbs on average decreased from 3% to 2%, vine cover from 4% to 2%, and graminoids increased from 3% to 10%. Deer browsing effects on vines were variable among sites, with vines decreasing as much as 10% at the LB site, but not much at all at other sites (WB, RT, and RR, Figure 3.3). Browsing effects on graminoid cover were variable among sites as well, with large increases in cover at TR and SH with deer browsing (11% and 16% respectively, Figure 3.3), and no changes at RT. Tree and shrub abundance varied a lot across sites, and no overall deer browsing effect was detected. Browsing increased shrub cover at SH by 11%, but decreased cover at RT and WB by 4% (Figure 3.3). The shrub growth form group was heavily influenced by the strong increase of autumn olive at Spring Hill. Most other shrub species declined.

Discussion

Based on the two non-mutually exclusive hypotheses regarding how resource availability levels can sometimes favor plants that can initiate a compensatory growth response to herbivory, and sometimes favor plants that are better defended against herbivores, I hypothesized that browsing effects on plant community structure, as described by species richness, diversity, abundance (percent cover), tree regeneration (sapling density and seedling cover), and biomass, and overall composition would vary with environmental conditions, both in magnitude and direction. Plant community structure was predicted to remain similar with deer browsing under low nutrient availability but change with browsing under high soil nutrient availability. Plant community composition, however, was predicted to change with deer browsing under low soil nutrient availability but to remain unaffected under high nutrient availability.

My reasoning was that under high nutrient availability, plants are more likely to tolerate browsing damage via growth responses, and communities might not change as a result. In resource-poor environments, however, plants cannot grow fast enough to outgrow deer browsing and selective browsing would shift community composition to more species with better plant defenses.

Deer browsing effects on plant community structure

Results revealed strong negative effects of deer browsing on some components of plant community structure, including plant percent cover, tree sapling density, and tree seedling cover. Contrary to prediction, browsing effects these components of plant community structure were only weakly associated with underlying environmental variables, specifically canopy litter chemistry. Although associations were weak, they were in line with my prediction; covariation between deer effects and litter chemistry suggests that browsing effects on percent cover and sapling density might be less negative at sites with lower nutrient availability (higher C:N litter). However, the direction of covariation between deer effects and litter chemistry effects on tree seedling cover was opposite, indicating browsing effects might be more negative on tree seedlings at sites with lower nutrient availability. The ecological importance of this variation in browsing effects with environmental conditions is likely small compared to the strong negative browsing effect overall, because associations between browsing and litter C:N were weak.

Variation in effects on plant percent cover and tree saplings and tree seedlings could potentially be explained by variation in deer browsing intensity. This study does not have the power to address this factor, however the classic but controversial (Fox 2013) intermediate disturbance hypothesis could explain my varying results (Moi et al. 2020). This hypothesis suggests that intermediate levels of disturbance (browsing in this case) result in increased diversity and/or richness, while low or high levels of browsing do not (Suominen et al. 2003). Although he intermediate disturbance hypothesis remains controversial (Fox 2013), empirical evidence in support has been found in terrestrial systems, including temperate forests (Moi et al.

2020). A recent meta-analysis by Gao et al. (2020) showed that grazing-richness and grazing-diversity relationships sometimes fit the intermediate disturbance hypothesis, but it depends on ecosystem type (Gao and Carmel 2020a, 2020b). Moreover, Gao et al. stress the importance of further clarifying interactions between grazing and aridity and vegetation type to improve the intermediate disturbance theory (Gao and Carmel 2020a).

Deer browsing did not have detectable effects on either plant species richness or diversity. Plant species richness was instead mostly associated with site location. The lack of browsing effects on plant species richness and diversity is in contrast to predictions, and somewhat surprising considering the large body of literature that shows strong effects of deer browsing on plant species diversity and richness (Rooney, 2001; Côté et al., 2004; Waller et al., 2009, Frerker et al. 2014, Bradshaw and Waller 2016). Especially when the abundance, measured as percent cover, of nearly a quarter of plant taxa at my sites was significantly impacted by deer browsing. Overall, tree seedlings and saplings, and forbs declined with deer browsing, while vines showed mixed responses, and graminoids and certain invasive shrubs increased. The strong decrease in cover of tree seedlings and saplings, and forbs compared to graminoids could be explained by the selective nature of deer browsing. Average diets of white-tailed deer consist mostly of green twigs and leaves (46%) and forbs (24%), and much less of graminoids (8%) (Turner 2004). Moreover, grasses generally tolerate browsing well and therefore often increase with herbivory (McNaughton 1976).

Specifically, browsing negatively impacted percent cover of several tree seedlings and saplings, including *A. saccharum, A. rubrum,* and *P. serotina*, as well as forbs and vines, including *C. lutetiana*, *Trillium sp., S. tamnoides*. In addition to percent cover of tree seedlings, sapling density declined dramatically as well with deer browsing. These negative browsing effects on tree seedling and sapling cover reveal a potential mechanism via which deer browsing could influence trajectories of forest succession. For example, studies have shown that deer are less abundant on tribal lands, and these forests are more diverse and regenerate more successfully (Waller and Reo 2018). Indigenous Nations have long managed their lands and wildlife in ways that reflect their cultural traditions and values, and because of different deer management strategies compared to public and private forestlands, ecological conditions on tribal lands differ relative to nontribal lands (Waller and Reo 2018).

Although most ground- and shrub-layer (including tree sapling) species declined with deer browsing, graminoids and a few invasive species increased in percent cover, including *Eleagnus umbellata* and *Celastrus orbiculatus*. *E. umbellata* (Autumn olive), is a common invasive shrub that has associations with nitrogen fixing bacteria and can increase soil N cycling rates (Goldstein et al. 2010). This relates to the hypothesis on how high resource availability levels can favor plants that can initiate a compensatory growth response to outgrow herbivory; Nitrogen fixing abilities in Autumn olive could greatly increase resource availability (Goldstein et al. 2010) and therefore Autumn olive possibly has greater abilities to outgrow herbivory. Not all invasive species were affected positively, for example the invasive shrub *Lonicera maackii* (honeysuckle) showed (non-significant) signs of decrease with deer browsing. *L. maackii* is known to be an important source of food for deer in early spring when other preferred woody species are scarce (Wright et al. 2019). Notably, some species disappeared completely with deer browsing, including *Trillium sp.,* an important indicator species of past and present deer browsing pressure (Koh et al. 2010).

My observations of decreasing tree seedling and sapling density and increasing presence of invasive species due to deer browsing are not unique (Bradshaw and Waller 2016). Invasive species tend to do well in areas with deer browsing, and both browsing and invasive species can have negative effects on native regeneration and understory growth (Woods 1993, Gorchov and Trisel 2003, Ward et al. 2018).

Deer browsing effects on plant community composition

In contrast to my predictions, effects of deer browsing on plant community composition were not detected at any site, and I did not detect associations between browsing effects and environmental variables. Plant community composition was however, strongly associated with site location, which explained nearly 70% of variation, and weakly associated with Litter C:N and soil C:N, together explaining only 18% of variation. The strong association between variation in plant community composition and site location is likely due to environmental factors not measured in this study. It should be noted that the ranges of C:N litter and soil were fairly narrow, potentially too narrow to conclusively confirm or contrast my predictions. I find it surprising that PERMANOVA models did not detect significant effects of deer browsing on plant communities (as indicated by the Hellinger transformed data), and I would argue these null results should be interpreted with caution given the evidence of significant effects of browsing on nearly 25% of plant species, major plant functional types, and overall percent cover, sapling density, and tree seedling cover, deer browsing surely has significant impacts on plant communities.

Significant browsing effects on structure but not composition of plant communities might be consistent with my predictions if all sites were relatively high in resource availability. I did not detect strong shifts in browsing effects on structure, and no effects at all on composition, but if resource availability simply did not vary enough at my sites, I would not expect shifts. As such, it would be interesting to see if patterns change when expanding the soil and litter C:N range to include sites with higher C:N ratios (less N availability). Notably, nearly 25% of identified plant taxa showed increases or decreases with deer browsing, highlighting that although no overall browsing effects on community composition were detected, deer browsing does have significant effects on plant communities.

Conclusions

Most research on deer browsing effects on plant communities in temperate forests has not considered interacting effects if deer browsing with other environmental factors. This study compared effects of deer browsing on plant communities across environmental gradients to test predictions on how resource availability might be associated with browsing effects on plant communities. Plant community structural measures were predicted to change with deer browsing under high resource availability but remain similar under low resource availability. In contrast, plant community composition was predicted to remain similar under high resource availability but change under low resource availability. Underlying these predictions is the idea that in resource-rich environments plants that tolerate browsing damage via growth responses are favored, and thus plant community composition might be less affected by browsing. Whereas plants in resource-poor environments might lack the potential to outgrow deer browsing, and selection might therefore favor species with better plant chemical or other defenses, thereby leading to greater shifts in community composition.

Results reveal that deer effects on plant communities were significant but did not vary with environmental factors. Browsing significantly decreased several plant structural measures, including plant percent cover, tree seedling cover, and sapling density, but had no detectable effects on plant community composition overall. Other important browsing effects on plant community composition, however, are reflected in significant changes in percent cover of nearly

25% of plant species and 3 plant functional types with deer browsing. My findings are consistent with predictions for environments with high nutrient availability but are inconclusive in addressing predictions for shifts in browsing effects across a wide enough range of nutrient availability.

Acknowledgements

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Figures

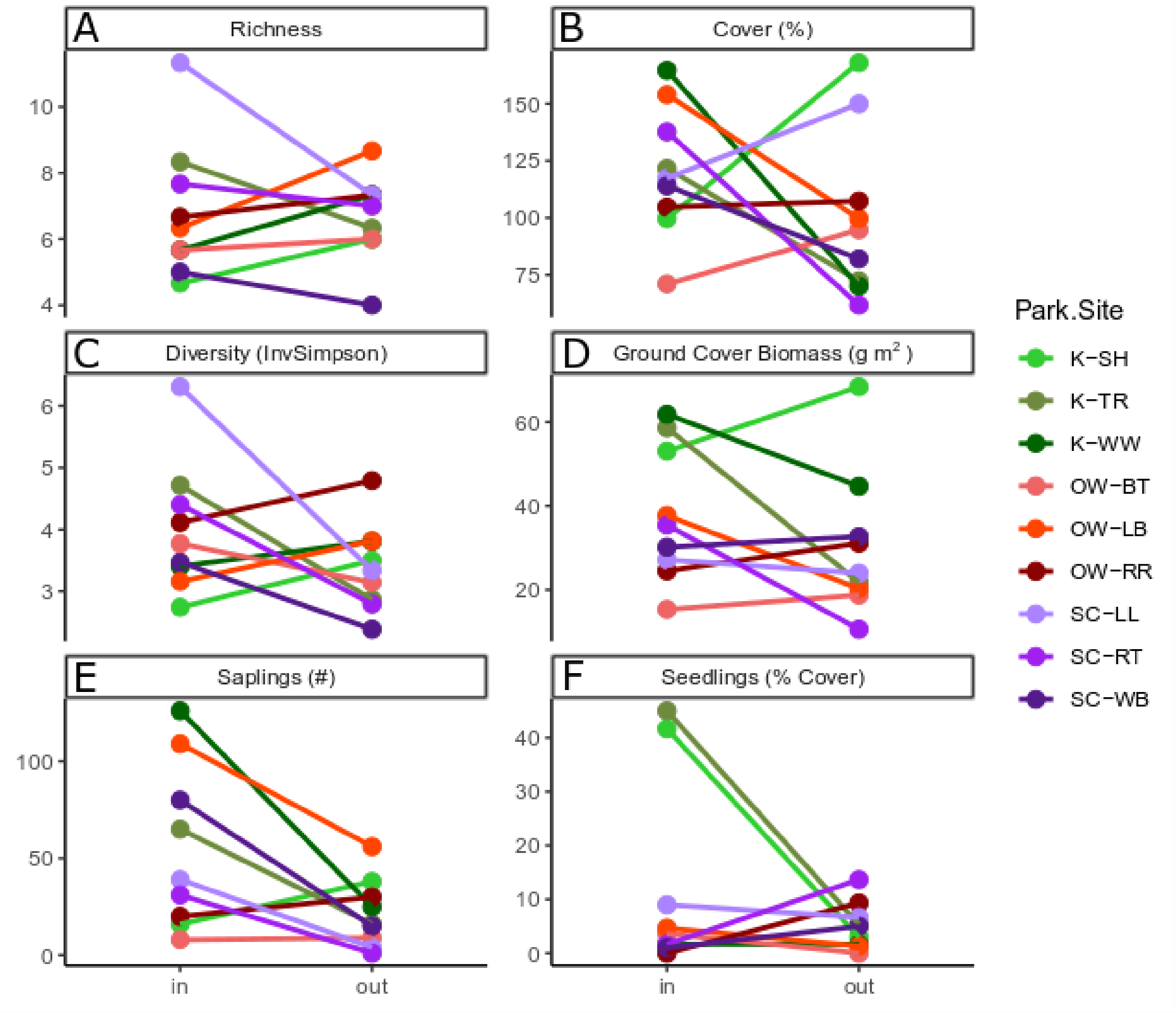

Figure 3.1 Plant community structure responses to deer browsing: A) Richness, B) % Cover, C) Diversity, D) Biomass, E) Sapling density, F) %seedlings. Responses to deer browsing are not uniform across sites. Overall deer significantly decreased percent cover, sapling density, and tree seedlings. Parks are abbreviated as follows: Kensington (K), Oakwoods (OW), and Stony Creek (SC). Sites are abbreviated as follows: Spring Hill (SH), Tamarack (TR), Wild Wing (WW), Big Tree (BT), Long Bark (LB), Railroad (RR), Lake Loop (LL), Return Trail (RT), and West Branch (WB).

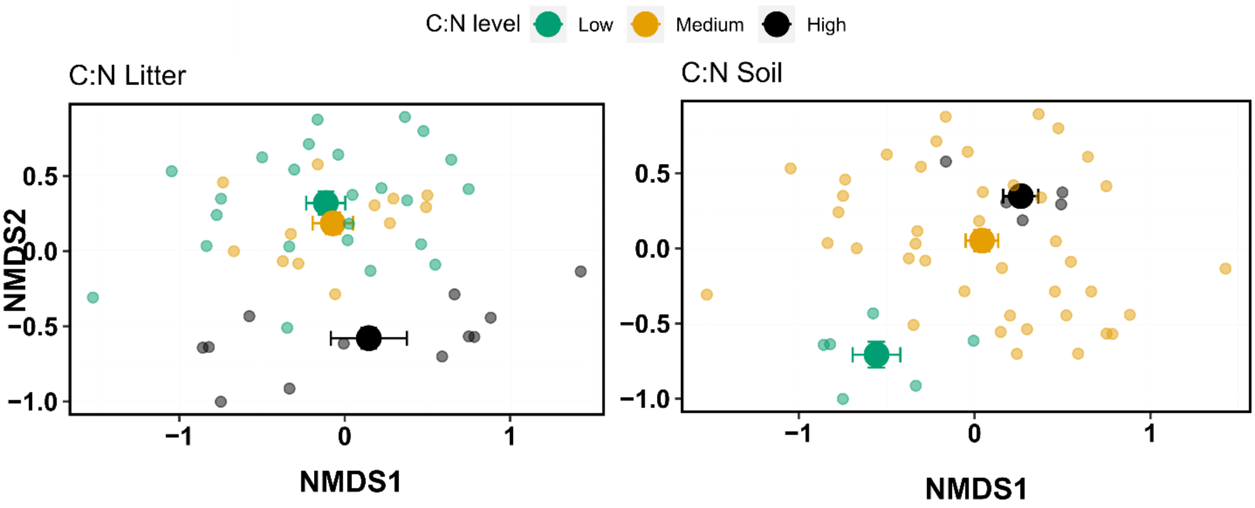

Figure 3.2 Plant community composition NMDS averages with standard error overlaid on original NMDS points for C:N litter and C:N soil. Significance is indicated by non-overlapping error bars. Continuous data on C:N were binned in three categories: High (black), Medium (yellow), and Low (green) for NMDS visualization purposes.

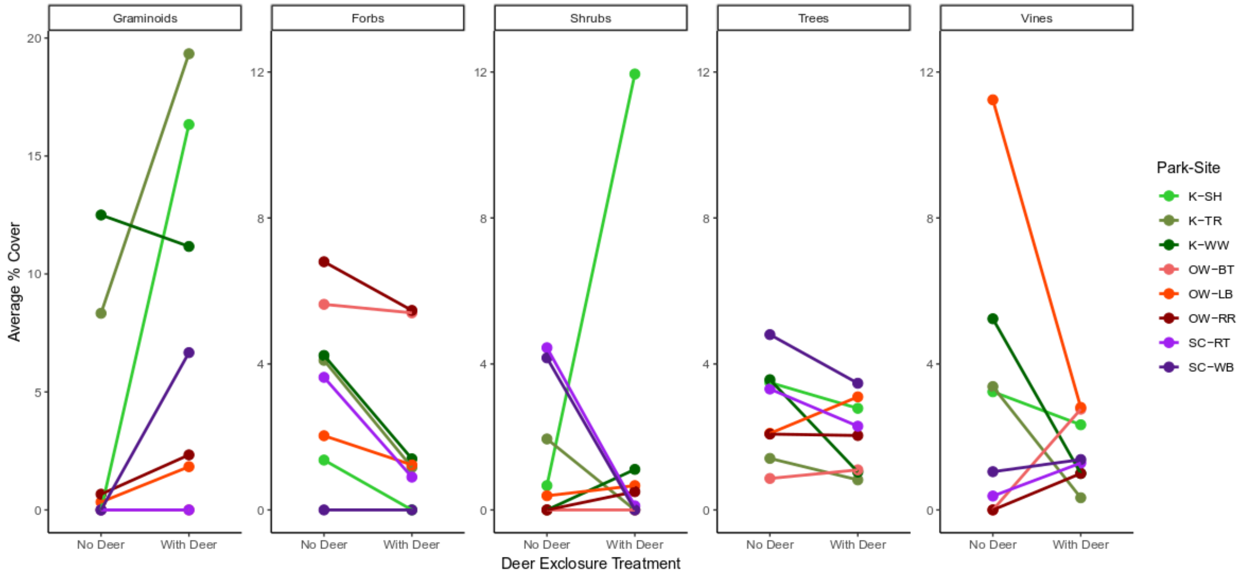

Figure 3.3 Percent cover of plants grouped by growth form, with and without deer browsing. Linear mixed effect model showed that deer browsing significantly decreases cover of forbs (p=0.005 and vines (p=0.05), and significantly increases cover of graminoids (p=0.01).

