

Nutrients influence diet selection in herbivores

By

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Natural selection should favor selective foraging in deer to choose among nutrients in plant communities to meet their physiological requirements. I designed experiments where I measured plant nutrients and white-tailed deer (*Odocoileus virginianus*) use on 1) 15 species of plants, and 2) 2 species of plants of manipulated nutrient concentrations. I tested the hypotheses: 1) deer use across plants would be influenced by plant nutrients, and 2) soil nutrients would influence plant selection by deer. My analyses indicated deer selected plants of low neutral detergent fiber and sulfur concentrations. However, deer selected for crude protein. I also confirmed soil nutrients indirectly influenced diet selection. Nutrient augmentation altered phosphorus concentrations within plants, and plant phosphorus concentration explained 47% of the variation in diet selection. My data indicated deer use both nutrient maximization and avoidance to balance diet selection and plants mediate the indirect effects of soil nutrients on herbivore diet selection.

## DEDICATION

I dedicate this thesis to my mother whose unwavering love and support have allowed me to accomplish my dreams. I will always be thankful for your sacrifices and patience throughout this journey. Words cannot describe how eternally grateful I am that you never lost faith in me and raised me to be the man I am today. I also want to thank my family, friends, and the many other people who have supported me on this journey. I very much appreciate you all.

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## TABLE OF CONTENTS

DEDICATION .....	ii
ACKNOWLEDGEMENTS .....	iii
LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
CHAPTER	
I. INTRODUCTION .....	1
LITERATURE CITED.....	3
II. INTEGRATING THE NUTRIENT MAXIMIZATION AND AVOIDANCE HYPOTHESES: HERBIVORE DIET SELECTION IS EXPLAINED BY ATTRACTION AND AVOIDANCE.....	4
INTRODUCTION .....	4
MATERIALS AND METHODS .....	8
Study Area .....	8
Experimental Design .....	8
Plant Sampling.....	9
Animal Sampling.....	10
Data Analysis.....	10
RESULTS.....	11
DISCUSSION.....	12
LITERATURE CITED.....	18
III. SOIL NUTRIENTS INDIRECTLY INFLUCENCE PLANT SELECTION IN WHITE-TAILED DEER.....	23
INTRODUCTION .....	23
MATERIALS AND METHODS .....	25
Study Area .....	25
Experimental Design .....	25
Plant Sampling.....	26
Animal Sampling.....	27
Data Analysis.....	27

RESULTS.....	27
DISCUSSION.....	28
LITERATURE CITED.....	34
IV. SYNTHESIS AND CONCLUSIONS.....	38

## LIST OF TABLES

2.1	Parameter estimates for standard least squares model predicting white-tailed deer ( <i>Odocoileus virginianus</i> ) plant selection based on nutritional qualities in Oktibbeha County, MS, February through June 2017. ....	15
3.1	Parameter estimates for generalized linear model predicting white-tailed deer ( <i>Odocoileus virginianus</i> ) plant selection based on soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of CaCO <sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer of a palatable plant (balansa clover, <i>Trifolium michelianum</i> ) and an unpalatable plant (winter wheat, <i>Triticum aestivum</i> ) in Oktibbeha County, MS, February through June 2017. ....	31

## LIST OF FIGURES

2.1	Visual representation of the constraints and assumptions of the nutrient maximization hypothesis (a) stating herbivore diet selection is driven by maximizing limiting nutrients and assuming that toxicity is not an important constraint given the possible intake of an animal, the nutrient avoidance hypothesis (b) contrastingly stating herbivore diet selection is driven by avoidance and assuming that limited nutrients are only limited because the toxicity constraint limits intake, and the nutrient balance hypothesis (c) stating that in nature, avoiding nutrient toxicity and acquiring limiting nutrients are necessary if both simultaneously exist as constraints.....	16
2.2	Experimental design quantifying white-tailed deer ( <i>Odocoileus virginianus</i> ) selection across plants of varying nutritional qualities. Fifteen plants were randomly assigned and planted in same size adjacent plots protected by electric fence and monitored with camera traps (i.e., Block). .....	17
3.1	Conceptual diagram of hypothesis: soil productivity indirectly affects white-tailed deer ( <i>Odocoileus virginianus</i> , hereafter deer) plant selection, and three associated predictions: A) soil productivity would influence deer plant selection but only when a palatable plant was present; box and whisker plot depicting effects of soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of CaCO <sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer on deer selection of a palatable plant (balansa clover, <i>Trifolium michelianum</i> ), B) nutrient addition would increase plant nutrient concentrations; box and whisker plot depicting effects of soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of CaCO <sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer on phosphorus concentrations of a palatable plant (balansa clover, <i>Trifolium michelianum</i> ), and C) changes in plant nutrients would explain deer plant selection; box and whisker plot depicting effects of increased plant phosphorus concentration on deer use of a palatable plant (balansa clover, <i>Trifolium michelianum</i> ) in Oktibbeha County, MS, February through June 2017. ....	32

3.2 Experimental design quantifying white-tailed deer (*Odocoileus virginianus*) plant selection across cool-season legume and cool-season cereal grain receiving treatments to manipulate nutritional quality. Balansa clover (*Trifolium michelianum*) and winter wheat (*Triticum aestivum*) were randomly assigned and planted separately in 4 equal-sized adjacent plots (i.e., block). Each plot received 1 amendment treatment: no nutrient addition (i.e., control), pH neutralization with addition of CaCO<sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer according to soil tests to manipulate intraspecific plant qualities. Each plot was protected by electric fence and monitored with camera traps in Oktibbeha County, MS, February through June 2017.....33

## CHAPTER I

### INTRODUCTION

The influence of nutrients on herbivore diet selection has been studied with the assumption that animals seek to maximize nutrient intake (i.e., nutrient maximization hypothesis; Berteaux et al. 1998, Ceacero et al. 2009, Lashley et al. 2015). More recently, the nutrient avoidance hypothesis has been postured as a mechanism of herbivore diet selection (Ceacero et al. 2015). Thus, herbivores may select a diet by avoiding certain nutrients to prevent toxicity. In reality, nutrient toxicity and limitation constraints likely co-occur frequently in nature indicating a new hypothesis to explain diet selection. Specifically, integrating the nutrient maximization and nutrient avoidance hypotheses is likely needed.