Tables

Table 3.1 Factor level criteria for each continuous environmental variable: N mineralization (g N g soil $^{-1}$ d^{-1}), CO_2 respiration (µmol C m^{-2} sec^{-1}), Litterfall (g m^{-2} yr^{-1}), C:N Soil, and C:N Litter.

Factor Level	**Litterfall** *(g m^{-2} yr^{-1})*	**C:N Soil**	**C:N Litter**
High	>3000	>16	>62
Medium	>=2500, <=3000	>=12, <=16	>=61, <=62
Low	<2500	<12	<61

Table 3.2 Linear mixed effects model summary for plant community structure. (****) indicates significance <0.0001, (***) indicates significance <0.001, (**) indicates significance <0.05, (*) indicates significance <0.1. Plant community structure data has coefficient estimates for significant factors. Deer browsing and other environmental factors have significant effects on plant community structure, and significant interactions effects between deer and environmental variables were found across structural measures. R^2 marginal provides variance explained only by fixed effects, and R^2 conditional provides the variance explained by the entire model, both fixed effects and random effects.

Model Variables	Plant Community Structure					
Fixed effects	**Richness**	**Diversity**	**Understory % Cover**	**Biomass**	**Sapling Density**	**Seedling % Cover**
Deer			-1318.6 (**)		-824.1(***)	-707.3(***)
C:N litter						340.4(****)
C:N soil				-0.2(**)		14.5(***)
Litterfall				-0.1(*)		-7.6(**)
Interactions						
Deer * C:N litter			23.2(**)		10.5(*)	-5.8(***)
Deer * C:N soil						
Deer * Litterfall					3.8(*)	
Random effects						
Site:Park	(*)				(****)	(***)
Park						
Model fit						
R^2 marginal	0.16	0.14	0.26	0.42	0.37	0.69
R^2 conditional	0.45	0.17	0.35	0.44	0.80	0.90

Table 3.3 Model summary for PERMANOVA analyses of plant community composition. (*) indicates significance p <0.1. The model R^2 is 0.4, explaining 40% of variance in plant community composition. P-values below 0.1 are considered ecologically significant.

Model variables	Df	SumOfSqs	R^2	F	Pr(>F)
Deer	1	0.6	0.05	0.9	0.56
C:N litter	1	1.0	0.09	1.5	0.07 *
C:N soil	1	1.0	0.09	1.4	0.09 *
Litterfall	1	0.7	0.07	1.1	0.33
Deer * C:N litter	1	0.2	0.02	0.4	0.99
Deer * C:N soil	1	0.3	0.02	0.4	0.99
Deer * Litterfall	1	0.6	0.05	0.9	0.63
Residual	10	6.7	0.6		
Total	17	11.1	**1**		
Total-Residual			**0.4**		

Table 3.4 All plant genus/species found at study sites, with significance indicator of deer browsing effects (*$p<0.1$,**$p<0.05$, ***$p<0.001$). Significance is indicated in bold, and represents an overall deer browsing effect when considering all sites. Most species were present at only a few sites, indicated by "present at site". Minimum and maximum percent cover, the average % cover of species at these sites, and associated plant growth forms are listed as well. Note that "trees" are only seedlings and saplings, not including overstory.

#	Genus	species	+ or -	Present at site	Min and Max	Average %cover deer	Average %cover no deer	Growth form
1	***Acer***	***sachharum*****	**-**	**SH,RT,WW**	**0-70**	**13**	**22**	**Tree**
2	***Acer***	***rubrum******	**-**	**WW,RR,BT, LL**	**0-25**	**0**	**7**	**Tree**
3	*Anemone*	*virginiana*		LL,WW,RT	0-6	1	1	Forb
4	*Bryophyte*			LL	0-14	0	6	Bryophyte
5	*Carya*	*Cordiformis*		WW	0-3	1	0	Tree
6	***Celastrus***	***orbiculatus*****	**+**	**SH,LL,WB**	**0-50**	**13**	**1**	**Vine**
7	***Circaea***	***lutetiana*****	**-**	**BT,LB,LL,RR,RT,SH,WW**	**0-33**	**2**	**6**	**Forb**
8	*Cornus*	*Florida*		WB	0-30	16	13	Tree
9	*Dioscorea*	*Villosa*		TR	0-20	0	7	Vine
10	***Elaeagnus***	***umbellata*****	**+**	**SH,TR**	**0-85**	**36**	**5**	**Shrub**
11	*Equisetum*	*hyemale*		WW,SH,LL	0-10	4	0	Forb
12	*Fraxinus*	*americana*		WB,TR,WW,LB,RT,LL,BT,RR,SH	0-55	17	13	Tree
13	*Fraxinus*	*pennsylvanica*		RT,LB,WW	0-45	16	12	Tree
14	*Fraxinus*	*sp.*		SH	0-2	1	0	Tree
	Fraxinus				0-55	16	12	Tree
15	*Galium*	*aparine*		WW	0-30	10	0	Forb
16	*Geranium*			BT,LB,RR,RT,TR,WW	0-60	7	12	Forb
17	***Graminoid***	*******	**+**	**LB,LL,RR,SH,TR,WB,WW**	**0-95**	**23**	**7**	**Graminoid**
18	*Hepatica*			TR	0-5	0	2	Bryophyte
19	*Hylodesmum*	*glutinosum*		TR	0-5	2	0	Forb
20	*Lonicera*	*maackii*		RT,LB,WW	0-80	0	10	Shrub
21	*Lonicera*	*tatarica*		WB	0-75	0	25	Shrub
22	*Lysimachia*	*nummularia*		LL	0-12	3	5	Vine
23	*Nabalus*	*altissimus*		TR	0-10	4	1	Forb
24	*Parthenocissus*	*quinquefolia*		LB,WW,SH,BT,TR,RT,RR	0-98	10	17	Vine
25	*Phragmites*	*australis*		WW	0-50	22	0	Graminoid
26	*Podophyllum*	*peltatum*		BT,LB,RR,TR	0-70	16	13	Forb
27	***Prunus***	***serotina*****	**-**	**BT,LB,SH,WB**	**0-20**	**1**	**5**	**Tree**
28	*Prunus*	*virginiana*		WB	0-25	0	8	Tree
29	*Prunus*	*sp.*		SH	0-2	1	0	Tree
	Prunus				0-25	1	5	Tree
30	*Quercus*	*alba*		BT,LB,LL,RT,TR,WW	0-8	2	2	Tree
31	*Rhamnus*	*cathartica*		LB,WW,SH,WB	0-38	0	5	Tree
32	*Ribes*	*cynosbati*		LB,LL	0-12	3	1	Shrub
33	*Rosa*	*multiflora*		LL	0-45	12	23	Shrub
34	*Rubus*	*occidentalis*		WW,TR,LL,RT	0-18	2	2	Shrub
35	*Sagittaria*			TR	0-4	2	2	Vine
36	*Smilacina*	*racemosa*		WW,BT,LB,LL,RR	0-75	1	8	Forb
37	***Smilax***	***tamnoides****	**-**	**LL,WB,LB**	**0-65**	**1**	**13**	**Vine**
38	*Tilia*	*americana*		WW,TR	0-8	1	2	Tree
39	***Toxicodendron***	***radicans*****	**-**	**LB,LL,RT,SH,TR,WW**	**0-40**	**3**	**8**	**Vine**
40	***Trillium***	*******	**-**	**BT,LL,RR,RT,WW**	**0-50**	**3**	**15**	**Forb**
41	*Ulmus*	*americana*		LL,LB,TR,RT	0-46	5	6	Tree
42	*Zanthoxylum*	*americanum*		WW,RR,SH,	0-90	3	15	Tree
43	*Zelkova*	*serrata*		WW	0-2	1	0	Tree

References

Alverson, W. S., D. M. Waller, and S. L. Solheim. 1988. Forests Too Deer : Edge Effects Forests Northern Wisconsin. Conservation Biology 2:348–358.

Bardgett, R. D., and D. a. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Begley-Miller, D. R., D. R. Diefenbach, M. E. McDill, P. J. Drohan, C. S. Rosenberry, and E. H. Just Domoto. 2019. Soil chemistry, and not short-term (1-2 year) deer exclusion, explains understory plant occupancy in forests affected by acid deposition. AoB PLANTS 11:1–15.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, F. Dentener, B. Emmett, J. W. Erisman, M. Fenn, F. Gilliam, A. Nordin, L. Pardo, and W. De Vries. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis. Ecological Applications 20:30–59.

De Boeck, H. J., C. M. H. M. Lemmens, S. Vicca, J. Van Den Berge, S. Van Dongen, I. A. Janssens, R. Ceulemans, and I. Nijs. 2007. How do climate warming and species richness affect CO2 fluxes in experimental grasslands? New Phytologist 175:512–522.

Bradshaw, L., and D. M. Waller. 2016. Impacts of white-tailed deer on regional patterns of forest tree recruitment. Forest Ecology and Management 375:1–11.

Chesson, P. 2000. Mechanisms of Maintenance of Species Diversity. Annual Review of Ecology and Systematics 31:343–66.

Côté, S. D., T. P. Rooney, J.-P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological Impacts of Deer Overabundance. Annual Review of Ecology, Evolution, and Systematics 35:113–147.

Craine, J. M., D. A. Wedin, and P. B. Reich. 2001. The response of soil CO2 flux to changes in atmospheric CO2, nitrogen supply and plant diversity. Global Change Biology 7:947–953.

Dias, A. T. C., J. van Ruijven, and F. Berendse. 2010. Plant species richness regulates soil respiration through changes in productivity. Oecologia 163:805–813.

DNR. 2010. Michigan deer management plan. http://www.michigan.gov/documents/dnre/WLD_Deer_Management_Plan_FINAL_5.8_320639_7.pdf?20150917085823.

Farrer, E. C., and K. N. Suding. 2016. Teasing apart plant community responses to N enrichment: the roles of resource limitation, competition and soil microbes. Ecology letters 19:1287–1296.

Fox, J. W. 2013. The intermediate disturbance hypothesis should be abandoned. Trends in

Ecology and Evolution 28:86–92.

Frerker, K., A. Sabo, and D. Waller. 2014. Long-Term Regional Shifts in Plant Community Composition Are Largely Explained by Local Deer Impact Experiments. PloS one 9:1–17.

Froyd, C. A., E. E. D. Coffey, W. O. van der Knaap, J. F. N. van Leeuwen, A. Tye, and K. J. Willis. 2014. The ecological consequences of megafaunal loss: Giant tortoises and wetland biodiversity. Ecology Letters 17:144–154.

Gao, J., and Y. Carmel. 2020a. Can the intermediate disturbance hypothesis explain grazing–diversity relations at a global scale? Oikos 129:493–502.

Gao, J., and Y. Carmel. 2020b. A global meta-analysis of grazing effects on plant richness. Agriculture, Ecosystems and Environment 302:107072.

Goldstein, C. L., K. W. J. Williard, J. E. Schoonover, S. G. Baer, J. W. Groninger, and J. M. Snyder. 2010. Soil and Groundwater Nitrogen Response to Invasion by an Exotic Nitrogen-Fixing Shrub. Journal of Environmental Quality 39:1077–1084.

Gorchov, D. L., and D. E. Trisel. 2003. Competitive effects of the invasive shrub, Lonicera maackii (Rupr.) Herder (Caprifoliaceae), on the growth and survuval of native tree seedlings:13–24.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in ecology & evolution 7:336–9.

Hobbs, N. T. 1996. Modifications of ecosystems by ungulates. The Journal of Wildlife Management 60:695–713.

Koh, S., D. R. Bazely, A. J. Tanentzap, D. R. Voigt, and E. Da Silva. 2010. Trillium grandiflorum height is an indicator of white-tailed deer density at local and regional scales. Forest Ecology and Management 259:1472–1479.

Landsman, A. P., and J. L. Bowman. 2017. Discordant response of spider communities to forests disturbed by deer herbivory and changes in prey availability. Ecosphere 8.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–9.

Legendre, P., and E. D. Gallagher. 2001. Ecologically Meaningful Transformations for Ordination of Species Data. Oecologia 129:271–280.

Leibold, M. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. The American Naturalist 134:922–949.

Mattson, D. A. H. and W. J. 1992. The Dilemma of Plants : To Grow or Defend Author (s): Daniel A . Herms and William J . Mattson Source : The Quarterly Review of Biology , Vol .

67 , No . 3 (Sep ., 1992), pp . 283-335 Published by : The University of Chicago Press Stable URL : http. The Quarterly Review of Biology 67:283–335.

Mcnaughton, S. J. 1985. Ecology of a Grazing Ecosystem : The Serengeti. Ecological Monographs 55:259–294.

McNaughton, S. J. 1976. Serengeti migratory wildebeest: facilitation of energy flow by grazing. Science (New York, N.Y.) 191:92–94.

Metcalfe, D. B., G. P. Asner, R. E. Martin, J. E. Silva Espejo, W. H. Huasco, F. F. Farfán Amézquita, L. Carranza-Jimenez, D. F. Galiano Cabrera, L. D. Baca, F. Sinca, L. P. Huaraca Quispe, I. A. Taype, L. E. Mora, A. R. Dávila, M. M. Solórzano, B. L. Puma Vilca, J. M. Laupa Román, P. C. Guerra Bustios, N. S. Revilla, R. Tupayachi, C. A. J. Girardin, C. E. Doughty, and Y. Malhi. 2014. Herbivory makes major contributions to ecosystem carbon and nutrient cycling in tropical forests. Ecology Letters 17:324–332.

Metcalfe, D. B., R. A. Fisher, and D. A. Wardle. 2011. Plant communities as drivers of soil respiration: Pathways, mechanisms, and significance for global change. Biogeosciences 8:2047–2061.

Moi, D. A., R. García-Ríos, Z. Hong, B. V. Daquila, and R. P. Mormul. 2020. Intermediate Disturbance Hypothesis in Ecology: A Literature Review. Annales Zoologici Fennici 57:67.

Popma, J. M.A. and Nadelhoffer K J. 2020 Deer browsing effects on temperate forest soil nitrogen cycling shift from positive to negative across fertility gradients. Canadian Journal of Forest Research. 50(12): 1281-1288

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ritchie, M. E., D. Tilman, and J. M. H. Knops. 1998. Herbivore effects on plant and nitrogen dynamics in oak savanna. Ecology 79:165–177.

Russell, F., D. Zippin, and N. Fowler. 2001. A Review of Effects of White-tailed Deer (Odocoileus virginianus) on Plants, Plant Populations and Communities. The American Midland Naturalist 146:1–26.

Sakata, Y., and M. Yamasaki. 2015. Deer overbrowsing on autumn-flowering plants causes bumblebee decline and impairs pollination service. Ecosphere 6:1–13.

Schäfer, D., D. Prati, P. Schall, C. Ammer, and M. Fischer. 2019. Exclusion of large herbivores affects understorey shrub vegetation more than herb vegetation across 147 forest sites in three German regions. PLoS ONE 14:1–16.

Suominen, O., J. Niemelä, P. Martikainen, P. Niemelä, and I. Kojola. 2003. Impact of reindeer grazing on ground-dwelling Carabidae and Curculionidae assemblages in Lapland.

Ecography 26:503–513.

Waller, D. M., and N. J. Reo. 2018. First stewards: Ecological outcomes of forest and wildlife stewardship by indigenous peoples of Wisconsin, USA. Ecology and Society 23.

Ward, J. S., S. C. Williams, and M. A. Linske. 2018. Influence of invasive shrubs and deer browsing on regeneration in temperate deciduous forests. Canadian Journal of Forest Research 48:58–67.

Woods, K. 1993. Effects of Invasion by Lonicera tatarica L . On Herbs and Tree Seedlings in Four New England Forests. The American Midland Naturalist 130:62–74.

Chapter 4 Deer Browsing Effects on Plant Chemical and Nutrient Concentrations

Abstract

Herbivores influence ecosystem nutrient dynamics in many ways, including altering the concentrations of nutrients and secondary defense chemicals in plant tissues, which can impact decomposition and nutrient cycling. Although much is known about how white-tailed deer (*Odocoileus virginianus*) alter temperate forest vegetation structure and composition, information about how they alter plant chemistry and ultimately forest nutrient dynamics is lacking. This detailed analysis of plant chemical composition in temperate forest understory plant communities shows that deer browsing alters plant chemistry and that plant species vary in their responses to browsing. Within plant species, concentrations of carbon (C), nitrogen (N), phenol, terpene, glycoside, and quinone sometimes increased, sometimes decreased, and sometimes did not change, in response to deer browsing. As a result, the chemical composition of leaf litter entering soils can change in response to increased deer browsing pressure. Together with inherent differences among plant species in chemical composition shown here, this work highlights the importance of both selective browsing and browsing induced defenses for ecosystem nutrient dynamics. Even though both hypothesized mechanisms appear to operate simultaneously, shifts in plant community composition due to selective browsing are likely the dominant driver of changes in the phytochemical landscape in temperate forests, more so than browsing-induced chemical changes within species. Although others have shown that plant defense responses change depending on nutrient supply, shifting from tolerance to induced resistance, I did not detect interacting effects of browsing and C:N in soil and litter on foliar

chemical composition. Overall, among-species differences were more important than environmental variables in determining plant chemical responses to browsing.

Introduction

Herbivory can alter patterns and rates of ecosystem nutrient cycling via complex trophic interactions (Hunter 2016). A familiar and important trophic interaction is the one between herbivores and primary producers. Selective foraging by herbivores, including white-tailed deer and other ungulates, changes plant community composition, which ultimately alters the chemical composition of plant tissues and the senesced tissues entering soils as leaf (and other) litter (Pastor et al. 1993, Olff and Ritchie 1998, Wardle 2002). Plant litter chemistry, and other traits, have major influences on decomposition rates across biomes (Cornwell et al. 2008). Changes in plant litter chemistry in response to herbivory are widespread and can serve to decrease palatability (Boeckler et al. 2011, Schuldt et al. 2014). These plant responses can also have consequences for decomposability of leaf litter; plants that are harder to digest by herbivores are often also more resistant to microbial decomposition (Grime et al. 1996, Cornelissen et al. 1999). For example, when herbivores browse on plants that are easy to digest and avoid plants with relatively greater chemical or structural defenses, plant communities can shift composition to becoming dominated by plants that produce recalcitrant leaf litter that decomposes more slowly and lower soil nutrient availability (Cornelissen et al. 1999, 2004, Cornwell et al. 2008). The chemical composition of foliage and leaf litter resulting from senesced leaves, including carbon and protein content as well as plant secondary chemistry, has a strong effect on abundance and activity of decomposers (Taylor et al. 1989). Defensive secondary chemicals, such as phenols and alkaloids, can influence browsing behavior of ungulates. For example, elk preferentially browse on aspen with relatively low phenol and glycoside concentrations compared to aspen with higher concentrations (Bailey et al. 2007). Similarly, this can be found on Isle Royale in Lake Superior where moose selectively browse on highly palatable (low C:N) deciduous species, driving the plant community towards being less palatable (high C:N). In the boreal forest on this large island, moose browsing decreases plant diversity and lowers rates of N cycling by selectively removing species with high C:N ratios (Pastor et al. 1993, Pastor and Cohen 1997). This example shows that selective browsing, which deer are known for as well (Waller et al. 2009), can alter the quality and abundance of leaf litter entering the soil and thus feed back to influence soil nutrient availability. Leaf litter chemistry drives decomposition and mineralization rates, and when leaf litter recalcitrance increases decomposition decreases (Cornelissen et al. 1999, 2004). As such, slow growing species with more recalcitrant tissues function to lower soil nutrient availability and foster conditions that put fast-growing species with higher nutrient demands at a competitive disadvantage (Cornelissen et al. 1999). This process is likely similar for deer browsing in temperate forest ecosystems, given that deer browse selectively like most ungulates and have been shown to alter plant community composition quite dramatically (Waller et al. 2009, Frerker et al. 2014). Deer have also been shown to decrease both leaf litter decomposition and soil nitrogen mineralization rates (Harrison and Bardgett 2003, 2004, Chollet et al. 2019). However, whether or not these processes are driven by alterations in leaf tissue chemistry is not well understood. Harrison and Bardgett (2003) suggest that deer decrease decomposition through the effects of browsing on litter quality, but no studies have shown consistent impacts of deer browsing on C:N ratios in leaf tissues and litter as has been shown for moose browsing (McInnes et al. 1992, Harrison and Bardgett 2003).

In addition to selective foraging, herbivory can also change the phytochemistry of plant tissue by inducing chemical defense mechanisms (Baldwin and Schultz 1983, Baldwin et al.

2002, Karban and Baldwin 2007, Lindroth and St Clair 2013, Benedek et al. 2019). These mechanisms include changes in elemental stoichiometry, production of plant secondary metabolites (PSM), and the production of volatiles to attract enemies of insect herbivores (Kessler and Baldwin 2002, Rhodes et al. 2017). Damage to living plant tissues can be reflected in plant litter quality and can slow decomposition rates (Findlay et al. 1996).

Deciduous trees show two main responses to herbivory: 1) premature leaf abscission and 2) induction of secondary compounds (Chapman 2006). Premature leaf abscission typically leads to improved litter quality and an acceleration of decomposition, while the induction of secondary compound production can decelerate decomposition. Herbivore-induced changes in leaf litter can thus have important consequences for nutrient cycling and productivity when reduced decomposition rates decrease nutrient availability to plants. Herbivore effects on ecosystem processes can vary from negative, to neutral, to positive. This variation in direction of herbivore effects can be explained by variations in the abilities of plant taxa to induce compensatory or defensive responses.

Phenols, which encompass a broad spectrum of plant secondary metabolites in plant leaf tissues, have been intensively studied with regard to defense against herbivores (Sunnerheim-sjoberg 1992, Hunter and Schultz 1993, Warbrick et al. 2020). Phenolic glycosides are well studied in aspen and are known to provide effective defense against ungulates (Wooley et al. 2008, Rhodes et al. 2017). Quinones, which are formed by oxidation of phenols, bind to leaf proteins and inhibit digestion of proteins by herbivores (Bhonwong et al. 2009). Terpenes also function as plant defense compounds (Gershenzon and Dudareva 2007). For example Scots pine needles with high terpene concentrations can negatively impact moose browsing (Danell et al. 1990), and Norway spruce trees with increased terpenes were colonized less by bark beetles

(Erbilgin et al. 2006). Ungulate browsing on balsam fir in eastern Canada, however, did not seem to relate to phenol levels in needles (Warbrick et al. 2020). Each of these chemical compounds can vary in presence and quantity, and mixtures of all compounds combined vary within and among species (Moore et al. 2014). Herbivores can alter the mixture of chemical compounds in plant communities, whether these compounds are induced defenses or constitutive. Additionally, changes in the amounts and types of defensive compounds in plant tissues, as well as compensatory growth responses, can vary across environmental gradients due to differences in resource availability (Mattson 1992, Bardgett and Wardle 2003, Moore et al. 2014, Burghardt et al. 2018).

Variation in plant tissue chemistry across time and space has been described as the “phytochemical landscape” (Hunter 2016). It is in this phytochemical landscape that trophic interactions take place and nutrient cycling patterns and rates can change. In this study I analyze variation in foliar chemistry of temperate forest understory plant communities that have been experimentally protected from (by experimental exclosures for 20 years) or subjected to deer browsing. I explore a temperate forest understory phytochemical landscape, *sensu* Hunter (2016), focusing on plant C and N concentrations and plant secondary compounds distributed across browsed and non-browsed landscapes as defined by my experimental and control plots. I examine chemical compositions of plant species in browsed and non-browsed plots, to test mechanisms of intra- and interspecific differences in plant chemistry. This approach allows for comparing two possible mechanisms by which deer browsing can shift plant community chemistry: induced chemical responses (changes within species) and selective browsing (differences between species).

Plants need to balance resource uptake for use in replacement of lost tissues or to sequester and protect resources from herbivores (Schultz et al. 2013). Physiological trade-offs between growth and defenses exist because resources must be diverted either way; plants must either outgrow herbivory fast enough, thereby directing resources away from the production of chemical and structural defenses, or vice versa, must allocate resources to chemical and structural defenses thereby diverting resources from producing foliage and other tissues (Mattson 1992). Based on this trade-off between investing in growth vs. production of defense compounds in response to herbivory (Mattson 1992), the level of defense will vary with resource availability; As resources become more limited, competition favors plants with high levels of defense compounds due to constraints in ability to compensate for herbivory with new growth. In resource rich habitats, in contrast, competition favors plants capable of compensatory growth, increased N, and low levels of defense compounds.

The objective of this research is to compare deer browsing effects on understory plant chemistry across environmental gradients. I used fenced exclosures along a soil fertility gradient to test for deer browsing effects on plant C, N, and defense compounds, and associations between browsing and environmental factors. I predicted that plant defense compounds would increase with deer browsing in low nutrient environments and that plant chemical communities would change as a result. In contrast, I predicted that plant defense compounds would remain similar under high nutrient environments. The underlying premise here is that environments with low resource availability favor plant species with higher levels of defense compounds that lack the ability to allocate resources to compensatory growth in response to browsing. Also, I predicted that plant leaf N will increase with deer browsing under high nutrient environments, but will decrease under low nutrient environments, because resource rich environments select for fast-growing plants with low levels of costly defenses that outcompete plants producing costly defense compounds that diminish potentials for compensatory growth. Testing these predictions provides insights into two possible mechanisms by which the phytochemical landscape (plant community chemistry) might change with deer browsing: 1) intraspecific variation: browsing-induced production of chemical defenses alter plant leaf chemistry, and 2) interspecific variation among plant species: selective browsing alters the quality and abundance of leaf litter in the plant community.

Methods

Study sites

I selected nine study sites within three Metroparks distributed across Oakland, Macomb, and Wayne counties in the greater Detroit Metro area of Michigan, USA (https://www.metroparks.com/about-us/park-maps/). These sites are representative of temperate forests in the US Midwest and Northeast in having experienced increases in deer populations since the 1970s, with densities reaching up to 20 deer per km^2 in some areas (Alverson et al. 1988, DNR 2010). The three sites in each Metropark are abbreviated as follows: Spring Hill (SH), Tamarack (TR), and Wild Wing (WW) are within the Kensington Metropark (K); Big Tree (BT), Long Bark (LB), and Railroad (RR) are within the Oakwoods Metropark (OW) ; Lake Loop (LL), Return Trail (RT), and West Branch (WB) are within the Stony Creek Metropark (SC). In June and July of 2017, I sampled within (non-browsed plot) and outside (browsed plot) of nine 10 m x 10 m experimental deer exclosures constructed in 1999, three in each of the three Metroparks as described above. These 20-yr-old exclosures are in dry-mesic and mesic forest types. Dry-mesic forests are oak or oak-hickory forest types in southern Lower Michigan and typically grow on sandy loam or loamy soils. Mesic forests in southern Michigan are typically dominated by American beech and sugar maple, growing on loamy upland soils. The exclosures are 2.5m tall and exclude ungulates (deer) while allowing small herbivores to move in and out. Mean annual temperature in southeastern Michigan is 9°C, mean annual rainfall is 81cm, mean annual snowfall is 130cm (U.S. Climate Data), and mean annual litterfall is 3422 kg dry mass ha^{-1} y^{-1}. White-tailed deer (*Odocoileus virginianus*) is the sole ungulate browser in this region, with densities of 8 and 16 deer per square kilometer at the Stony Creek and Oakwoods and 16 deer per square kilometer at Kensington parks. Deer populations are estimated and actively managed by Huron Clinton Metroparks (Ryan Colliton, personal communication 2018).

Vegetation surveys

Vegetation surveys were performed inside and outside deer exclosures to test for the effects of deer browsing on plant communities (Chapter 3). Control plots were established outside and nearby each exclosure to ensure similar slope, aspect, and habitat. Within each control (browsed) and exclosure (non-browsed for 20 years) plot I randomly placed three 1m x 1m frame quadrat locations within each plot. All plant species in the ground layer and understory were identified within each quadrat. I focused on ground layer vegetation, including woody and non-woody species, and understory vegetation because these layers are accessible to browsing deer. Tree seedlings and saplings were included in the vegetation surveys; tree stems of 1 m in height or higher were considered saplings and those under 1 m were considered seedlings. Tree stems with a diameter at breast height of > 9cm were considered adult tree and excluded from vegetation surveys.

Percent cover was visually estimated for each species or taxa (or growth form in the case of graminoids), and bare ground if there was no vegetation. Percent cover of all species was then summed per quadrat, sometimes resulting in values above 100% if vegetation was overlapping.

Fresh leaves of the three most dominant species in each plot (browsed, and non-browsed), were collected in June and July 2017 with gloved hands and were flash frozen in liquid N. Because not all species were equally dominant or present in browsed and non-browsed plots, I sometimes collected different species inside and outside exclosures within the same forest stand. Therefore, some but not all species have paired samples from browsed and non-browsed plots. This approach was employed to maximize chemical information at the plot level given the limited budget to process samples. Frozen leaves were stored in a cooler on dry ice in the field and at -80 ˚C in the laboratory. Leaves were lyophilized prior to grinding in a ball mill for further chemical analyses. Plant species richness was calculated by summing the number of species present in a quadrat. Total plant percent cover was calculated by summing the percent cover of all plant species within a quadrat. Plant species diversity (inverse Simpson) was calculated using the vegan package in R. Plot level richness, cover, and diversity were calculated by averaging the three quadrats.

Leaves

Canopy leaf litterfall (kg ha^{-1} yr^{-1}) was estimated using five, 0.31 m^2 litter baskets (Popma and Nadelhoffer 2020) within each browsed (n = 9) and non-browsed plot (n = 9). Leaves were collected once in October and once in December (2018), separated by species, and dried for 48h at 80˚C in our University of Michigan laboratory. Collection baskets were 66 cm x 46 cm, modified from commercial laundry baskets by drilling drainage holes in the bottoms.

Canopy leaf litter was sorted by species and dried for 48h at 80°C to analyze leaf C:N for each individual species. Leaves were ground for 2 minutes to a fine powder prior to C:N, %C, and %N analyses. Site-level C:N ratios were calculated by first multiplying the mass of each represented species by the %C and %N in the litter of that species, resulting in grams of C and N in litter of each species at each site. Site level litter C:N ratios were then calculated by summing the masses of C and N of all the species at each site, and dividing total grams C by total grams N.

Environmental variables

I collected one composite soil sample inside and outside of each deer exclosure (n = 9) in June 2017. Each composite sample consisted of five 2.5 cm diameter soil cores sampled to a depth of 10 cm. Composite soil samples were used for determining soil C:N using a CN Analyzer (University of Michigan Biological Station Analytical Lab, Pellston, MI, USA). Fresh soils were stored at 4°C and prepared within 5 days of collection for C:N analyses.

Chemical analyses

Lyophilized, homogenized understory leaf tissue samples (*ca*. 20 mg) were extracted 3x in 300 µL HPLC-grade methanol (Fisher OPTIMA), in an ultrasonic bath (cycle time 15 mins). Ice was added to the bath to ensure that samples remained chilled throughout extraction, and the laboratory lighting was subdued any time the samples were not stored in an opaque container. Between extractions, the samples were centrifuged at 16,000 x g, and the supernatant was collected and brought to 1.0 mL with HPLC-grade methanol. Sample extracts were stored at -80 ^{o}C prior to further processing as described below.

A 100 µL aliquot of each supernatant solution was transferred to a certified-clean amber GC vial and was evaporated to dryness under vacuum at 30 ^{o}C. To the vial, 900 uL of fresh HPLC-grade acetonitrile (Fisher OPTIMA) and 100 µL of 95% N-Trimethylsilyl-N-methyl trifluoroacetamide (MSTFA; ACROS Organics) were added. The vials were vortexed until the extract was re-dissolved and the MSFTA was incorporated into the acetonitrile. Vials were then capped and the contents were refluxed at 65 ^{o}C for one hour. Preliminary testing showed that MSTFA produced substantially more derivatized phenols than N,O-

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) reagents both with- and without trimethylsilyl chloride catalyst (Sigma Aldrich) in these samples. No appreciable differences in derivatized products or abundances were found when refluxing 1, 2, 4, or 10 hours and products were found to be stable for >48 h at room temperature.

Samples were analyzed for semi-volatile components using a Thermo Scientific Trace 1310 GC and ISQ LT mass spectrometer. Data acquisition and analyses were performed using Thermo Scientific/Dionex Chromeleon (v.7.2; Dionex Corporation Sunnyvale, CA). 1 µL aliquots were injected, with a 1:10 split, into a constant flow of helium gas set to 1.5 mL min^{-1} onto a 45 m x 0.25 mm x 0.25 µm TG-5 MS column (Thermo Scientific). GC parameters were: inlet temperature 300 ^{o}C; initial temperature 100 ^{o}C, held for 1 minute then ramped at 4 ^{o}C to 270 ^{o}C then held at 270 ^{o}C for 16 minutes. The MS transfer line was held at 300 ^{o}C and the ion source at 320 ^{o}C. MS scanning began at 5 minutes runtime, just after the solvent front and excess MSTFA finished eluting; scanning was from *m/z* 50-500 for 0.2 s/scan. Peaks were integrated manually, baseline spectral correction performed for each individual peak using the average of 21 spectra from within the peak (14 at FWHM and 7 from the apex) and 9 spectra from the nearest detected baseline. Spectra were queried using the NIST 2014 EI GC/MS spectral database. Periodic re-injection of samples throughout the run showed no appreciable degradation of sample constituents and the MS tune stability was assured by means of infusing calibration gas directly into the ion source every 24 hours.

A second, 10 µL aliquot was diluted to 2 mL total volume in ultrapure water and was analyzed for total phenolic content using the Folin-Ciocalteau method optimized for use on a segmented flow analyzer (Seal Analytical AA3 automated colorimeter; Seal Analytical Meqoun, WI). Briefly, the manifold employed was identical to that commonly utilized for *ortho*-phosphate analysis (Seal Analytical Application #G-175-96). Samples were infused into the manifold at 1.6 mL/min with a Sample:Wash ratio of 1:3. The 0.2N Folin-Ciocalteau reagent (Sigma Aldrich) with 1% by volume Dowfax 2A1 (Dow Chemical Company, Midland, MI) was infused immediately after segmentation air at 0.1 mL/min. A delay coil (10-turn) enabled mixing of the reagent and sample, thereafter saturated sodium carbonate solution was infused at 0.1 mL/min. Following the infusion of sodium carbonate, samples entered another 10-turn mixing coil and were subsequently incubated at 37 ºC for 10 minutes before encountering a 10-turn delay loop (to return samples to room temperature), and then colorimetric analysis at 660 nm. Gallic acid was used as the calibration solution, and yielded a linear regression ($r^2 > 0.995$) over the range of 2-200 mg/L. To correct for chlorophyll absorbance at this same wavelength, samples were also run with ultrapure water in place of Folin-Ciocalteau reagent, and the resulting absorbances subtracted from the raw absorbances run with reagent in place.

Data analyses

Effects of browsing, environmental factors, and plant species identity on chemical richness, chemical diversity, %C, %N, C:N, total phenols, and other chemical constituents as described below were analyzed using linear mixed effects models (lmer function in R, R Core Team 2019). I ran two separate models for each response variable: 1) effects of browsing and environmental gradients, and their interactions were analyzed using linear mixed effects models. This model tested predictions on deer browsing effects on plant chemistry. 2) Effects of browsing, species identity, and their interactions were analyzed using linear mixed effects models. This second model tested predictions on intra- and interspecific variation in chemical responses to deer browsing. Due to lack of power and paired samples the models were split into two, instead of combining all factors into one. Site locations were included as random effects to account for variation among sites. Within species differences in all response variables were analyzed by running a linear model (lm function in R) for each species for which I had samples from both browsed and non-browsed plots. Data from American elm (*Ulmus americana*) were log transformed to equalized variance before running the linear model.