In addition to understanding how nutrients influence diet selection, previous literature evaluating whether animals can alter intraspecific plant selection is scant. Several studies have linked soil productivity to changes in plant communities and that diet, health, morphology, and fitness of many cervid species vary with soil productivity (Gill 1956; Gaillard et al. 1996; Strickland and Demarais 2000; Herfindal et al. 2006; Jones et al. 2010; Lehoczki et al. 2011). Those difference in diet selection may occur in part because of how soil productivity influences intraspecific plant quality, but variables affecting diet selection are confounded in nature. I designed 2 experiments to test a key prediction of my new hypothesis (i.e., nutrient balance hypothesis) that integrates the

nutrient maximization and nutrient avoidance hypotheses, and to determine if herbivores are capable of making selections based on nutrients even within a plant species. To test the nutrient balance hypothesis, I designed a cafeteria style experiment with fifteen cool-season plant and monitored 14 nutritional qualities of the plants and herbivore selection. That design allowed me to test the prediction that herbivore selection would be partially explained by attraction to some nutrients and avoidance of others. To determine if animals could make selections based on fluctuating nutritional quality within a plant species, I designed a nutrient addition experiment where I manipulated soil productivity to cause intraspecific variation in plant quality and determine if changes in plant quality affected plant selection. These experiments collectively will improve our understanding of mechanisms affecting diet selection and how those mechanisms can be utilized to inform wildlife management.

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CHAPTER II  
INTEGRATING THE NUTRIENT MAXIMIZATION AND AVOIDANCE  
HYPOTHESES: HERBIVORE DIET SELECTION IS EXPLAINED BY  
ATTRACTION AND AVOIDANCE

**INTRODUCTION**

Optimal foraging theory was postured to explain animal foraging behavior with a key prediction that animals strive to maximize a tradeoff between energy intake and expenditure (MacArthur and Pianka 1966). Support for this theory has demonstrated that balancing time with energy intake is essential to animal fitness (Gillette et al. 2000; Sinervo 2013). Large herbivores alter bite size, cropping rates, and patch choice to optimize intake rate of digestible plants during grazing events (Distel et al. 1995; Kenny and Black 1984). Large herbivores may also prioritize diet quality over quantity to minimize digestive constraints when available daily grazing time is restricted (Ginane and Petit 2005). When forage quality is held constant, handling time becomes the key factor in selection as demonstrated by clam size selectivity of common crow (*Corvus caurinus*; Richardson and Verbeek 1987) and mussel size selectivity by Oystercatchers (*Haematopus ostralegus*; Meire and Ervynck 1986). When handling time is held constant but forage quality varies, selection for limiting nutrients becomes the key factor, as demonstrated with white-tailed deer (*Odocoileus virginianus*) selection of energy over protein in mixed rations with similar handling times (Berteaux et al. 1998).

Nutrient availability fluctuates frequently in natural environments for many reasons (e.g., seasonal changes, plant senescence, and rainfall; Bormann and Likens 1967; Himmelblau and Amasino 2001; Lashley and Harper 2012). As such, natural selection should favor highly selective and flexible foraging behaviors in herbivores to navigate the heterogeneous matrix of nutrients in plant communities and allow animals to seek out and exploit limiting nutrients (Hättenschwiler et al. 2008). Evidence from a variety of study systems have provided support for this selectivity and flexibility in diet selection. For example, Ceacero et al. (2009) observed captive Iberian red deer (*Cervus elaphus hispanicus*) selected forages high in Na and Co when made deficient in feeding trials. Similarly, wild populations of white-tailed deer have the ability to select plants high in phosphorus (Lashley et al. 2015), protein (Dostaler et al. 2011), or energy (Berteaux et al. 1998) when limiting, and retract or expand the breadth of selected plants to accommodate fluctuations in resource availability such as caused by drought (Lashley and Harper 2012). Likewise, when many nutrients are limiting, diet selection should maximize an array of limiting nutrients. For example, McNaughton (1990) reported seasonal movements of African migratory grazers were linked to a wide range of forage nutrients (i.e., Ca, Cu, N, Na, Zn, Mg, P, and Ca/P balance). Contrastingly, when no nutrients are limited, forage qualities may poorly explain diet selection. For example, Vangilder et al. (1982) analyzed white-tailed deer rumen samples and were unable to detect any nutritional quality that explained diet selection.

Although the majority of studies test predictions related to maximizing intake of limited nutrients (i.e., nutrient maximization hypothesis), recent experiments provide evidence that avoidance of toxicity is a more important consideration in diet selection

(i.e., nutrient avoidance hypothesis; Ceacero et al. 2015). For example, Ceacero et al. (2015) determined that Iberian red deer avoided toxic levels of sulfur even when other essential nutrients are limiting. They posed that avoiding toxicity may supersede selection for limiting nutrients because toxicity has a more acute negative effect on fitness than deficiency. Thus, animal foraging behavior seems to be flexible enough to accommodate the fluctuations in nutrient availability and physiological state of the animal and thus may vary widely even within an herbivore species.

Given the flexibility of foraging behavior, it is conceivable that herbivores may actually maximize a tradeoff between acquiring limiting nutrients while avoiding nutrient toxicity (hereafter nutrient balance hypothesis). This is a fundamental shift in thought from previous hypotheses because this hypothesis predicts that animals will maximize intake of limiting nutrients until mixing a diet that reaches toxicity in another nutrient. Thus, abundant nutrients are more likely to regulate the consumption of limiting nutrients rather than vice versa as is the conventional line of thought. However, most studies consider too few nutrients to test the nutrient balance hypothesis (e.g., 4 - Nelms 1996; 2 - Berteaux et al. 1998; 2 - Dostaler 2011). Moreover, even when many nutrients are considered, quantifying diet selection in wild populations is notoriously difficult and often researchers utilize indirect measures of selection such as bite counts (Lashley et al. 2011; Lashley and Harper 2012), microhistological surveys (Alipayo et al. 1992; Marrero and Nogales 2005; Lashley et al. 2015; Jung et al. 2015; Lashley et al. 2016), estimates of plant biomass removal (Lunceford 1986), or captive animal observations (Nelms 1996; Ceacero 2009; Ceacero 2015). However, each of those measures have associated biases making accurate estimates difficult (Lashley et al. 2016). Captive populations allow more

controlled experimental manipulations of nutrient availability and precise measures of animal selection, but may be limited in inferential power to predict how environmental stressors and resource heterogeneity affect diet selection in wild populations (Spalinger et al. 1997). Thus, a robust experimental design that allows manipulation of a wide variety of nutrients, and a more direct quantification of diet selection in wild herbivore populations is needed to test the nutrient balance hypothesis.