Mass spectrometer analyses yielded 674 individual compounds across 181 samples. I removed 496 rare compounds that were present in less than 5% of samples. The remaining 178 compounds contained many compounds that were very similar to one another, oftentimes only being a slightly different derivative. Due to this overlap in compounds, I aggregated the 178 compounds into 18 groups: acid, alcohol, amino alcohol, amino acid, catechin, gluconolactone, glyceride, glycoside, ketone, lipid, metabolite, nitrile, nucleic acid, quinone, siloxane, sterol, sugar, and terpene. Grouping of compounds was done using tentative identifications from the NIST database. Although not all groups of compounds are active against herbivory, all compounds were included in chemical community analyses to test for browsing effects on overall plant chemical communities.

Effects of browsing, environmental factors, and plant species identity on four specific groups of chemical compounds were analyzed using linear mixed effects models (lmer function in R). I chose four groups of compounds that are involved in plant defenses against herbivores: 1) phenols, 2) terpenes, 3) glycosides, and 4) quinones. Phenols were measured separately and were calibrated to reflect actual concentrations ($\mu g\ mg^{-1}$). As I was not able to calibrate the other chemical compounds, these data were analyzed using areas under curves as proxies for relative amounts instead of actual amounts. As such, data on terpenes, glycosides, and quinones are shown as relative comparisons and not as actual concentrations. Species differences in phenol concentrations were analyzed using log transformed phenol data.

I analyzed main effects of deer browsing and five environmental factors on chemical composition, as well as interactions between deer browsing and each environmental factor, using a PERMANOVA model. PERMANOVA models were executed with the adonis2() function in the **vegan** package in R (R Core Team 2019). Chemical compounds were grouped into 19 classes: acid, alcohol, amine, amino alcohol, amino acid, catechin, gluconolactone, glyceride, glycoside, ketone, lipid, metabolite, nitrile, nucleic acid, quinone, siloxane, sterol, sugar, and terpene. The response variable was my chemical community dissimilarity index. To create the dissimilarity index, I transformed chemical compound abundance data using a Hellinger transformation, followed by the calculation of Euclidian distances. I chose the Hellinger transformation because it is particularly suited for abundance data with many zeros (Legendre and Gallagher 2001). Effects of sites (n=8, excluding the lake loop site) were accounted for as random effects because I was mainly interested in environmental effects. PERMANOVA analyses were performed on n=8 sites, excluding one site referred to as “Lake Loop”. Data from this site in the Stony Creek forest were excluded because leaf litter C:N average here was more than 2 standard deviations outside the distribution of other plots, likely due to the presence of large amounts of *Tilia americana* (American basswood) leaf litter at this site but at none of the other sites. Additionally, similar PERMANOVA analyses were performed separately to test for within and among species differences. Within species differences were tested with separate models for each species collected from browsed and non-browsed plots to test differences in chemical community composition due to deer browsing.

Chemical composition data were plotted using non-metric-dimensional scaling (NMDS) scores of the chemical compositions of the two deer browsing treatments (browsed and not browsed) and along environmental gradients. NMDS scores were calculated using the metaMDS function in the vegan package in R. In order to visualize effects of continuous environmental variables on chemical composition, I transformed data from continuous environmental variables into three discrete factor variables: High, Medium, Low. Sites were assigned to High, Medium, or Low bins based on histogram distributions of site-level environmental averages for the following variables: C:N Soil, C:N Litter, and Litterfall. Plotting histograms of site averages for each variable allowed me to bin sites into High, Medium, or Low, using natural breaks in distributions (Chapter 3, Table 1).

Site-level phenolic content was calculated by weighting phenolic concentration using the average percent cover of individual species. Phenolic data were analyzed for the three most dominant species in each plot. I multiplied the phenol concentration of each leaf sample by the average percent cover in a plot for the corresponding species. I then summed the averages of this product for each species in the plot to estimate the plot level phenolic content. A linear model (lm in R) was used to test for browsing and site effects on site phenol content. GC Mass Spec data were not subjected to a site-level analyses, because I was not able to calibrate other compounds and weight them accordingly.

Results

Deer browsing effects on foliar nitrogen and C:N

Deer browsing and the three other environmental factors had no overall effect on either %N or C:N in understory plant leaf tissues (Figure 4.1, Table 4.1), but species showed significant variations in their C:N responses (p=0.01, Table 4.1). Foliar %N varied significantly among species (p<0.001), from a low of 1.6% in red maple (*A. rubrum*) without deer browsing to a high of 4.3% in autumn olive (*E. umbellata*) with browsing (Figure 4.1). Browsing decreased foliar %N in American elm (*U. americana*) (p=0.01) and increased %N in autumn olive foliage (p=0.05) (Figure 4.1). C:N ratios varied significantly among species (p<0.0001, Table 4.1), from a low of 11.1 in autumn olive to a high of 30.0 in red maple. C:N ratios increased with browsing in American elm and Virginia creeper (*P. quinquefolia*) (p=0.001 and p=0.03 respectively) but decreased in autumn olive (p=0.03). Furthermore, deer browsing effects on C:N ratios significantly varied significantly among species (p=0.01, Table 4.2).

Deer browsing effects on plant defense compounds

Although overall plant leaf total phenolic content (µg mg^{-1}) was not affected by deer browsing, my analysis showed a weak, but significant, interaction between deer browsing and canopy litterfall (Table 4.1). Browsing effects were more positive with increasing canopy litterfall (Table 4.1, coefficient estimate 9.5e-04). Total phenol concentrations also differed among plant species (p<0.001, Figure 4.2) ranging from 0.5 µg mg^{-1} in non-browsed American elm foliage to 2.9 µg mg^{-1} in both browsed American elm and sugar maple (*A. saccharum*). Browsing effects within species varied (p=0.004); autumn olive, sugar maple, and American elm increased in total phenols with deer browsing, while Virginia creeper decreased (Figure 4.2). To weigh the total amount of phenol each species contributes to the ecosystem, I plotted total phenol against percent cover for each species (Figure 4.3). Species with high phenolic content and low percent cover contribute lower amounts of phenol to the phytochemical landscape than do species that are high in phenolic content and percent cover. Similarly, species with low phenol concentrations, but with relatively high percent cover values contribute more phenols to the chemical landscape than do species that are low in phenol concentration and with low percent cover values. Species that plot on the top right corner of this graph, in this case sugar maple, are the most important at the landscape scale, followed by species in the top left or bottom right.

I analyzed relative amounts of chemical defense compounds, including terpene, glycoside and quinone, based on the areas under GC mass spectrometer curves (See Methods). As expected, plant species differed in chemical defense compounds, and in their responses to browsing (Table 4.1). Foliar terpene was the only group of defense compounds affected by deer browsing, surprisingly showing an overall decrease, but with variations in response among species (Table 4.1, Figure 4.4). Ash (*Fraxinus sp.*) had the highest terpene levels, while autumn olive, Virginia creeper, poison ivy (*T. radicans*), geranium, and may apple (*P. peltatum*) all showed relatively low terpene levels (Figure 4.4). Canopy litterfall C:N was negatively associated with terpene, and positively associated with glycoside, but these associations were weak (Table 4.1). Overall, deer browsing, and environmental factors did not influence foliar glycoside content. However, glycoside content did vary significantly among plant species ($p<0.0001$, Figure 4.5) and plant species showed variation in glycoside responses to deer browsing ($p<0.0001$, Figure 4.5). Glycoside levels were relatively high in May apple, ash, and Virginia creeper (Figure 4.5). Quinones were not abundant and were only detectable in red maple, American elm, geranium, poison ivy, and sugar maple, with levels being highest in red maple (Figure 4.6).

Plant chemical richness and diversity

Linear mixed effects models showed significant variations in chemical compound richness and diversity based on sites, plant species, and deer browsing (Table 4.1, Figure 4.7). Overall chemical richness decreased under deer browsing, but this response was not uniform across sites (Figure 4.7). Furthermore, variation in chemical richness was strongly associated with site location regardless of deer browsing treatment, as shown by highly significant random effects (Table 4.1). Chemical compound richness at LB (53) and RR (53) was higher than at SH

(38). Similarly, diversity of chemical compounds varied between sites (p<0.0001, Figure 4.7), but surprisingly no browsing effects were detected. There was an apparent trend of increasing chemical diversity with deer browsing, but the overall effect of deer browsing was not statistically significant (Figure 4.7). Chemical diversity was highest at LL (13), and the remaining 8 sites did not differ. Interactions between deer browsing and overstory litterfall were significant; specifically, browsing effects on richness showed positive associations (trending less negative) with increased litterfall (p=0.02, Table 4.1).

Species differed in chemical richness (p=0.003) as calculated using the 178 most common individual chemical compounds identified in foliage at these sites; May apple and ash showed the highest foliar chemical richness in their leaves (59 and 58 respectively), and were significantly different from American elm (38), graminoids (37), and autumn olive (35). Chemical richness in sugar maple (41), poison ivy (40), and trillium (40) was also lower than in American elm and graminoids (Figure 4.8). Within species differences were found in the chemical richness of only one genus, ash (*Fraxinus sp.*), which increased from 53 to 64 individual chemical compounds with deer browsing (p=0.005). Variation in chemical compound diversity between species was significant (p<0.0001), and browsing effects differed between species (p=0.0003). Ash (*Fraxinus sp.*) showed the highest Inverse Simpson of value 13.4, followed by enchanter's nightshade (*Circaea lutetiana*) at 12.0. The other ten species (May apple, American elm, graminoids, poison ivy, autumn olive, trillium, red maple, sugar maple geranium, Virginia creeper) were significantly lower, ranging between 3.5 and 8.5. Deer browsing only affected chemical compound diversity in autumn olive, increasing it from 4.6 to 7.9 (p=0.003, Figure 4.8).

Plant chemical composition

Plant chemical composition was affected by deer browsing, soil C:N, and litter C:N (Table 4.1, Figure 4.9). Overall, the PERMANOVA model explained a small portion of chemical composition (Table 4.1, R^2=0.1). The strongest predictor in the model was deer browsing (R^2=0.03). In contrast to predictions, PERMANOVA analyses did not detect any variation in deer browsing effects with other environmental factors. To visualize the effects on chemical composition, I plotted NMDS scores of chemical communities (Table 4.1, Figure 4.9). The stress value of NMDS was acceptable at 0.2.

Plant chemical community composition also varied based on plant species identity (Figure 4.10). Moreover, the direction and strength of browsing effects on plant chemical communities differed among species as well (Figure 4.11). PERMANOVA analyses showed that American elm, ash, autumn olive, sugar maple, and Virginia creeper all had different within-species chemical communities when comparing browsed to non-browsed leaves (Figure 4.11).

Site level chemistry

Species differed in phenolic concentration, and deer browsing had effects on phenolic concentrations in some but not all species. Similarly, the presence of some, but not all, species within plant communities was affected by deer browsing (Chapter 3, Table 3.4). To estimate site level phenolic content, I took into account the phenolic content and the average percent cover of each species, in browsed and non-browsed plots. Deer browsing did not affect site level phenolic content (p=0.16), but among site differences in phenolic content were significant (p=0.003, Figure 4.12). SH and LB, had relatively higher phenol content than RT, WW, and LL (Figure 4.12). No differences were found among sites or browsing treatments when comparing site-level %N or C:N.

Discussion

The goal of this study was to provide new insights into two possible mechanisms by which the phytochemical landscape (plant community chemistry) might change with deer browsing: 1) intraspecific variation: browsing-induced production of chemical defenses alter plant leaf chemistry, and 2) interspecific variation among plant species: selective browsing alters the quality and abundance of leaf litter in the plant community.

I detected evidence for both mechanisms: Plant chemical composition varied strongly with species identity and plant species varied with respect to how their chemical compositions changed in response to deer browsing. Overstory litter C:N was the only environmental factor among those examined that had a significant direct effect on changes in chemical composition, but interactive effects between deer browsing and both litter C:N and litterfall were detected (Table 4.1). Of the defensive compounds analyzed here, phenol and terpene were affected by deer browsing; terpene decreased with browsing but this affect covaried with litter C:N, and browsing effects on phenol covaried positively with litterfall. Chemical richness decreased with browsing, but variation in richness was most strongly influenced by site location and plant species identity. Overall, differences in plant chemistry varied most strongly among plant species, and less so with deer browsing and other environmental factors.

Plant nitrogen and C:N

I predicted that plant leaf N would increase with deer browsing under high nutrient environments, but would decrease under low nutrient environments, because resource rich environments select for fast-growing plants with low levels of costly defenses that outcompete plants producing costly defense compounds that diminish potentials for compensatory growth. Contrary to these predictions, variations in plant N and C:N varied most strongly with species identity, and showed no general or overall detectable responses to either deer browsing or environmental factors. Some individual species did vary in response to deer browsing. Changes in foliar N and C:N were most striking in autumn olive (*E. umbellata*) an exotic invasive species, in which foliar N concentration increased in response to browsing, and American elm (*Ulmus americana*) which decreased in foliar percent N. Browsing also increased percent cover of autumn olive, suggesting that deer herbivory leads to a competitive advantage in this species, potentially linked to N concentrations. The mechanism for this advantage is unclear based on this study, and it could be a direct or indirect result of deer browsing. Either way, the phytochemical landscape, *sensu* Hunter (2016), is likely affected by the combined increase in percent cover and N of autumn olive.

Foliar C:N is another measure of plant tissue quality, affecting both herbivores and decomposers. The idiosyncratic nature of C:N responses at the ecosystem level to deer browsing that I observed in this study have also been reported by others (Harrison and Bardgett 2003, Bardgett and Wardle 2010). My results show this inconsistency is likely caused by differences among species in response to browsing, more so than by induced changes in plant tissue quality. Relative abundances of plant species at a site affect how ecosystem C:N is affected by deer browsing. Herbivore-induced changes in plant tissues have been shown to persist in senesced litter (Findlay et al. 1996, Chapman 2006). As litter C:N is an important driver of litter decomposition (Cornwell et al. 2008), therefore, significant browsing effects on plant community C:N will likely have consequences on decomposition rates and nutrient cycling in soils.

Plant defense compounds

I predicted that plant defense compounds would increase with deer browsing in low nutrient environments and that plant chemical communities would change as a result. In contrast, I predicted that plant defense compounds would remain similar under high nutrient environments. The underlying premise here is that environments with low resource availability favor plant species with higher levels of defense compounds that lack the ability to allocate resources to compensatory growth in response to browsing. Variation in plant defensive compounds was most strongly correlated with plant species identify, and chemical responses to browsing varied among plant species. Contrary to my predictions, effects of browsing were not found to correlate strongly with environmental factors. Variations within and among species suggests temperate forest understory plants can produce defensive compounds in different amounts both constitutively and in response to browsing. Phenol concentrations in American elm, sugar maple (seedlings), and autumn olive all increased with deer browsing, while concentrations in Virginia creeper decreased. Increased phenolic concentration did not however, always relate to increased performance (percent cover). Among the species in this study for which deer browsing increased foliar phenolic contents, only autumn olive increased in abundance with deer. These intra- and inter-specific differences in plant secondary metabolites can have wide effects on ecosystem functioning, including soil biota (Coq et al. 2018), litter decomposition (Austin and Ballaré 2010), soil N mineralization and C respiration (Kanerva and Smolander 2008), and microbial communities (Schweitzer et al. 2007).

Plant genetic frameworks can be important in understanding intraspecific variation in plant defenses, and the consequences for soil functioning (Classen et al. 2007, Burghardt et al. 2018, Coq et al. 2018). I detected intraspecific variation in chemical defenses against browsing in a few understory species, but not all. Most species had no detectable response in phenol levels to browsing. The lack of induced plant defenses however, does not mean a plant is not equipped to respond to browsing. This could instead indicated a tolerance rather than a resistance strategy, or interspecific variation rather than intraspecific variation (Warbrick et al. 2020). Interspecific variation in plant chemistry also contributes significantly to a change in local chemical landscape due to shifts in relative abundance after selective foraging by herbivores (Pastor and Naiman 1992). Interspecific variation in responses to herbivory can often be explained by associated metabolic costs and other trade-offs (Moore et al. 2014). Constitutive production of plant defense compounds is costly, therefore inducible defenses might be more common in plants that are not under continuous herbivory (Mithofer and Boland 2012).

Species vary in phenol concentrations, but relative abundances of species vary across sites, and species abundances change with browsing. Site-level phenolic content, one piece of the phytochemical landscape, can be assessed by looking at shifts in phenol concentrations and shifts in plant species abundances due to deer browsing. Selective foraging shifts plant community composition, and thereby changes the chemical landscape and ultimately affect nutrient cycling (Pastor and Naiman 1992, Wardle 2002). Furthermore, my work showed some species respond to browsing by increasing and some by decreasing phenol concentrations. By combining phenolic concentrations and abundance data from long-term non-browsed and browsed plots, I did not detect differences in site-level phenol content. Similarly, foliar percent N and C:N remained unchanged at the site-level. From this preliminary comparison I cannot conclude that deer browsing affects plant chemical landscapes, however, variation in phytochemical landscapes among sites was significant. This variation among sites could add to the difficulty in finding consistent general patterns in deer browsing effects. Further research should carefully consider species abundance data and chemistry data to make site-level comparisons.