I designed a cafeteria-style field experiment where I manipulated the availability of nutrients with 15 plants to test the nutrient balance hypothesis in a wild population of white-tailed deer (hereafter deer). To improve on previous limitations in quantification of diet selection in wild populations, I directly monitored deer use of plants with camera traps rather than relying on indirect measures of selection. Conceptually, diet selection driven by maximizing limiting nutrients assumes that toxicity is not an important constraint given the possible intake of an animal (Figure 1a). That is, the animals can consume as much as physiologically possible to meet the limiting nutrient constraint. Contrastingly, diet selection driven by avoidance assumes that limited nutrients are only limited because the toxicity constraint limits intake (Figure 1b). That is, diet selection is simply avoidance of plants with high levels of toxic nutrients which allows animals to increase forage intake and as an artifact of increased intake, meet the limiting nutrient constraints. However, in nature, avoiding nutrient toxicity and acquiring limiting nutrients are necessary if both simultaneously exist as constraints (Figure 1c), and in many cases, actively avoiding toxicity while maximizing intake of limiting nutrients may be necessary through diet selection. That is, only maximizing limiting nutrients may result in reaching toxicity in abundant nutrients while only avoiding abundant nutrients

may result in deficiencies in limiting nutrients. To test the nutrient balance hypothesis, I tested a key prediction that deer diet selection should be partially explained by attraction to some nutrients and partially by avoidance of others if animals indeed selectively forage to meet both constraints.

## **MATERIALS AND METHODS**

### **Study Area**

I conducted this study at the Andrews Forestry and Wildlife Laboratory, a 220-ha research property, located in the Interior Flatwoods soil region of Oktibbeha County, Mississippi. The property is comprised of about 10-ha of agronomic fields with the remaining 210-ha being dominated by loblolly pine (*Pinus taeda*) forest.

### **Experimental Design**

I designed a cafeteria-style experiment in a randomized block design (n=4) by planting 15 cool-season plants varying in nutritional quality in each block. Also, each block contained a fallow plot with no planting. I randomized placement of plants and the fallow plot to ensure estimates were not biased by microsite conditions (e.g., moisture, shade, or slope gradients, game trails). Each of the plots was approximately 0.02 ha in size (Figure 2) and protected from herbivory by electric fencing. The 15 plants were common agronomic cool-season crops: ladino white clover (*Trifolium repens*), durana white clover (*T. repens*), crimson clover (*Trifolium incarnatum*), arrowleaf clover (*Trifolium vesiculosum*), balansa clover (*Trifolium michelianum*), berseem clover (*Trifolium alexandrinum*), red clover (*Trifolium hirtum*), winter wheat (*Triticum aestivum*), bob oats (*Avena sativa*), rye (*Secale cereal*), rape (*Brassicas spp.*), turnips (*Brassicas*

*spp.*) chicory (*Cichorium intybus*), ryegrass (*Lolium multiflorum*), and Austrian winter peas (*Pisum sativum subsp. arvense*). Before planting, fields were cultivated (e.g., herbicide application, mowed, disked, tilled) and soils were amended with lime and fertilizer according to soil analyses performed at Mississippi State University's Soil Lab. The electric fencing was designed to exclude deer from the plants to allow all to establish before beginning trials. This is an important strength of the design because the plants may become available at different rates which could influence diet selection if some plants are not yet available or already depleted upon trial initiation.

To test our hypothesis, plant samples were collected and protective fences were removed from 2 blocks per each of 4 trials, for a period of 14 days. Thus, I temporally replicated the experiment with each trial beginning 2-4 weeks after the previous trial was concluded. I used a 14-day period because that time period was short enough that no plots were depleted but long enough to ensure appropriate samples sizes were obtained to make accurate and precise estimates of behavior (Lashley et al. 2018). This was a unique design because the plants were maturing at different rates and because forage maturity is important to the nutritional quality of the plant (Lashley et al. 2014a). Temporal replication effectively allowed us to reorganize relative nutritional qualities of the same 15 plants over time. All plots within each block including the fallow plot were monitored with motion-triggered camera traps set to a 1 minute interval between pictures.

### **Plant Sampling**

To measure forage availability and nutritional qualities, I sampled all forage biomass  $\geq 4$  cm in height within a randomly placed 1 m quadrat. I followed forage handling protocol presented by Lashley et al. (2014a) where samples were dried in a

convection oven at a temperature of 47°C to constant mass. Once dry, samples were weighed to the nearest gram and shipped to Dairy One Forage Lab (<http://dairyone.com>) for nutrient analysis, which is certified by the National Forage Testing Association. I measured 14 nutritional parameters that might influence diet selection based on previous literature: crude protein (CP), protein solubility (Prot. Solub), neutral detergent fiber (NDF), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and sulfur (S); (McDowell 1992; Robbins 1993).

### **Animal Sampling**

I tallied deer detections and posture of each deer. Posture was recorded as Alert, Actively Feeding, or Searching. Similar to previous experiments using camera traps to monitor foraging behavior, I used the actively foraging posture as an indication of forage selection (Lashley et al. 2014b; Biggerstaff et al. 2017). Although animals could be handling plants during other behaviors, I considered our metric of use as a conservative measure of foraging because other non-feeding behaviors are difficult and less likely during this posture.

### **Data Analysis**

To test the hypothesis that attraction and avoidance were simultaneously influencing diet selection, I used a multistep model selection process with Standard Least Squares (SLS) Regression. To ensure results were not biased by failed plots (i.e., too little biomass to be attractive), I plotted the distribution of biomass for each and removed the 25% quartile for each forage type considering them failed plots. As a result, chicory and

Austrian winter pea plots were removed because of crop failure as a result of extended drought. I then quantified the severity of multicollinearity between variables using the Variance Inflation Factor (VIF) and removed variables with  $VIF \geq 5$  (Belsley et al. 2005; Craney and Surles 2002). Variables were removed until  $VIF < 5$  for all variables because nutrient values were highly skewed (bound at zero with positive skew). I adjusted the deer use data by fallow plot use to account for random visitation not associated with selection and log-transformed use to meet assumptions of normality. I used trial as a random effect to control for varying deer physiological requirements over time (Kammermeyer and Miller 2006). I fit the final model using deer use as the response variable, and forage nutritional qualities as predictor variables. I used an alpha level of  $< 0.05$  to determine significance. I used JMP 13 (SAS Institute Inc., Cary, NC) for all analyses.