Plant chemical richness and diversity

Individual plants and plant communities with greater defensive chemical diversity are thought to be better defended against herbivore attacks (Moore et al. 2014). I found significant variation in chemical diversity and richness both among species and with deer browsing. Chemical diversity in Autumn olive (*E.* umbellata) increased, as did chemical richness in Ash species (*Fraxinus sp.*). Autumn olive increased not just in chemical diversity, but also N concentration, in response to browsing and is a strong competitor that increases significantly in abundance in the presence of deer browsing (Table 4.3). Other species with relatively high chemical diversity included ash and enchanter's nightshade. While autumn olive simultaneously showed increased percent cover and chemical diversity, enchanter's nightshade decreased in percent cover with high chemical diversity. The ecological implication is therefore not uniform, more diverse defensive chemicals sometimes, but not always, equated to better defense against herbivores or came at the expense of growth and reproduction. Deer browsing decreased chemical compound richness, but species varied and not all species responded similarly. Autumn olive and graminoids, both strong competitors in the presence of deer, had low chemical compound richness. At the site level, plant communities varied in chemical richness and diversity, but I detected no effects of deer browsing. Similar to leaf N, among-species differences in richness and diversity of defense chemicals were more significant than within-species responses to browsing.

Plant chemical composition

Chemical composition in understory plant leaves correlated most strongly with plant species identity, and varied to a lesser extent with deer browsing and soil and litter C:N. Additionally, individual species also responded differently to deer browsing; both the direction and magnitude of browsing effects on chemical communities varied among species.

Environments are variable, in space and time, and this variation likely drives differences in chemical composition within and among plant species (Moore et al. 2014). Although underlying environmental gradients can be important drivers of plant chemistry and defense strategies within and among species (Findlay et al. 1996, Bardgett and Wardle 2003, LeBauer and Treseder 2008, Burghardt 2016), I did not detect strong correlations between chemical composition and environmental factors. Plasticity of individual species responses to herbivory can result in differential induction of defense compounds across environmental gradients (Burghardt et al. 2018). While most research has focused on insect herbivory, within-species variation in defense compounds has been shown Scotts pine, where individuals with higher terpene concentrations were browsed less by moose (Danell et al. 1990).

Plant defense strategy can change depending on nutrient supply, shifting from tolerance to induced resistance (Burghardt 2016). However, I did not detect interacting effects between environmental gradients and deer browsing on plant chemical compounds. Chemical composition was affected by both deer browsing and environmental factors, but no interactions were detected. Overall, among-species differences were more important than environmental variables in determining plant chemical responses to browsing.

Could the impacts of deer browsing on plant chemistry described here lead to changes in soil nutrient cycling as detected by Popma and Nadelhoffer (2020)? Herbivore-induced changes in plant tissues have been shown to persist in senesced litter (Findlay et al. 1996, Chapman 2006), but this is not always the case (Frost and Hunter 2008). Work on insect herbivores has shown that induction of chemical defenses can take place both at the site of attack or can occur systemically (Kessler and Baldwin 2002). Furthermore, plants can reallocate resources away from the site of attack to store N in roots and stems while increasing C in leaves (Frost and

Hunter 2008). Ungulates forage differently than insects, but variability in the mode of herbivore defenses is important to consider, nonetheless. Identifying whether the effects of deer browsing on temperate forest understory plant tissues persist in senesced litter is important to truly link browsing effects on plants to browsing effects on ecosystem processes such as decomposition and nutrient cycling. A recent decomposition experiment on a forested island off the west coast of Canada showed that litter decomposition is slower with deer browsing (Sitka black-tailed deer, *Odocoileus hemionus sitkensis*), and the authors suggested this is due to lower litter quality (Chollet et al. 2019). Chollet et al. attribute the decrease in litter quality they observed to documented shifts in plant communities, but whether induced defenses within species at their site contributed to changes in litter quality is unclear. Other ungulate browsing studies have found contradicting results, with slower decomposition in browsed Birch (*Betula pubescens*) in the Scottish Highlands (Harrison and Bardgett 2003) and increased decomposition rates in grazed tundra (Olofsson and Oksanen 2002). In addition to aboveground chemical changes in plant tissues, roots belowground can also respond chemically to herbivory (Hamilton and Frank 2001). Root responses to deer browsing are therefore an important topic to explore in future research efforts.

Conclusion

This work shows that deer can change plant community chemistry by selective foraging, due to variation among species in plant chemistry, and by stimulating browsing-induced chemical responses in some species. Selective browsing by deer is known to dramatically alter plant communities and forest successional pathways (Waller and Alverson 1997, Rooney 2001, Russell et al. 2001, Rooney and Waller 2003, Côté et al. 2004, Frerker et al. 2014). Effects of deer browsing and species identity on leaf chemistry responses were both significant, but species identity was significant across all measured responses while browsing was not. Therefore, although both hypothesized mechanisms appear to operate simultaneously, shifts in plant community composition due to selective browsing likely serve as the dominant driver of changes in the phytochemical landscape in temperate forests, more so than browsing-induced chemical changes within species. Although others have shown that plant defense responses change depending on nutrient supply, shifting from tolerance to induced resistance, I did not detect interacting effects of browsing and C:N ratios in litter and soil on foliar chemical composition. Although site-level chemistry did not show significant changes in phenol content, other (senesced) litter quality measures should be considered in this comparison, as well as a more accurate weighting based on biomass estimates. My conclusions should therefore be interpreted with caution until more complete measures are made.

Acknowledgements

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Figures

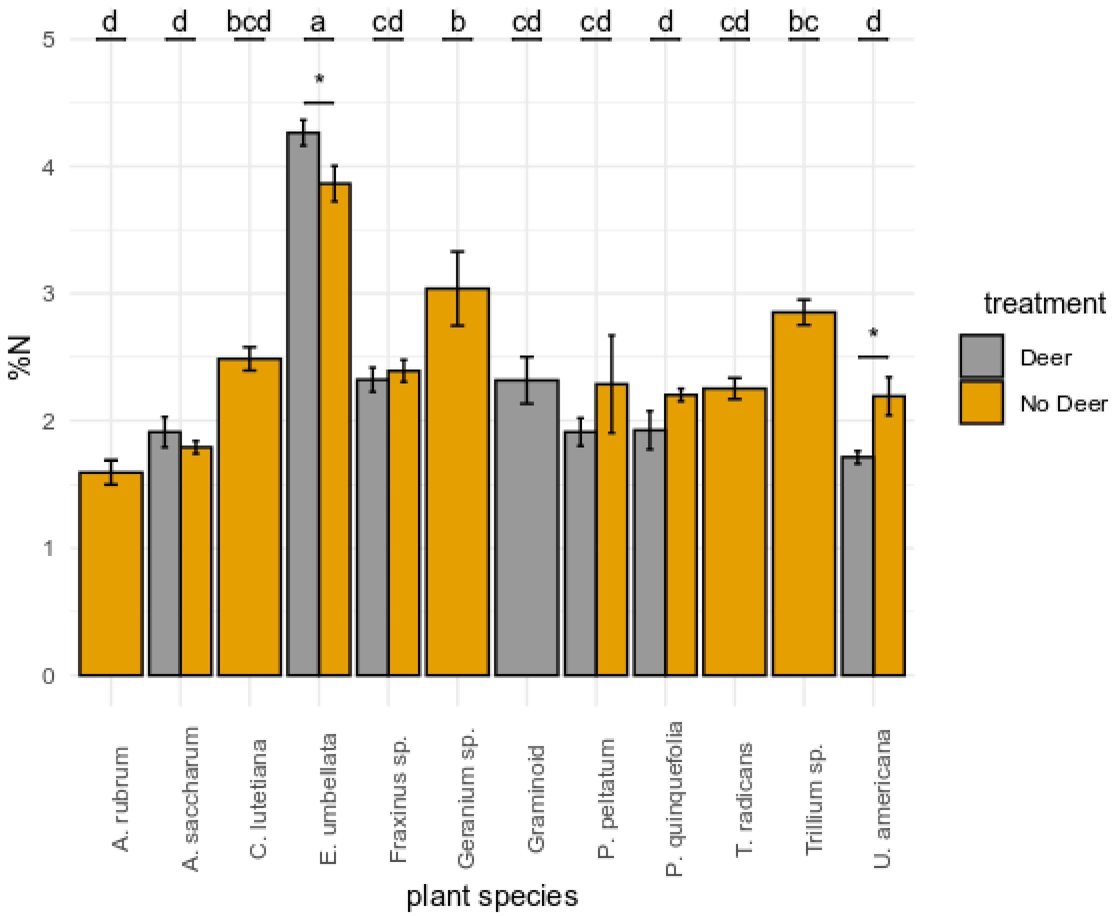

Figure 4.1 %N in plant leaf tissues with and without deer browsing in the twelve most common plant species across all sites. Significance level α=0.05, overall effect of browsing on %N was analyzed using a mixed effect model, with sites as random effects. No overall effect was found. Browsing effects on individual species were analyzed using a linear model (anova), significance was found in two species and is indicated with a *: *U. americana* decreased %N with browsing ($p<0.01$) and *E. umbellata* increased %N with browsing ($p<0.05$). %N also varied between species, species with different letters were significantly different.

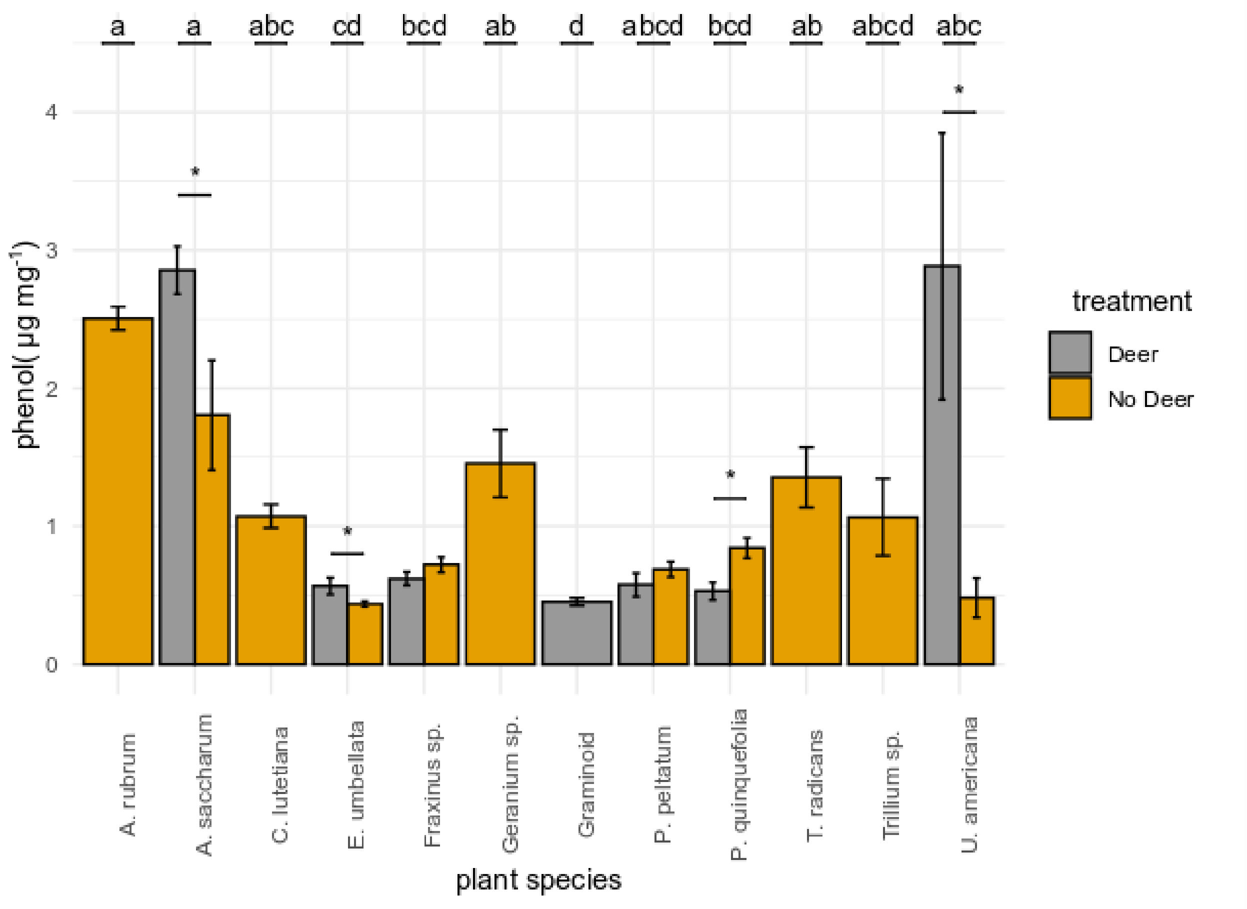

Figure 4.2 Total phenol (µg mg^{-1}) in plant leaves with and without deer browsing, in the twelve most common species. Significance level α=0.05, overall effect of browsing on phenol was analyzed using a mixed effect model, with sites as random effects. No overall effect of browsing was found, but species differed. Browsing effects within individual species were analyzed using a linear model, significance was found in four species: *A. saccharum, E. umbellata, P. quinquefolia,* and *U. americana*.

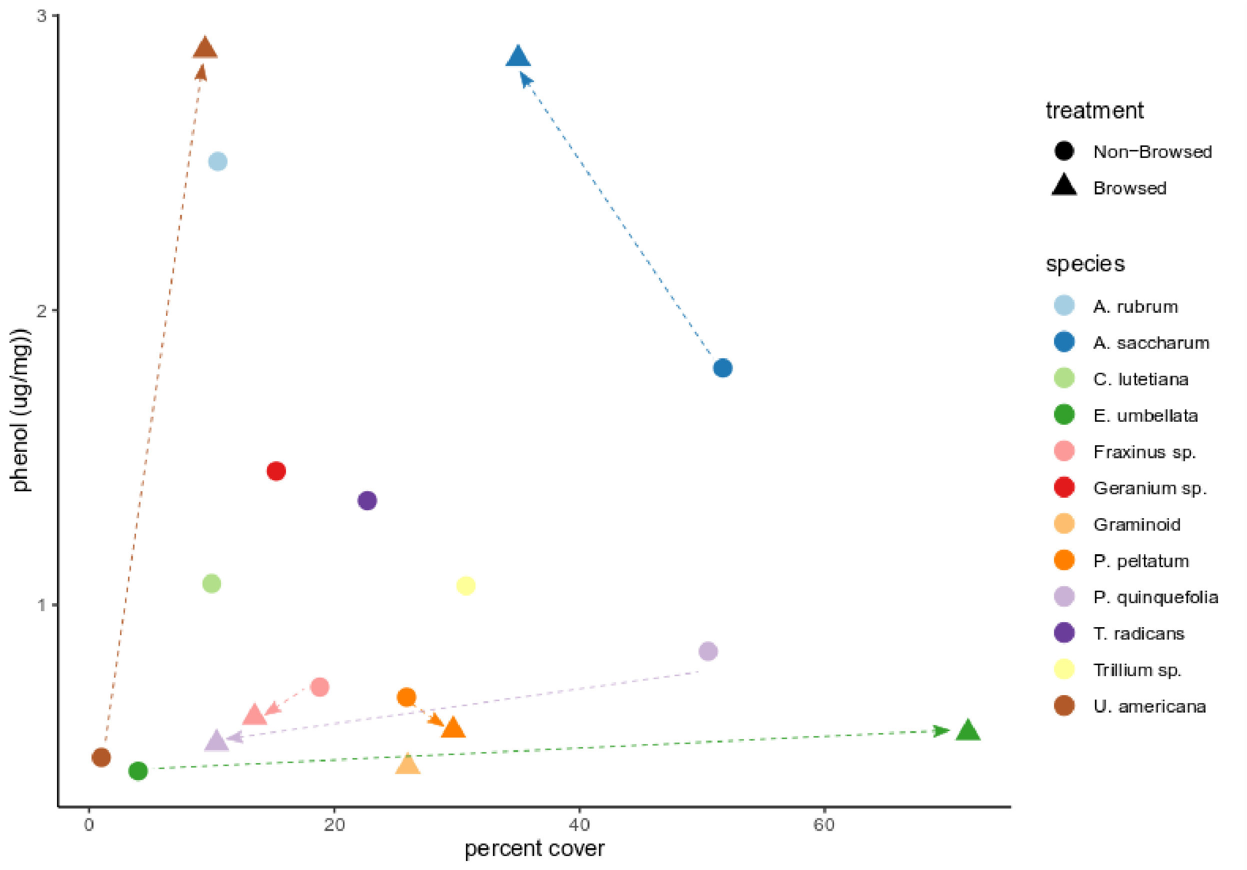

Figure 4.3 Total phenol and percent cover per species. Twelve taxa were found overall, five taxa were found only in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Phenol concentration varied among species, and not all species respond similarly to deer browsing.

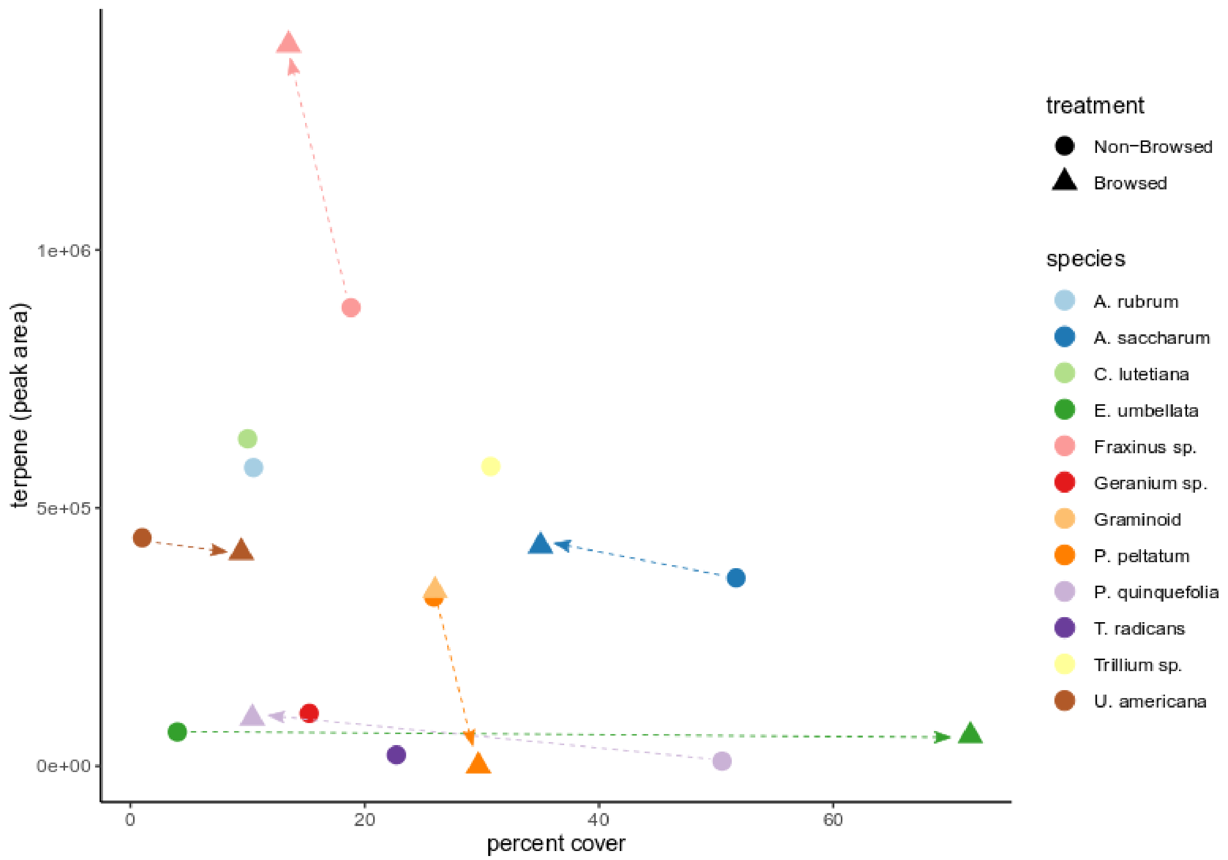

Figure 4.4 Relative terpene amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative terpene amount varies among species, and not all species respond similarly to deer browsing.

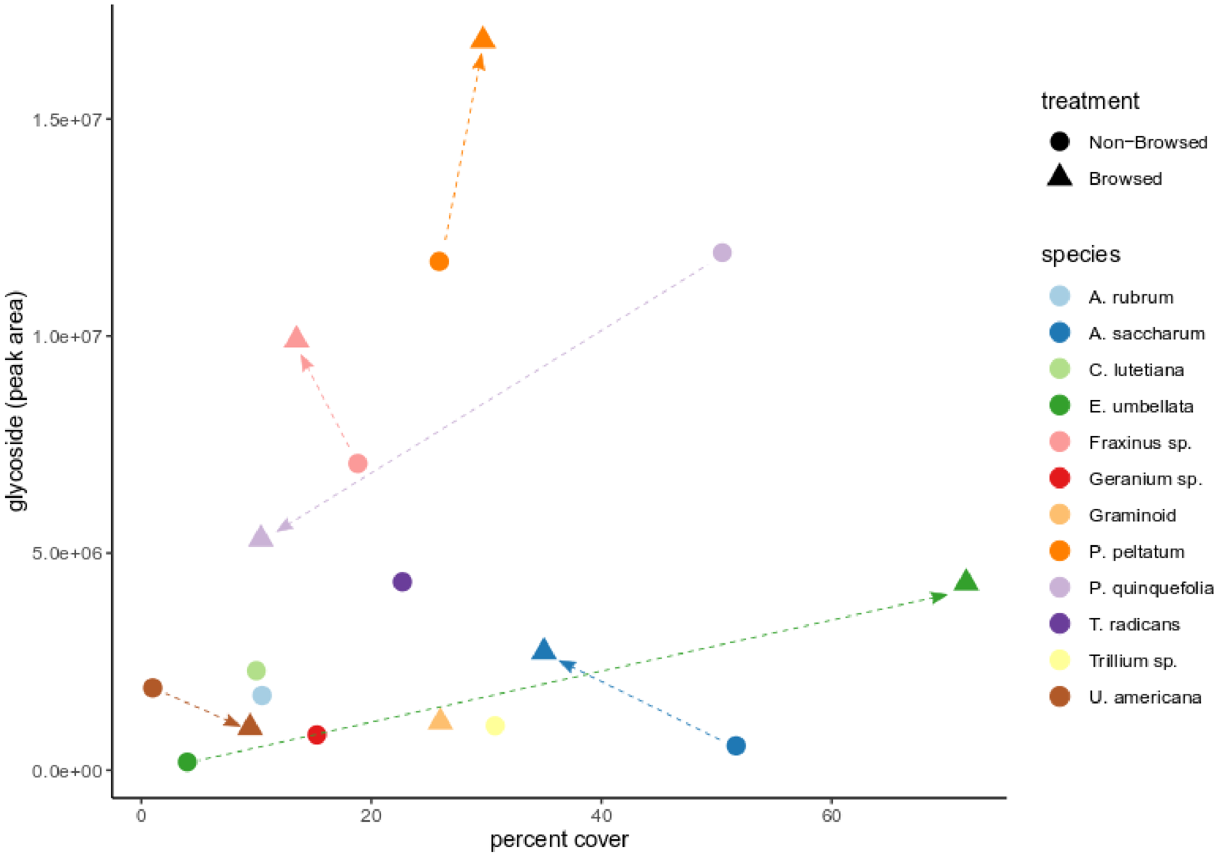

Figure 4.5 Relative glycoside amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative glycoside amount varies among species, and not all species respond similarly to deer browsing.

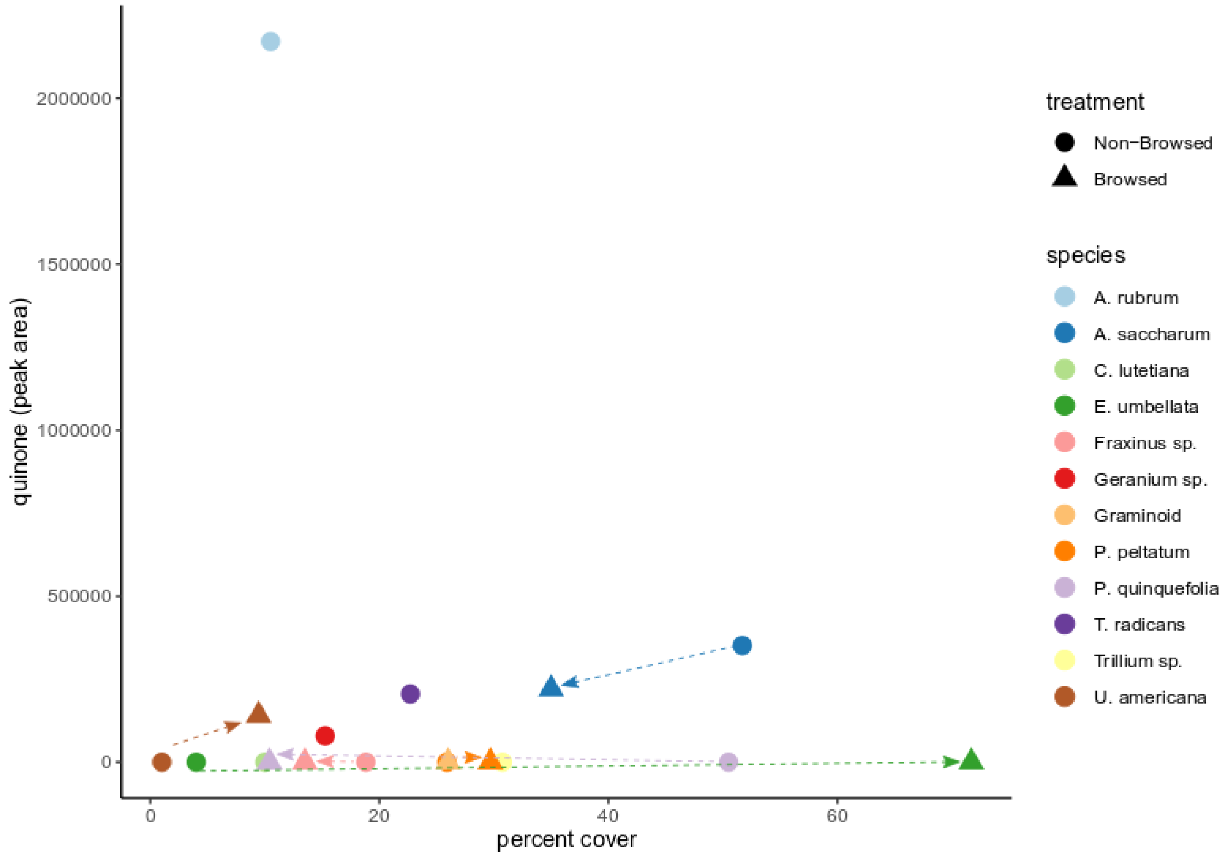

Figure 4.6 Relative quinone amount and percent cover per species. Twelve taxa were found overall, five taxa were only found in non-browsed plots, one taxon only in browsed plots, and six taxa in both non-browsed and browsed plots. Relative quinone amount varies among species, and not all species respond similarly to deer browsing.

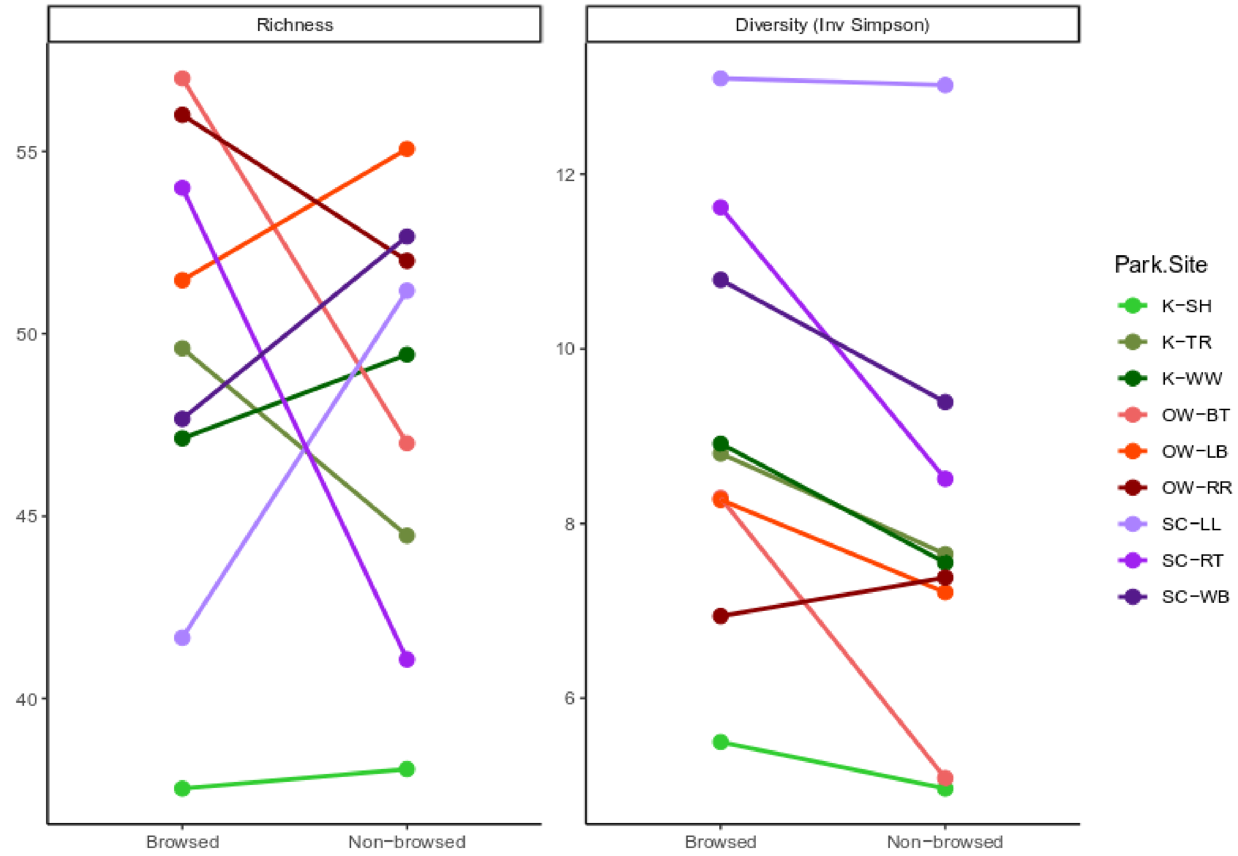

Figure 4.7 Chemical compound richness and diversity with and without deer browsing, across 9 study sites. Chemical richness varied at the site level ($p<0.0001$), and browsing effects varied ($p=0.04$); Chemical compound richness at LB (53) and RR (53), was higher than at SH (38). Diversity of chemical compounds varied between sites ($p<0.0001$), but browsing had no effect.

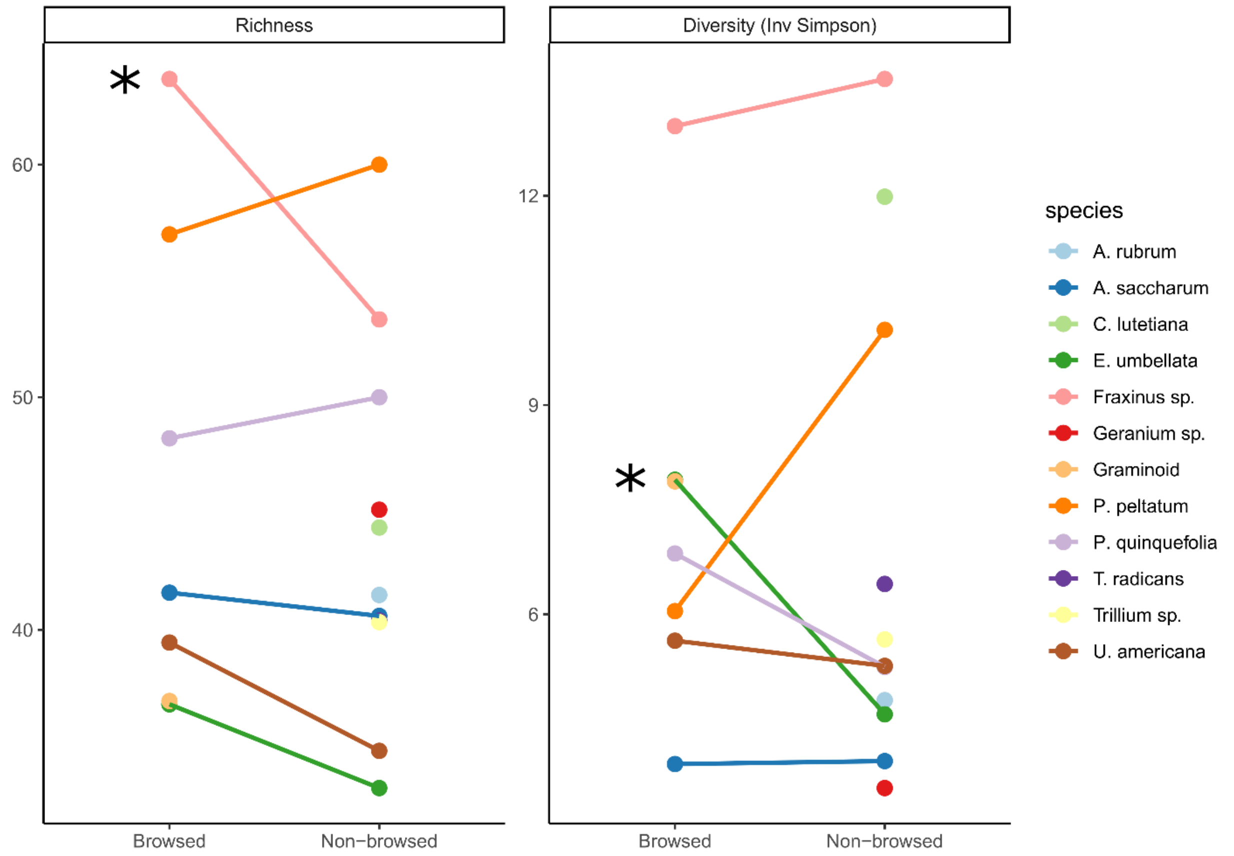

Figure 4.8 Chemical compound richness and diversity with and without deer browsing in 12 different plant species that were found inside and outside exclosures. Asterix (*) indicates significant browsing effect. Richness significantly increased with browsing in *Fraxinus sp.* (p=0.005). *P. peltatum* and *Fraxinus* had highest foliar chemical richness in their leaves (59 and 58 respectively), and were significantly different from *U. americana* (38), *graminoids* (37), and *E. umbellata* (35). diversity increased with browsing in *E. umbellata* (p=0.003). *Fraxinus* and *C. lutetiana* had higher diversity (13.4 and 12.0) than the other 10 species.