## **RESULTS**

I collected 13,758 photographs containing 18,527 deer. Of those, 54% of the deer photographed were in the feeding posture and classified as a use event. Plant nutritional qualities explained over half of the variation in deer use ( $R^2 = 0.68$ ; Table 1). Due to VIF's  $\geq 5$ , I removed Ca, Mg, and Fe from our model. Deer use was explained by attraction to CP ( $p = 0.0285$ ) and the avoidance of S ( $p = 0.0276$ ) and NDF ( $p = 0.0004$ ; Table 1). Thus, deer maximized the tradeoff between obtaining the limited quantities of digestible protein while minimizing the intake of potentially toxic sulfur.

## DISCUSSION

Diet selection was positively influenced by a potentially limited nutrient (i.e., CP) and negatively influenced by a potentially toxic nutrient (i.e., S) supporting our proposed nutrient balance hypothesis. Had I only evaluated two or three nutrients, as is common in the literature, I may have misinterpreted our results. For example, if I had only considered CP and fiber content such as done in Donstaler et al. (2011), I too would have concluded that nutrient maximization was driving selection in full support of the nutrient maximization hypothesis. Similarly, if I had only considered mineral nutrients, I would have concluded that nutrient avoidance was driving diet selection in full support of the nutrient avoidance hypothesis (Ceacero et al. 2015). However, as suggested by Ceacero et al. (2015), considering a wide range of plant nutritional qualities allowed us to determine both forces were simultaneously influencing diet selection.

Deer selected highly digestible plants low in S. Although the level of S that results in toxicity has not been reported in wild ungulates, Drewnoski et al. (2014) concluded that 2000 mg/kg were toxic to cattle (*Bos taurus*) and Zinn et al. (1997) reported the same in domestic ruminants. Moreover, Spears et al. (2011) reported a linear decrease in average daily gains and dry matter intake when dietary S increased from 0.12 to 0.46%. Other studies involving domestic ruminants have reported S concentrations above 0.2% resulted in lower concentrations of Cu and Se, possibly leading to severe diseases (Spears et al. 2011; Cammack et al. 2010; Ivancic and Weiss 2001; McBride 2007). I expect the effects of S toxicity in deer to be comparable to those of domestic ruminants; thus, deer may avoid consuming large concentrations of S when foraging. Our data show precisely this behavior as several of our plants consistently exceeded 0.2% S

(e.g., rape-0.47%, turnips-0.46%) and were subsequently avoided in our experiment. Diet selection for acquiring limiting nutrients must compensate for this avoidance secondarily, as animals will avoid toxicity even at the cost of deficiency (Ceacero et al. 2015).

Deer in my study likely increased selection of plants as NDF decreased because NDF is a measure of forage bulkiness which correlates with intake (Mertens 1987). Other studies reported similar decreased voluntary intake as forage NDF increased in domestic ruminants, attributable to the overall decrease in digestibility (Mertens 1987; Harper and McNeill 2015). And, Donstaler et al. (2011) reported attraction to highly digestible forages in captive white-tailed deer. Thus, deer in my study likely avoided high NDF to increase intake rates.

The positive selection for CP corresponds to other preference studies in ruminants (Danell et al. 1994; Tixier et al. 1997; Deguchi et al. 2001; Dostaler et al. 2011). Selection of CP would support increased protein demands to meet physiological needs during the spring season of white-tailed deer (Robbins 1993) and would have increased in demand over the course of my experiment because protein requirements are heightened in the third trimester of gestation (Parker et al. 2005). Thus, using the trial period as a random effect was an important consideration in my design given the expected changes in physiological requirements of the herbivore. After controlling for that effect, CP still emerged as an attractive limiting nutrient.

My data provide support for the nutrient balance hypothesis and as predicted, deer diet selection was explained by attraction to some nutrients and avoidance of others. Future research using a similar design could advance our knowledge of diet selection behavior further by incorporating more populations with varying nutritional constraints to

determine how these processes change as nutritional limitations change in the environment. Understanding how wildlife populations respond to differing nutrient limitations could aid in conservation of those species by informing management decisions to target nutritional constraints.

Table 2.1 Parameter estimates for standard least squares model predicting white-tailed deer (*Odocoileus virginianus*) plant selection based on nutritional qualities in Oktibbeha County, MS, February through June 2017.

Term	Estimate	Standard Error	DFDen	t Ratio	Prob> t
Intercept	5.4039	2.6026	53.9000	2.0800	0.0426
Biomass	0.0026	0.0054	57.5600	0.4900	0.6241
Crude Protein	0.1754	0.0780	56.2100	2.2500	<b>0.0285</b>
Protein Solubility	0.0085	0.0404	53.5800	0.2100	0.8338
Neutral Detergent Fiber	-0.0929	0.0248	58.7600	-3.7500	<b>0.0004</b>
Phosphorus	-5.8525	4.0412	57.4600	-1.4500	0.1530
Potassium	0.6874	0.5050	56.8600	1.3600	0.1789
Sodium	5.6961	3.0806	55.7300	1.8500	0.0698
Zinc	-0.0042	0.0158	58.3900	-0.2600	0.7932
Copper	-0.1388	0.1259	58.7600	-1.1000	0.2749
Manganese	0.0002	0.0007	58.8100	0.2800	0.7797
PPM Molybdenum	0.2448	0.2409	58.8600	1.0200	0.3138
Sulfur	-5.9447	2.6290	56.7100	-2.2600	<b>0.0276</b>

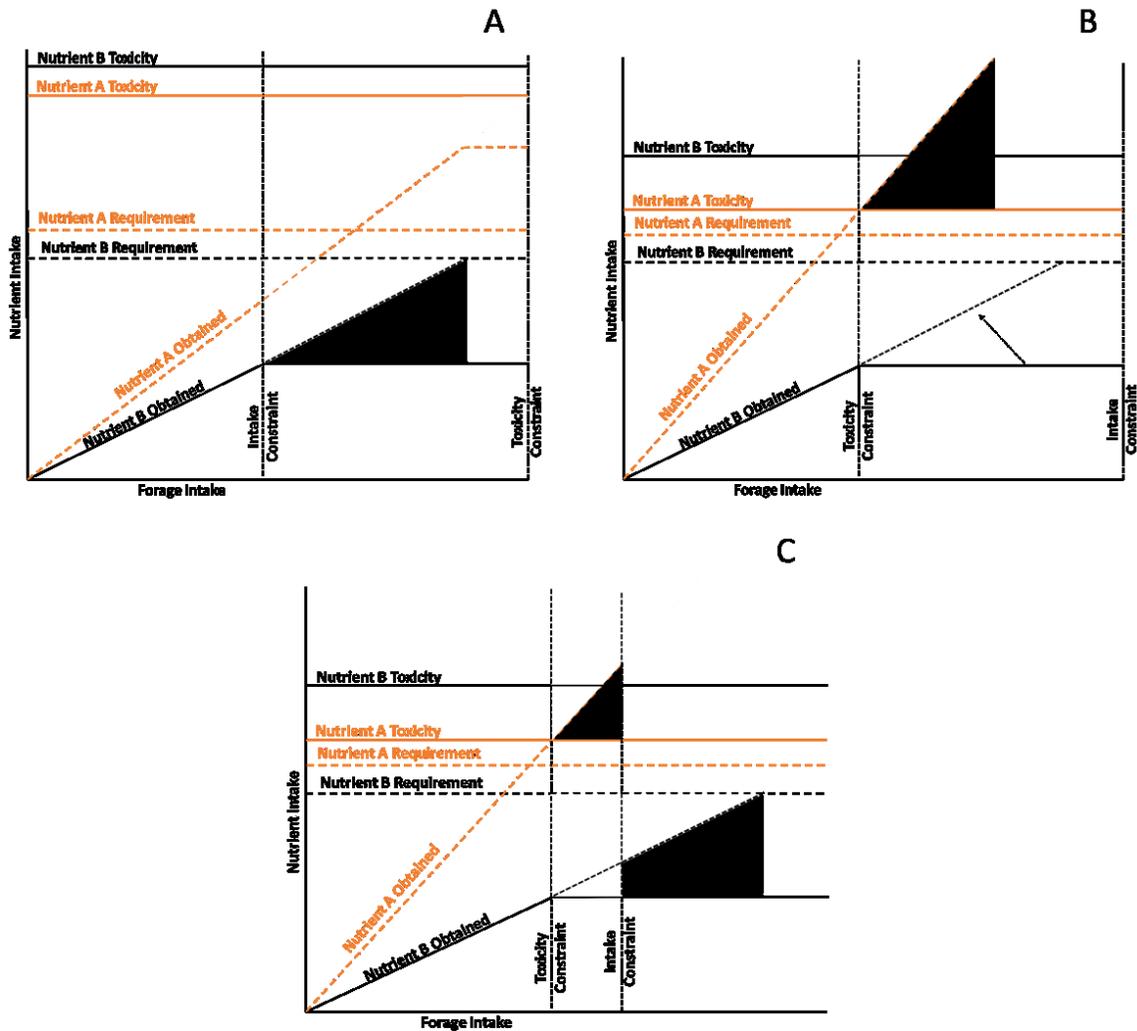


Figure 2.1 Visual representation of the constraints and assumptions of the nutrient maximization hypothesis (a) stating herbivore diet selection is driven by maximizing limiting nutrients and assuming that toxicity is not an important constraint given the possible intake of an animal, the nutrient avoidance hypothesis (b) contrastingly stating herbivore diet selection is driven by avoidance and assuming that limited nutrients are only limited because the toxicity constraint limits intake, and the nutrient balance hypothesis (c) stating that in nature, avoiding nutrient toxicity and acquiring limiting nutrients are necessary if both simultaneously exist as constraints.

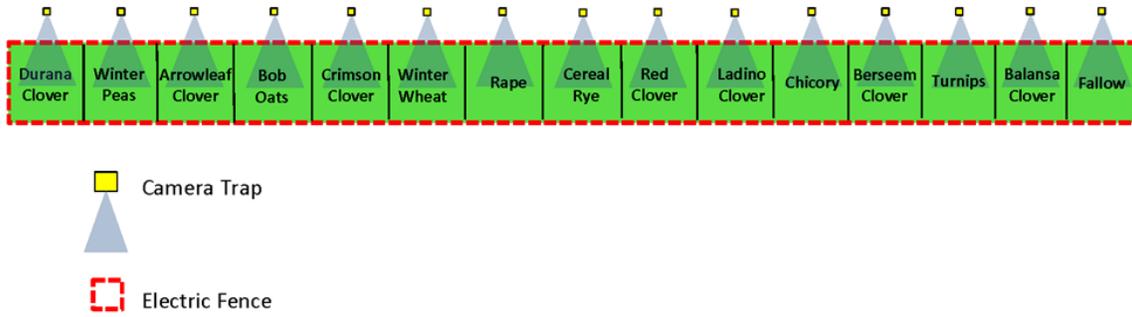


Figure 2.2 Experimental design quantifying white-tailed deer (*Odocoileus virginianus*) selection across plants of varying nutritional qualities. Fifteen plants were randomly assigned and planted in same size adjacent plots protected by electric fence and monitored with camera traps (i.e., Block).

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CHAPTER III  
SOIL NUTRIENTS INDIRECTLY INFLUENCE PLANT SELECTION IN WHITE-  
TAILED DEER

**INTRODUCTION**

Soil productivity is a measure of primary productivity that a given soil type can sustain based on nutrient availability (Keddy et al. 1997). Soil productivity ultimately shapes plant communities (Venterink et al. 2001) by regulating plant species abundance, composition, and tissue chemistry (Demchik and Sharpe 2001; Juice et al. 2006; Jones et al. 2008). Soil nitrogen (N), phosphorus (P), and potassium (K) are the primary nutrients responsible for regulating these community characteristics (Bridgham et al. 1996; Verhoeven et al. 1996; Van Duren and Pegtel 2000). Nitrogen availability is perhaps most important because it is often limited and strongly influences species composition by promoting plants with adaptations to use alternative nitrogen sources when soil N is low and plants adapted to exploit soil N when it is abundant (Tamm 1991; Aerts and Chapin 1999). Because of the direct influences of soil productivity on plant communities, soil productivity has a strong indirect influence on nutrient availability to herbivores (Biere 1995; Blair et al. 1977; Chapin 1980; Cowan et al. 1970; Fraser and Grime 1998).