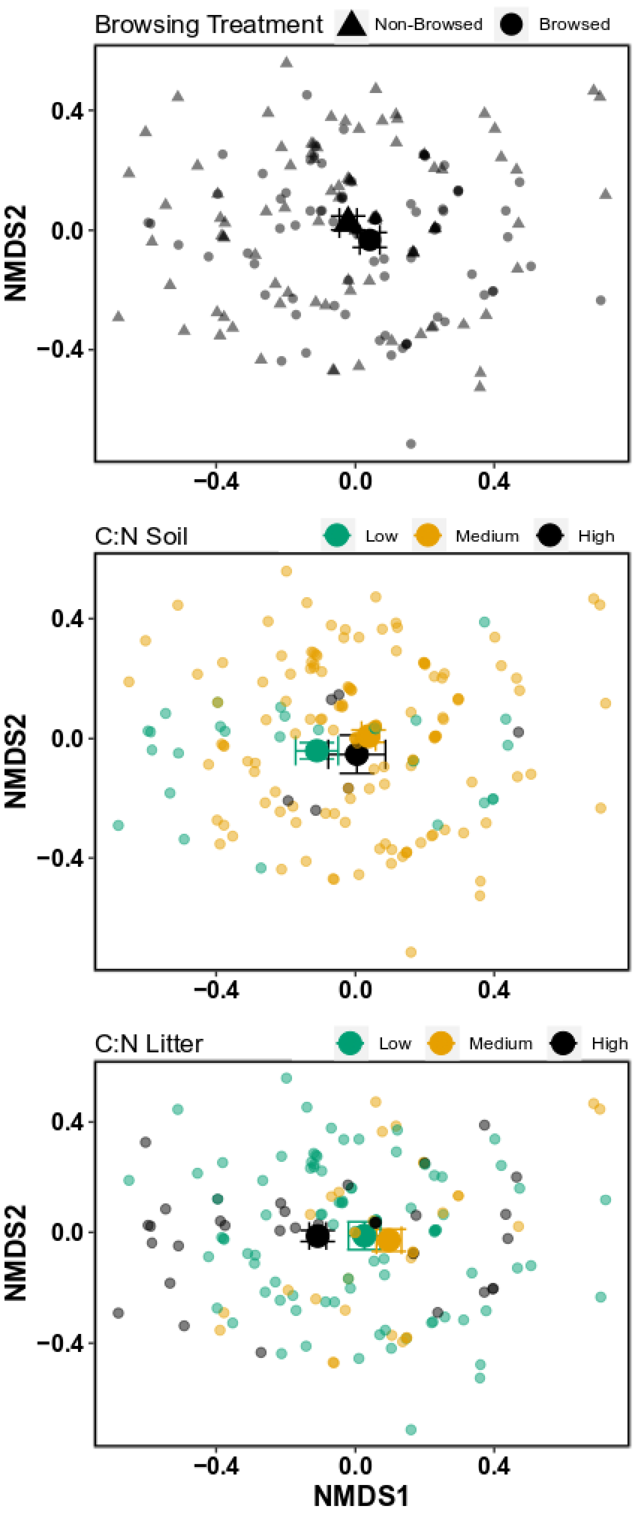

Figure 4.9 Chemical community composition NMDS averages with standard error overlaid on original NMDS points for deer browsing treatment, C:N soil and C:N litter. Deer browsing treatment is "Non-browsed" and "Browsed. Continuous values for C:N were grouped into three levels: low (green), medium (yellow) and high (black).

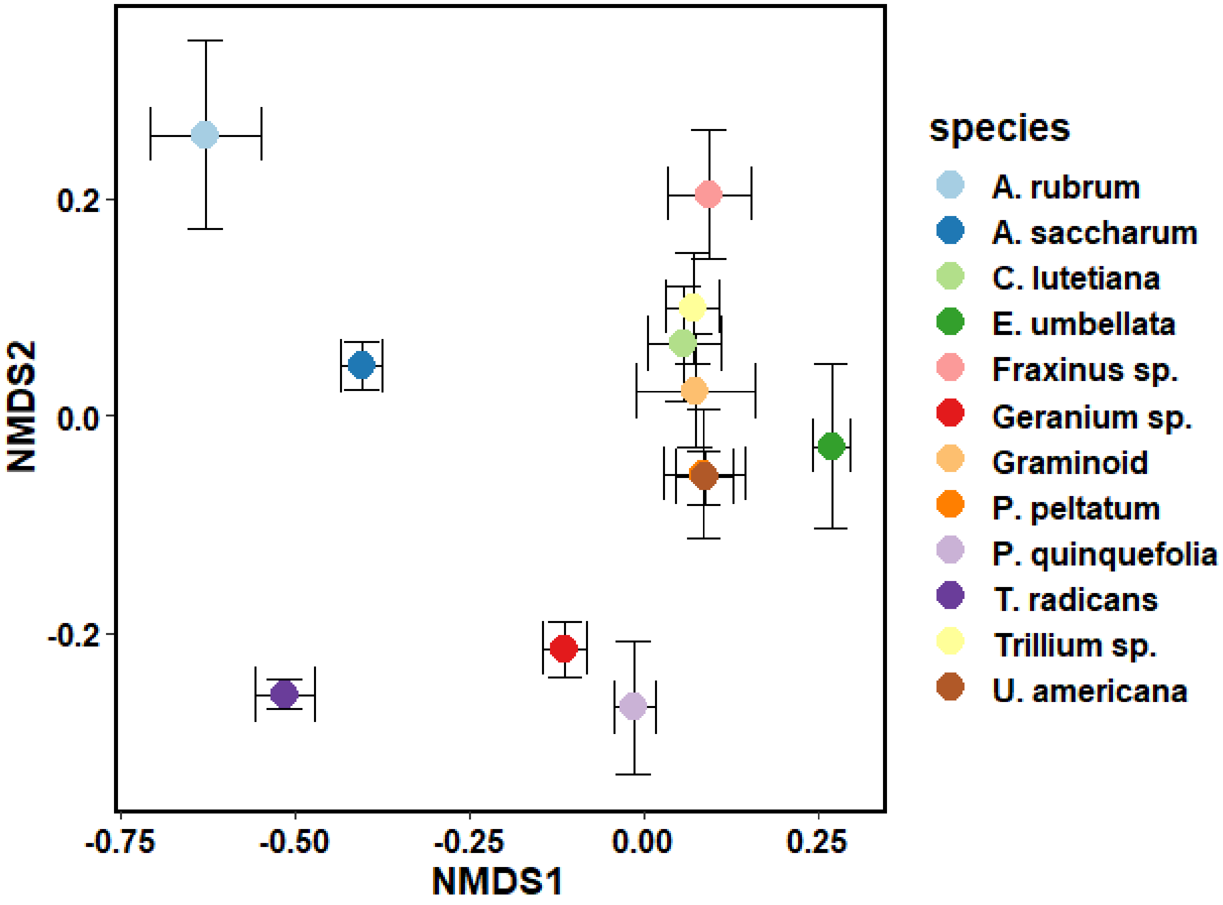

Figure 4.10 Average NMDS points for community composition of chemical compounds with standard error bars. PERMANOVA models show differences among twelve most common plant species (p=0.001).

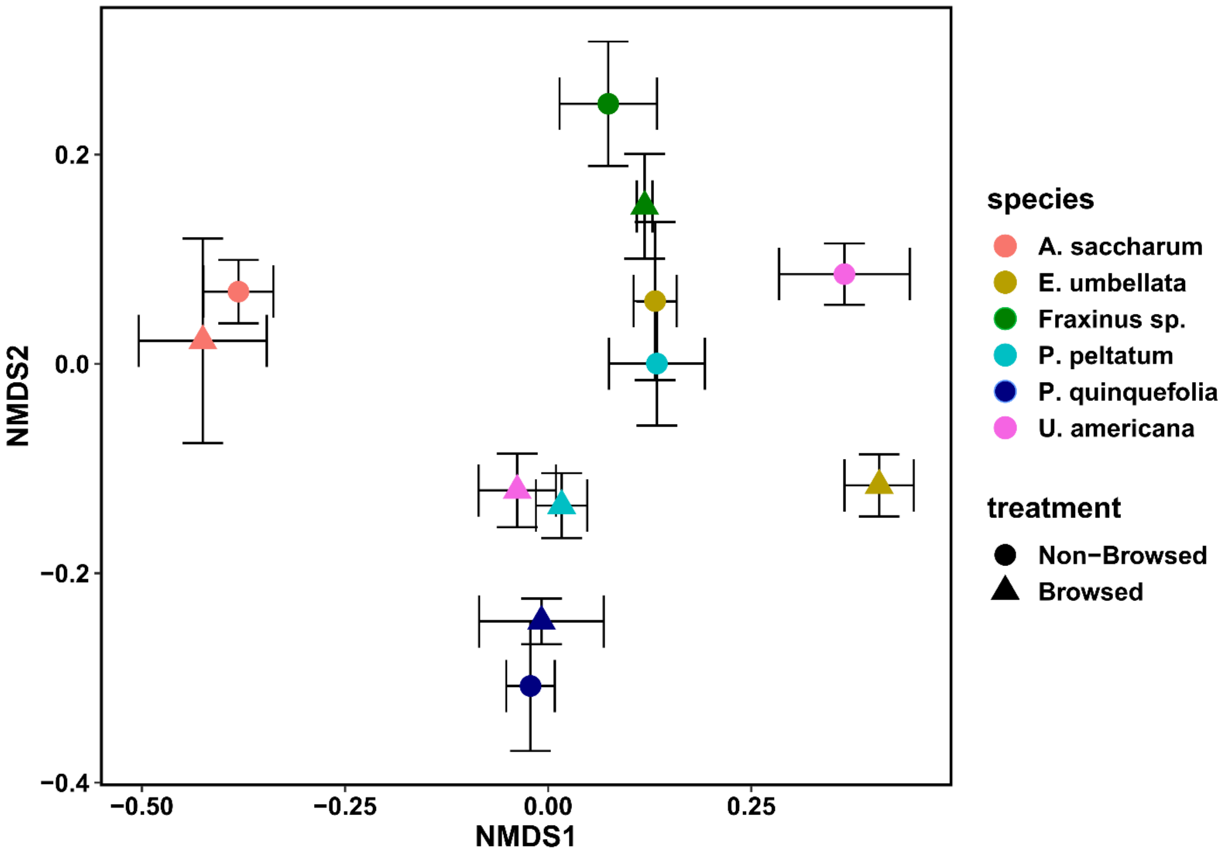

Figure 4.11 Average NMDS points for community composition of chemical compounds with standard error bars. Deer browsing effects on plant chemical communities differ among species (p=0.001).

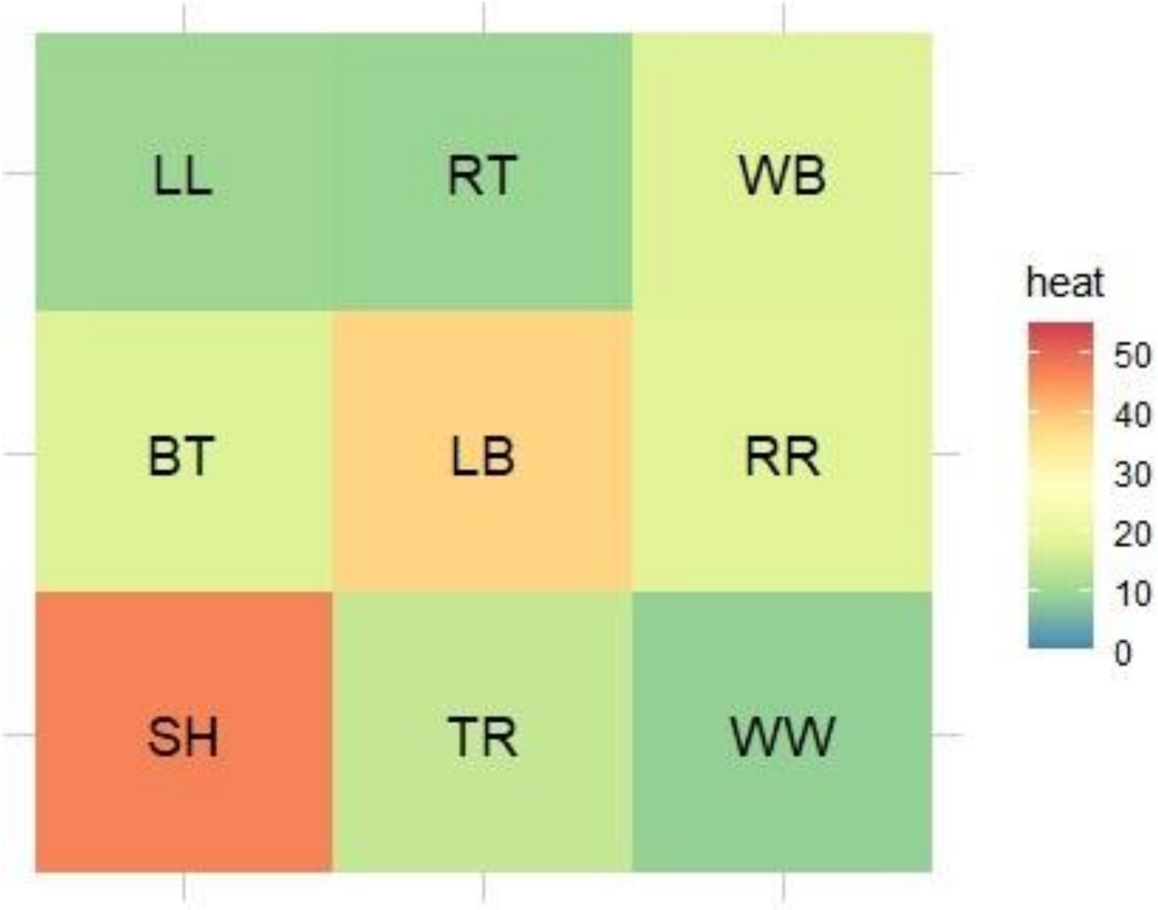

Figure 4.12 Total phenol load per site. Total phenol load represents a weighted sum of phenolic concentration per species based on percent cover at each site. A linear model did not detect significant browsing effects, but sites varied significantly (p=0.003). SH and LB were significantly higher in total phenolic levels than the other 6 sites.

Tables

Table 4.1 Linear mixed effects model summary for plant tissue chemical content and composition. (****) indicates significance <0.0001, (***) indicates significance <0.001, (**) indicates significance <0.05, (*) indicates significance <0.1. Phenol and Richness results have coefficient estimates for significant factors, indicate strength and direction of effect. Quinone has a “+” to indicate direction of effect. Linear mixed models were used to analyze %N, C:N, richness, diversity, Phenol, terpene, glycoside, and quinone contents were analyzed using linear models.

Mixed effects model (LMER)	**%N**	**C:N**	**Phenol**	**Terpene**	**Glycoside**	**Quinone**	**Chemical Richness**	**Chemical Diversity**
Deer				-**			-9.6*	
CN.litter				-*	+*			
CN.soil								
Litterfall								
Interactions								
Deer*CN.litter				+*				
Deer*CN.soil								
Deer*Litterfall			+9.5e-04*				+3.0***	
Total								
Random effects								
(1|site:park)						***	****	****
Linear model (LM)								
Deer		*					***	*
Species	****	****	****	****	****	****	****	****
Deer * species		***	****	*	****		**	****
Random effects								
(1|site:park)								

Table 4.2 Model summary for PERMANOVA analyses of chemical community composition. Significance level p<0.1 is indicated with *, p<0.01***. The model R2 is 0.1. explaining 10% of variance in chemical community composition. P-values below 0.1 are considered ecologically significant.

Model variables	Df	SumOfSqs	R^2	F	Pr(>F)
Deer	1	0.9	0.03	5.9	0.003 ***
C:N litter	1	0.3	0.01	2.2	0.086 *
C:N soil	1	0.8	0.03	5.3	0.003 ***
Litterfall	1	0.3	0.01	1.8	0.125
Deer * C:N litter	1	0.1	0.01	0.6	0.582
Deer * C:N soil	1	0.1	0.01	0.4	0.771
Deer * Litterfall	1	0.3	0.01	1.7	0.136
Residual	169	26.2	0.90		
Total	**176**	**29.0**	**1**		
Total-Residual			**0.10**		

Table 4.3 Plant species leaf %C and C:N, and the effect of deer on each species. Species marked with a "*" were significantly affected by browsing ($p<0.05$). Deer browsing decreased %C overall ($p=0.006$), but effects varied between species. %C increased in *U. americana* ($p=0.05$), and decreased in *E. umbellata* ($p=0.01$). C:N increased in *U. americana* ($p=0.001$) and *P. quinquefolia* ($p=0.03$), and decreased in *E. umbellata* ($p=0.03$).

Plant species	**%C**		**C:N**	
	Deer	**No Deer**	**Deer**	**No Deer**
Acer rubrum	*NA*	47.9	*NA*	30.1
Acer saccharum	47.2	47.1	25.0	26.4
Circaea lutetiana	*NA*	41.3	*NA*	16.7
***Elaeagnus umbellata**	**47.4**	**48.1**	**11.1**	**12.5**
Fraxinus sp.	45.9	46.0	20.5	19.9
Geranium sp.	*NA*	45.2	*NA*	16.1
Graminoid	44.6	*NA*	21.0	*NA*
Podophyllum peltatum	45.6	45.6	24.0	21.5
***Parthenocissus quinquefolia**	43.2	43.1	**24.2**	**19.7**
Toxicodendron radicans	*NA*	44.7	*NA*	20.0
Trillium	*NA*	43.0	*NA*	15.2
***Ulmus americana**	**45.8**	**44.7**	**27.0**	**20.8**

Table 4.4 Plant species leaf %N and the effect of deer on each species' percent cover. %N values marked with a "*" were retrieved from the TRY plant database (Kattge and Knoll 2019).

Plant species %cover affected by deer	**Deer effect**	**%N**
Acer saccharum	-	1.8
Acer rubrum	-	1.6
Celastrus orbiculatus	+	2.7*
Circaea lutetiana	-	2.5
Elaeagnus umbellata	+	3.9
Graminoid	+	1.4*
Prunus serotina	-	2.3*
Smilax tamnoides	-	*NA*
Toxicodendron radicans	-	2.3
Trillium	-	2.9

References

Alverson, W. S., D. M. Waller, and S. L. Solheim. 1988. Forests Too Deer : Edge Effects Forests Northern Wisconsin. Conservation Biology 2:348–358.

Austin, A. T., and C. L. Ballaré. 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. Pnas 107:2–6.

Bailey, J. K., J. A. Schweitzer, B. J. Rehill, D. J. Irschick, T. G. Whitham, and R. L. Lindroth. 2007. Rapid shifts in the chemical composition of aspen forests: An introduced herbivore as an agent of natural selection. Biological Invasions 9:715–722.

Baldwin, I. T., A. Kessler, and R. Halitschke. 2002. Volatile signaling in plant – plant – herbivore interactions : what is real ? Current opinion in biotechnology 5:1–4.

Baldwin, I. T., and J. C. Schultz. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. Science:277–279.

Bardgett, R. D., and D. a. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Bardgett, R. D., and D. A. Wardle. 2010. Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change. OUP Oxford.

Benedek, K., J. Bálint, I. Máthé, G. Mara, and T. Felföldi. 2019. Linking intraspecific variation in plant chemical defence with arthropod and soil bacterial community structure and N allocation. Plant Soil 444:383–397.

Bhonwong, A., M. J. Stout, J. Attajarusit, and P. Tantasawat. 2009. Defensive role of tomato polyphenol oxidases against cotton bollworm helicoverpa armigera and beet armyworm spodoptera exigua. Journal of Chemical Ecology 35:28–38.

Boeckler, G. A., J. Gershenzon, and S. B. Unsicker. 2011. Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. Phytochemistry 72:1497–1509.