Detangling the effects of soil productivity on diet selection is difficult because plant community structure and animal nutritional demands are confounding factors linked to soil productivity. In addition to the large literature base linking soil productivity to

changes in plant communities, several studies have also demonstrated that diet, health, morphology, and fitness of many cervid species vary with soil productivity (Gill 1956; Gaillard et al. 1996; Strickland and Demarais 2000; Herfindal et al. 2006; Jones et al. 2010; Lehoczki et al. 2011). This is problematic for understanding how soil productivity affects diet selection because the structure of plant communities and the nutritional demands of animals contribute to plant selection (Vangilder et al. 1982; Ceacero et al. 2009; Ceacero et al. 2015; Dykes 2018). Thus, to fully understand how soil productivity affects diet selection, an experiment designed to manipulate soil productivity while controlling for changes in plant communities and animal nutritional demands is needed.

I designed a nutrient-addition experiment where I manipulated soil productivity to determine how intraspecific plant selection was influenced within a population of white-tailed deer (*Odocoileus virginianus*; hereafter deer). Because I used a single plant species and my manipulated gradient in soil productivity was accessible by the same population, my design enabled us to control for influences of plant community structure and nutritional demands. Moreover, I conducted an additional paired trial with the same manipulation but without the presence of a palatable plant to ensure any observed changes in behavior were not directly linked to nutrient additions. I hypothesized that soil productivity would indirectly influence deer plant selection. I tested three predictions from that hypothesis: A) soil productivity would influence deer plant selection but only when a palatable plant was present, B) nutrient addition would increase plant nutrient concentrations, and C) changes in plant nutrients would explain deer plant selection (Figure 1).

## **MATERIALS AND METHODS**

### **Study Area**

This study was conducted at the Andrews Forestry and Wildlife Laboratory located in the Interior Flatwoods soil region of Oktibbeha County, Mississippi. The property totals 220-ha comprised of about 210-ha of loblolly pine (*Pinus taeda*) forest and 10ha of forest openings.

### **Experimental Design**

I established experimental blocks in forest openings. Each block (n=4) contained 9 plots in which I randomized treatments. To prepare plots for planting, I used broad-spectrum herbicide, mowing, disking, and tilling to remove competing vegetation. Soil subsamples from each experimental plot were collected and combined for a single soil sample from each experimental block. Soil analyses were completed at the Mississippi State University Extension Soils Laboratory (<http://extension.msstate.edu/lawn-and-garden/soil-testing>). I used a randomized block design to account for any biases associated with microsite conditions (e.g., moisture, shade, deer behavior, topography). Each block included four plots of balansa clover (*Trifolium michelianum*) and four plots of winter wheat (*Triticum aestivum*). Treatments included a plot with no nutrient addition (control), pH neutralization with addition of CaCO<sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and a combination of pH neutralization and nutrient addition (lime and fertilizer, Figure 2). Nutrient addition volumes were determined by recommendations from laboratory results for peak soil productivity conditions in the respective block and thus varied across blocks. In addition, I monitored deer use of a fallow plot within each block to quantify deer use not associated with plantings or nutrient addition treatments.

All plants were protected from herbivory by electric fencing until March 2017 when balansa clover was actively growing and palatable but winter wheat had senesced and was subsequently unpalatable. The use of a palatable and unpalatable plants allowed us to detangle direct and indirect effects of the nutrient addition treatments on deer use. In other words, I was able to determine if nutrient addition itself influenced deer behavior directly. Fences were removed from two blocks in early March to allow herbivore access for a two-week feeding bout, electric fencing was reestablished and then removed from the other two blocks for a two week foraging bout at the end of March. Deer use was monitored with motion-triggered camera traps set with a 1 minute delay between pictures.

### **Plant Sampling**

Immediately before allowing herbivore access to plants, I sampled plant biomass within a randomly placed 1 m diameter sampling quadrat at an above ground height of  $\geq$  4 cm to measure available biomass. Following Lashley et al. (2014) forage handling protocol, I dried plant samples to constant mass in a convection oven at a temperature of 47°C. Samples were weighed to the nearest gram and then shipped to Dairy One Forage Lab (<http://dairyone.com>) for nutrient analysis which is certified by the National Forage Testing Association. I measured 14 nutritional parameters that might influence diet selection based on previous literature: crude protein (CP), protein solubility (Prot. Solub.), neutral detergent fiber (NDF), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and sulfur (S); (McDowell 1992; Robbins 1993).

## **Animal Sampling**

I recorded the sex, age, and posture of each deer in each photograph. Posture was recorded in all pictures as Alert, Actively Feeding, or Searching (Lashley et al. 2014; Cherry et al. 2015; Schuttler et al. 2017; Biggerstaff et al. 2017). Each deer with the head down posture was classified as a use event in each photograph (Lashley et al. 2014; Biggerstaff et al. 2017). Thus, in group sizes  $> 1$ , a single picture could yield  $> 1$  use event.

## **Data Analysis**

I used the GLM function to perform linear regressions relating deer use to treatment with and without a palatable forage present. I used a Welch's *t*-test due to unequal variance and sample size to ensure plant biomass was similar between the plots with and without a palatable forage present. I transformed response variables to address the assumptions of normality when necessary. I performed an analysis of variance to test the effect of treatment on plant quality. I used linear models to evaluate the influence of plant nutrients on deer use. An alpha level of  $\leq 0.05$  was used to determine significance. All statistical analyses were completed in R (Version 3.4.1, [www.r-project.org](http://www.r-project.org)).

## **RESULTS**

I collected 3,664 photographs of deer containing a total of 5,318 individuals. Of those, 48% of the deer photographed were in the feeding posture and classified as a use event. Biomass did not differ between the palatable and unpalatable forage (two-sample *t*-test,  $df = 21.671$ ,  $t = 1.2686$ ,  $p = 0.2181$ ) ensuring that differences in deer use between the palatable and unpalatable forage were not attributable to availability of cover. Deer

use was influenced by all treatments in the palatable forage but had no effect in the unpalatable forage indicating soil productivity indirectly affects deer use (Figure 1 A; Table 1). Plant biomass (ANOVA,  $df = 3,12$ ,  $F = 4.416$ ,  $p = 0.026$ ), P (ANOVA,  $df = 3,12$ ,  $F = 8.704$ ,  $p = 0.00244$ ; Figure 1B), and Mg (ANOVA,  $df = 3,12$ ,  $F = 4.441$ ,  $p = 0.0256$ ) were affected by nutrient addition treatment in the palatable forage. I removed Mg from the model to explain deer use because of multicollinearity with P ( $R^2=0.79$ ). Phosphorus ( $p = 0.019$ ) but not biomass ( $p = 0.739$ ,  $R^2 = 0.002$ ) had a positive influence on deer plant selection and P concentration explained almost half of the variation in plant selection ( $R^2 = 0.47$ ; Figure 1C).

## DISCUSSION

Soil productivity indirectly influenced diet selection by altering P concentrations in plant tissues. Increased plant P concentrations may have been a direct effect of P addition or indirectly the addition of N to the soil depending on which nutrient is more limiting to plant growth (Tessier and Raynal 2003). My results are consistent with the Thornly model of photosynthate partitioning that nutrient addition favored photosynthate partitioning of xylem-mobile nutrients to the leaf organs effectively causing elevated P concentrations in leaf organs (Marschner et al. 1996). The fact that I did not observe an increase in leaf N is likely an indication that my plants were P limited which is also supported by my relatively high N:P (i.e., 12:1) in plant leaves from control treatments. Also, because the plants had access to full irradiance, photosynthetic capacity was likely already maximized without nutrient additions, and because leaf N is highly correlated with leaf photosynthetic capacity, I therefore should not have expected leaf N to be affected by nutrient additions (Evans 1989). Because P is often a limiting nutrient in

terrestrial ecosystems (Elser et al. 2007; Vitousek et al. 2010), and therefore to herbivores, it may be the most important nutrient in herbivore diet selection (Vangilder et al. 1982; Grasman and Hellgren 1993; Hewitt 2011; Lashley et al. 2015). Thus, coupling my results with previous experiments suggest that factors affecting P availability in leaf organs may determine the probability of herbivore selection across many terrestrial landscapes (Campo and Dirzo 2003; Santiago et al. 2012).

Soil productivity indirectly influences herbivore foraging behavior which may partially explain differential herbivore effects on plants across that gradient. Several studies suggests soil productivity gradients are important when considering herbivore effects on plant diversity (Grubb 1992; Ritchie et al. 1998; Ritchie and Olf 1999), regeneration, and propagule transport (Olf and Ritchie 1998). Large herbivores can increase plant diversity across productivity gradients by affecting seed dispersal, regeneration success, and soil nutrients through excrements (Steinauer and Collins 1995; Olf and Ritchie 1998). However, plant diversity may decrease across a soil productivity gradient when large herbivore densities are high relative to the productivity of the soil (Milchunas et al. 1988). My data indicate that some differences in herbivore effects on plant communities across gradients in soil productivity may be indirect because of shifting herbivore behavior.

There is ample evidence that herbivores can discriminate between high and low quality plant species (Awmack and Leather 2002; Ceacero et al. 2009; Ceacero et al. 2015) and the ability to differentiate between plants of varying quality is necessary to maximize fitness (Gillette et al. 2000; Sinervo 2013). Postingestive feedback has been proposed as the mechanism enabling ungulates to discriminate between species of

different quality (Provenza 2005). However, this mechanism has not been thoroughly investigated for intraspecific diet selection. Within plant species, visual and chemical cues may confound how animals respond to the postingestive feedback. Because intraspecific plant quality can vary widely with soil productivity, future research is needed to develop a basic understanding of how herbivores determine intraspecific changes in nutritional quality to improve our understanding of herbivore-plant interactions.

Table 3.1 Parameter estimates for generalized linear model predicting white-tailed deer (*Odocoileus virginianus*) plant selection based on soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of CaCO<sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer of a palatable plant (balansa clover, *Trifolium michelianum*) and an unpalatable plant (winter wheat, *Triticum aestivum*) in Oktibbeha County, MS, February through June 2017.

	Term	Estimate	Std. Error	z value	Pr(> z )
Palatable	(Intercept)	3.2189	0.2000	16.094	<0.001
	Fertilizer	2.3924	0.2023	11.828	<0.001
	Lime	0.9555	0.2124	4.498	<0.001
	Lime/Fertilizer	1.9601	0.2035	9.632	<0.001
Unpalatable	(Intercept)	-1.63E+01	1.49E+03	-0.011	0.991
	Fertilizer	1.56E+01	1.49E+03	0.010	0.992
	Lime	-3.30E-10	2.10E+03	0.000	1.000
	Lime/Fertilizer	1.94E+01	1.49E+03	0.013	0.990