Burghardt, K. T. 2016. Nutrient supply alters goldenrod’s induced response to herbivory. Functional Ecology:1769–1778.

Burghardt, K. T., M. A. Bradford, and O. J. Schmitz. 2018. Acceleration or deceleration of litter decomposition by herbivory depends on nutrient availability through intraspecific differences in induced plant resistance traits. Journal of Ecology 106:2380–2394.

Chapman, S. 2006. Herbivory differentially alters plant litter dynamics of evergreen and deciduous trees. Oikos 114:566–574.

Chollet, S., M. Maillard, S. Grayston, and J. Martin. 2019. Deer slow down litter decomposition by reducing litter quality in a temperate forest. Peer Community in Ecology:1–32.

Classen, A. T., S. K. Chapman, G. Whitham, S. C. Hart, and G. W. Koch. 2007. Genetic-based plant resistance and susceptibility traits to herbivory influence needle and root litter nutrient dynamics. Journal of Ecology:1181–1194.

Coq, S., J. Nahmani, R. Resmond, J. Segrestin, J. François, D. Patrick, and S. Elena. 2018. Intraspecific variation in litter palatability to macroarthropods in response to grazing and soil fertility. Functional Ecology 32:2615–2624.

Cornelissen, J. H. C., N. Pérez-harguindeguy, S. Díaz, J. Philip, B. Marzano, M. Cabido, F. Vendramini, B. Cerabolini, S. Diaz, J. P. Grime, and M. Cabid. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phylotogist 143:191–200.

Cornelissen, J. H. C., H. M. Quested, D. Gwynn-Jones, R. S. P. Van Logtestijn, M. a H. De Beus, a. Kondratchuk, T. V. Callaghan, and R. Aerts. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. Functional Ecology 18:779–786.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. Van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Côté, S. D., T. P. Rooney, J.-P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological Impacts of Deer Overabundance. Annual Review of Ecology, Evolution, and Systematics 35:113–147.

Danell, K., R. Gref, and R. Yazdani. 1990. Effects of mono- and diterpenes in scots pine needles on moose browsing. Scandinavian Journal of Forest Research 5:535–539.

DNR. 2010. Michigan deer management plan. http://www.michigan.gov/documents/dnre/WLD_Deer_Management_Plan_FINAL_5.8_320639_7.pdf?20150917085823.

Erbilgin, N., P. Krokene, E. Christiansen, G. Zeneli, and J. Gershenzon. 2006. Exogenous application of methyl jasmonate elicits defenses in Norway spruce (Picea abies) and reduces host colonization by the bark beetle Ips typographus. Oecologia 148:426–436.

Findlay, S., M. Carreiro, V. Krischik, and C. G. Jones. 1996. Effects of Damage to Living Plants on Leaf Litter Quality. Ecological Applications 6:269–275.

Frerker, K., A. Sabo, and D. Waller. 2014. Long-Term Regional Shifts in Plant Community

Composition Are Largely Explained by Local Deer Impact Experiments. PloS one 9:1–17.

Frost, C. J., and M. D. Hunter. 2008. Insect herbivores and their frass affect Quercus rubra leaf quality and initial stages of subsequent litter decomposition. Oikos 117:13–22.

Gershenzon, J., and N. Dudareva. 2007. The function of terpene natural products in the natural world. Nature Chemical Biology 3:408–414.

Grime, J. P., J. H. H. C. Cornelissen, K. Thompson, and J. G. Hodgson. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. Oikos 77:489–494.

Hamilton, E. W., and D. A. Frank. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82:2397–2402.

Harrison, K. A., and R. D. Bardgett. 2003. How browsing by red deer impacts on litter decomposition in a native regenerating woodland in the Highlands of Scotland. Biology and Fertility of Soils 38:393–399.

Harrison, K. A., and R. D. Bardgett. 2004. Browsing by red deer negatively impacts on soil nitrogen availability in regenerating native forest. Soil Biology and Biochemistry 36:115–126.

Holeski, L. M., S. C. Mckenzie, E. L. Kruger, J. J. Couture, K. Rubert-Nason, and R. L. Lindroth. 2016. Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (Populus tremuloides) to browsing by a keystone forest ungulate. Journal of Ecology 104:850–863.

Hunter. 2016. The Phytochemical Landscape. Linking Trophic Interactions and Nutrient Dynamics. Princeton University Press.

Hunter, M. D., and J. C. Schultz. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. Oecologia 94:195–203.

Kanerva, S., and A. Smolander. 2008. Original article How do coniferous needle tannins influence C and N transformations in birch humus layer? European Journal of Soil Biology 44:1–9.

Karban, R., and I. T. Baldwin. 2007. Induced Responses to Herbivory. University of Chicago Press.

Kattge, J., and H. Knoll. 2019. TRY plant trait database – enhanced coverage and open access. Global Change Biology 26:119–188.

Kessler, A., and T. Baldwin. 2002. Plant Responses to Insect Herbivory: The Emerging Molecular Analysis. Annual review of plant biology 53:299–328.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89:371–9.

Legendre, P., and E. D. Gallagher. 2001. Ecologically Meaningful Transformations for Ordination of Species Data. Oecologia 129:271–280.

Lindroth, R. L., and S. B. St Clair. 2013. Adaptations of quaking aspen (Populus tremuloides Michx .) for defense against herbivores. Forest Ecology and Management 299:14–21.

Mattson, D. A. H. and W. J. 1992. The Dilemma of Plants : To Grow or Defend Author (s): Daniel A . Herms and William J . Mattson Source : The Quarterly Review of Biology , Vol . 67 , No . 3 (Sep ., 1992), pp . 283-335 Published by : The University of Chicago Press Stable URL : http. The Quarterly Review of Biology 67:283–335.

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

Mithofer, A., and W. Boland. 2012. Plant Defense Against Herbivores : Chemical Aspects. Annual review of plant biology 63:431–450.

Moore, B. D., R. L. Andrew, C. Külheim, and W. J. Foley. 2014. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. New Phytologist 201:733–750.

Olff, H., and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. Trends in Ecology and Evolution 13:261–265.

Olofsson, J., and L. Oksanen. 2002. Role of Litter Decomposition for the Increased Primary Production in Areas Heavily Grazed by Reindeer : A Litterbag Experiment. Oikos 96:507–515.

Pastor, J., and Y. Cohen. 1997. Herbivores, the functional diversity of plants species, and the cycling of nutrients in ecosystems. Theoretical Population Biology 51:165–179.

Pastor, J., B. Dewey, R. J. Naiman, P. F. Mcinnes, Y. Cohen, and N. Mar. 1993. Moose Browsing and Soil Fertility in the Boreal Forests of Isle Royale National. Ecology 74:467–480.

Pastor, J., and R. J. Naiman. 1992. Selective foraging and ecosystems processes in boreal forests. American Naturalist 139:690–705.

Popma, J. M.A. and Nadelhoffer K J. 2020 Deer browsing effects on temperate forest soil nitrogen cycling shift from positive to negative across fertility gradients. Canadian Journal of Forest Research. 50(12): 1281-1288

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rhodes, A. C., V. Anderson, and S. B. St Clair. 2017. Ungulate herbivory alters leaf functional traits and recruitment of regenerating aspen. Tree Physiology 37:402–413.

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

Rooney, T. P., and D. M. Waller. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. Forest Ecology and Management 181:165–176.

Russell, F., D. Zippin, and N. Fowler. 2001. A Review of Effects of White-tailed Deer (Odocoileus virginianus) on Plants, Plant Populations and Communities. The American Midland Naturalist 146:1–26.

Schuldt, A., T. Assmann, H. Bruelheide, W. Durka, D. Eichenberg, and H. Werner. 2014. Functional and phylogenetic diversity of woody plants drive herbivory in a highly diverse forest. New Phylotogist 202:864–873.

Schultz, J. C., H. M. Appel, A. P. Ferrieri, and T. M. Arnold. 2013. Flexible resource allocation during plant defense responses. Frontiers in Plant Science 4:1–11.

Schweitzer, J. A., J. K. Bailey, R. K. Bangert, S. C. Hart, and T. G. Whitham. 2007. The Role of Plant Genetics in Determining Above- and Below-ground Microbial Communities. Pages 107–120 *in* M. J. Bailey, A. K. Lilley, T. M. Timms-Wilson, and P. T. N. Spencer-Phillips, editors. Microbial Ecology of Aerial Plant Surfaces. CAB International.

Sunnerheim-sjoberg, K. 1992. Multivariate Study of Moose Browsing in Relation to Phenol Pattern in Pine Needles. Journal of Chemical Ecology 18.

Taylor, B. R., D. Parkinson, W. F. J. Parsons, and N. Feb. 1989. Nitrogen and Lignin Content as Predictors of Litter Decay Rates : A Microcosm Test. Ecology 70:97–104.

Waller, D. M., and W. S. Alverson. 1997. Ecological Aspects of Management: The White-tailed deer. Wildlife Society Bulletin 25:217–226.

Waller, D. M., S. Johnson, R. Collins, and E. Williams. 2009. Threats posed by ungulate herbivory to forest structure and plant diversity in the Upper Great Lakes Region-with a review of methods to assess those threats. Page Natural Resource Report NPS/GLKN/NRR–2009/102. Fort Collins, Colorado.

Warbrick, M., A. Kroeker, and P. Nosko. 2020. Antiherbivore chemicals in emerging and previous-year foliage of balsam fir saplings responding to prior simulated browsing. Plant Ecology 221:487–500.

Wardle, D. A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press.

Wooley, S. C., S. Walker, J. Vernon, and R. L. Lindroth. 2008. Aspen Decline, Aspen Chemistry, and Elk Herbivory: Are They Linked? Rangelands 30:17–21.

Chapter 5 Conclusion

In this dissertation, I evaluated the effects of deer browsing on temperate forests across environmental gradients. Although plant community responses to herbivores are well understood, direction and magnitude of effects on ecosystem processes and properties remain difficult to predict (Milchunas and Lauenroth 1993, Bardgett and Wardle 2003, Buchkowski et al. 2019). My goal was to elucidate some of the inconsistencies found in how herbivores affect ecosystem processes. To do so I used experimental deer exclosures in Southeastern Michigan, spanning co-occurring gradients of productivity and fertility. I first investigated effects of deer browsing on soil carbon (C) and nitrogen (N) cycling (Chapter 2, (Popma and Nadelhoffer 2020)). The strength and direction of browsing effects on N and C cycling shifted from positive to negative across a fertility gradient. Deer browsing increased net N mineralization rates in high nutrient environments and decreased N mineralization rates in low nutrient environments, whereas browsing decreased soil respiration rates in high nutrient environments and increased respiration rates in low nutrient environments. The contrasting patterns of deer browsing effects on respiration and net N mineralization rates might be explained by microbial community responses to changes in N inputs. At high nutrient sites, plants might tolerate browsing by increasing productivity. This increased productivity could lead to more N-rich plant detritus entering the soil decomposition cycle, which could reduce microbial C respiration (Frey et al. 2014). The importance of environmental gradients in mediating browsing effects on soil C and N cycling I found fits with existing acceleration and deceleration frameworks in which resource availability determines the direction of indirect herbivore effects on ecosystem processes (Bardgett and Wardle 2003, Côté et al. 2004, Schmitz et al. 2015).

I then explored potential underlying mechanisms of deer browsing effects on ecosystem nutrient cycling. To do so, I investigated variations in the effects of browsing on plant communities (Chapter 3) and plant chemistry (Chapter 4) across an environmental gradient. Deer effects on plant communities were significant but did not vary with environmental factors. Browsing significantly decreased several plant structural measures, including plant percent cover, tree seedling cover, and sapling density, but had no detectable effects on plant community composition overall. Other important browsing effects on plant community composition, however, were reflected in significant changes in percent cover of nearly 25% of plant species and 3 plant functional types with deer browsing.

Effects of both deer browsing and species identity on leaf chemistry responses were significant, but species identity was significant across all measured responses while browsing was not. Therefore, although both hypothesized mechanisms appear to operate simultaneously, shifts in plant community composition due to selective browsing are likely the dominant driver of changes in the phytochemical landscape in temperate forests, more so than browsing-induced chemical changes within species. Although others have shown that plant defense responses change depending on nutrient supply, shifting from tolerance to induced resistance, I did not detect interacting effects of browsing and C:N ratios in litter and soil on foliar chemical composition.

Differential effects of browsing on plant community composition could explain the variation in nutrient cycling responses to browsing. This dissertation addresses the inconsistencies of herbivore effects on ecosystem processes, adding insights from temperate forest ecosystems to the existing frameworks on trophic interactions.

Further questions and considerations

Based on recent studies, context-dependent effects on nutrient cycling could also be explained by abiotic factors such as soil moisture, soil texture, and temperature (Dodge et al. 2020, Sitters et al. 2020). Grassland soil C and N pools increased with herbivory at warmer temperatures, and diminished at colder temperatures (Sitters et al. 2020). Soil properties themselves can also be altered by ungulate browsing, and mediate browsing effects on nutrient cycling (Dodge et al. 2020).

However, browsing effects on plant litter quality that alter nitrogen dynamics in litter do not always influence plant growth (Buchkowski et al. 2019). To determine how browsing effects on plant litter dynamics will affect plant growth, it is important to consider the balance between mineralization and immobilization during microbial decomposition (Cherif and Loreau 2013), and the carbon/nutrient limitation of decomposers (Zou et al. 2016). Browsing effects on litter N dynamics can be buffered by existing soil organic N pools (Zou et al. 2016). For there to be strong feedbacks between browsers, nutrient cycling, and plant growth, mediated via litter decomposition, browsing pressure needs to be consistent from year-to-year. Furthermore, browsing can have significant effects on microbial communities with potential consequences for soil C and N cycling and storage (Cline et al. 2017, Burke et al. 2019). Interestingly, research on enzyme activity has shown that soil microbial communities can recover within short periods after deer exclusion (Woods et al. 2019).

From a conservation and management perspective it is important to explore effects of varying deer densities. Complete removal of deer is not realistic in most cases, and more research with different deer densities is necessary to determine whether recovery is possible at reduced deer densities. To determine whether and to what extent reducing deer densities can facilitate soil recovery will need further investigation. The timescales at which deer browsing affects soil processes can vary and therefore recovery times might vary as well. Direct effects of waste products and trampling likely occur on shorter timescales than indirect effects of browsing induced vegetation shifts.

Understanding factors that contribute to changes in forest C and N cycling continues to be critically important, especially considering predicted scenarios of climate change. Overall, my dissertation work provides support and insights into how interactions between above- and belowground processes are important drivers of ecosystem functioning. Notably, my work highlights the importance environmental factors in causing variability in feedbacks between above- and belowground processes.

References

Bardgett, R. D., and D. a. Wardle. 2003. Herbivore-mediated linkages between aboveground and belowground communities. Ecology 84:2258–2268.

Buchkowski, R. W., O. J. Schmitz, and M. A. Bradford. 2019. Nitrogen recycling in coupled green and brown food webs: Weak effects of herbivory and detritivory when nitrogen passes through soil. Journal of Ecology 107:963–976.

Burke, D. J., S. R. Carrino-Kyker, A. Hoke, S. Cassidy, L. Bialic-Murphy, and S. Kalisz. 2019. Deer and invasive plant removal alters mycorrhizal fungal communities and soil chemistry: Evidence from a long-term field experiment. Soil Biology and Biochemistry 128:13–21.

Cherif, M., and M. Loreau. 2013. Plant-herbivore-decomposer stoichiometric mismatches and nutrient cycling in ecosystems. Proceedings of the Royal Society B 280:20122453.

Cline, L. C., D. R. Zak, R. A. Upchurch, Z. Freedman, and A. R. Peschel. 2017. Soil microbial communities and elk foraging intensity: implications for soil biogeochemical cycling in the sagebrush steppe. Ecology Letters:202–211.

Côté, S. D., T. P. Rooney, J.-P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological Impacts of Deer Overabundance. Annual Review of Ecology, Evolution, and Systematics 35:113–147.

Dodge, V. J., V. T. Eviner, and J. H. Cushman. 2020. Context-dependent effects of a reintroduced ungulate on soil properties are driven by soil texture , moisture , and herbivore activity. Ecology and Evolution:10858–10871.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., and Wickings, K. 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry **121**(2): 305–316. doi:10.1007/s10533-014-0004-0.

Milchunas, D., and W. Lauenroth. 1993. Quantitative Effects of Grazing on Vegetation and Soils Over a Global Range of Environments. Ecological Monographs 63:327–366.

Popma, J. M.A. and Nadelhoffer K J. 2020 Deer browsing effects on temperate forest soil nitrogen cycling shift from positive to negative across fertility gradients. Canadian Journal of Forest Research. 50(12): 1281-1288

Schmitz, O.J., Buchkowski, R.W., Burghardt, K.T., and Donihue, C.M. 2015. Functional Traits and Trait-Mediated Interactions: Connecting Community-Level Interactions with Ecosystem Functioning. *In* Advances in Ecological Research, 1st edition. Elsevier Ltd. doi:10.1016/bs.aecr.2015.01.003.

Sitters, J., E. R. J. Wubs, E. S. Bakker, T. W. Crowther, P. B. Adler, S. Bagchi, J. D. Bakker, L. Biederman, E. T. Borer, E. E. Cleland, N. Eisenhauer, J. Firn, J. M. H. Knops, A. S. Macdougall, R. L. Mcculley, J. L. Moore, B. Mortensen, P. L. Peri, S. M. Prober, C. Riggs, A. C. Risch, M. Schütz, and E. W. Seabloom. 2020. Nutrient availability controls the impact of mammalian herbivores on soil carbon and nitrogen pools in grasslands. Global Change Biology 26:2060–2071.

Woods, M. J., E. Roberson, D. Cipollini, and M. A. Rúa. 2019. White-tailed deer and an invasive shrub facilitate faster carbon cycling in a forest ecosystem. Forest Ecology and Management 448:104–111.

Zou, K., E. Thebault, G. Lacroix, and S. Barot. 2016. Interactions between the green and brown food web determine ecosystem functioning. Functional Ecology 30:1454–1465.