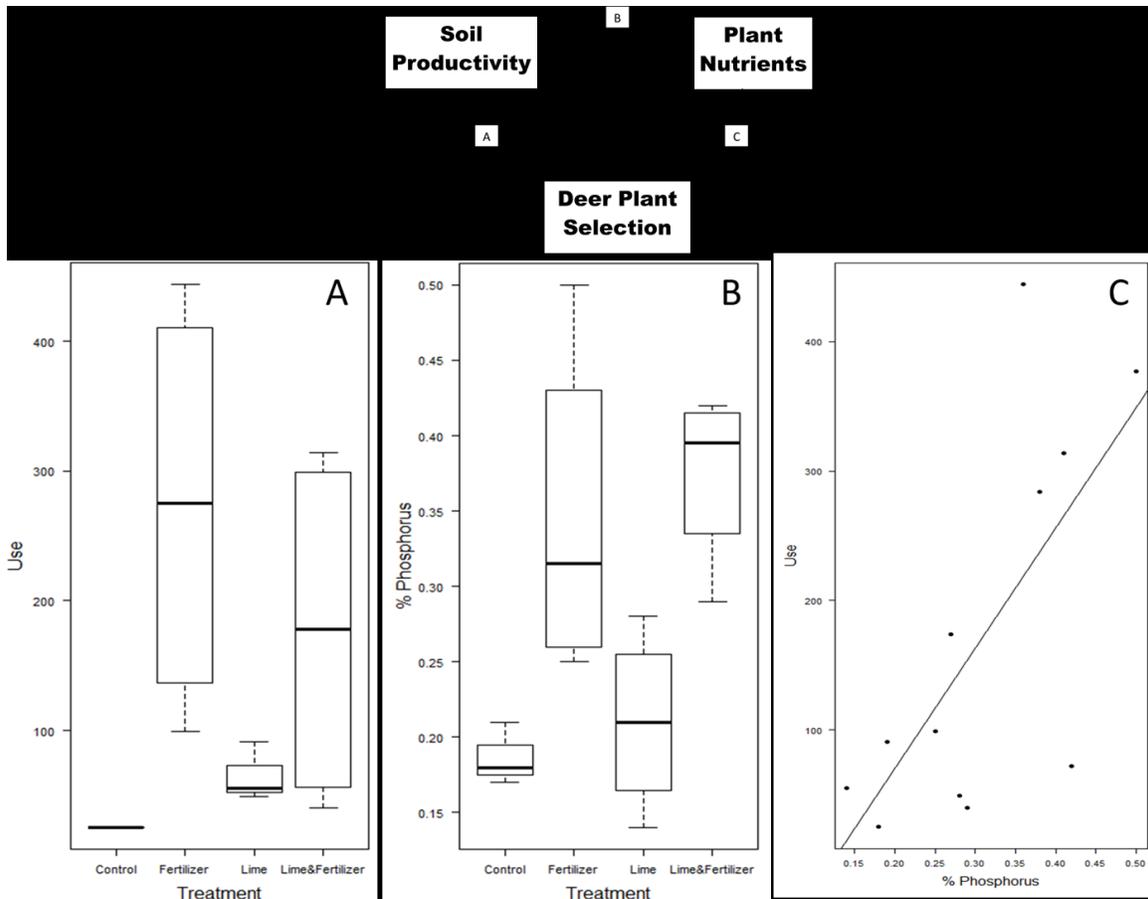


Figure 3.1 Conceptual diagram of hypothesis: soil productivity indirectly affects white-tailed deer (*Odocoileus virginianus*, hereafter deer) plant selection, and three associated predictions: A) soil productivity would influence deer plant selection but only when a palatable plant was present; box and whisker plot depicting effects of soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of  $\text{CaCO}_3$  (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer on deer selection of a palatable plant (balansa clover, *Trifolium michelianum*), B) nutrient addition would increase plant nutrient concentrations; box and whisker plot depicting effects of soil amendment treatments: no nutrient addition (i.e., control), pH neutralization with addition of  $\text{CaCO}_3$  (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer on phosphorus concentrations of a palatable plant (balansa clover, *Trifolium michelianum*), and C) changes in plant nutrients would explain deer plant selection; box and whisker plot depicting effects of increased plant phosphorus concentration on deer use of a palatable plant (balansa clover, *Trifolium michelianum*) in Oktibbeha County, MS, February through June 2017.

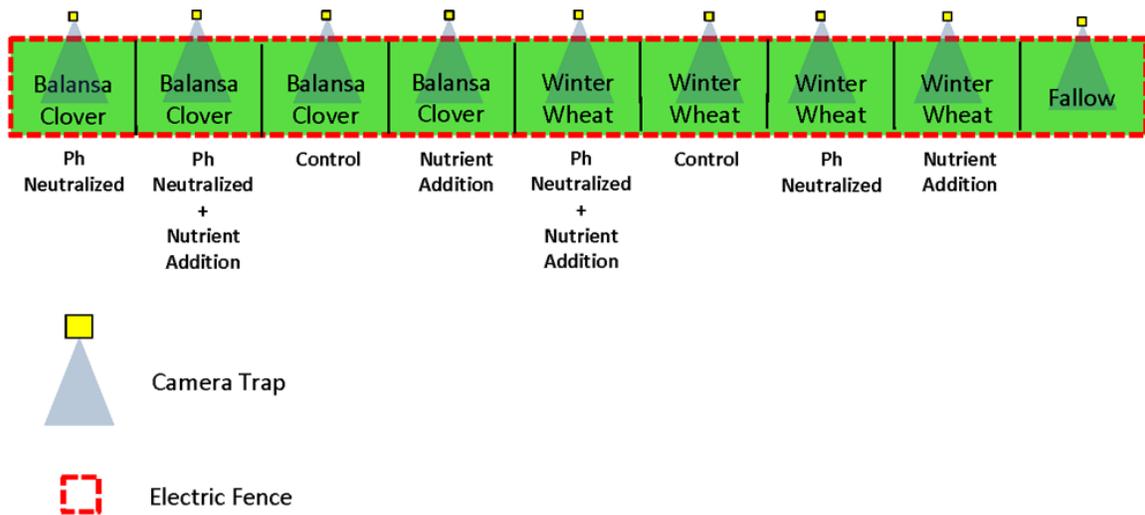


Figure 3.2 Experimental design quantifying white-tailed deer (*Odocoileus virginianus*) plant selection across cool-season legume and cool-season cereal grain receiving treatments to manipulate nutritional quality. Balansa clover (*Trifolium michelianum*) and winter wheat (*Triticum aestivum*) were randomly assigned and planted separately in 4 equal-sized adjacent plots (i.e., block). Each plot received 1 amendment treatment: no nutrient addition (i.e., control), pH neutralization with addition of CaCO<sub>3</sub> (i.e., lime), nutrient addition of N, P, and K (i.e., fertilizer), and lime and fertilizer according to soil tests to manipulate intraspecific plant qualities. Each plot was protected by electric fence and monitored with camera traps in Oktibbeha County, MS, February through June 2017.

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## CHAPTER IV

### SYNTHESIS AND CONCLUSIONS

Deer selected plants high in crude protein while avoiding large concentrations of sulfur and neutral detergent fiber supporting my proposed nutrient balance hypothesis. Further, my nutrient addition experiment provided additional evidence that deer are capable of discriminating across intraspecific plants of differing nutritional quality. Manipulations of soil productivity altered plant quality (e.g., phosphorus and biomass) and subsequently influenced diet selection. My results indicate that herbivore diet selection is a reflection of the nutritional constraints of the population in a given environment. Thus, diet selection can inform habitat management decisions to target environmental constraints thus improving conservation efforts for wildlife in the future.