LITTLE AND LARGE HERBIVORES AS INDICATORS OF TROPHIC TRANSFER: DO TRACE NUTRIENTS DRIVE POPULATIONS?

A Thesis

by

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ABSTRACT

Trace minerals, such as copper, iron, and zinc, are essential for reproduction, growth, and immunity of mammalian herbivores and the growth of their populations. I examined the relationship between supplies of essential minerals on the landscape and hepatic stores of those minerals in two wild herbivores to assess the effects of weather, soils, plants, animal attributes (i.e., sex), and population processes (i.e., density) on the transfer of trace minerals from soils to the animal. Soils, grasses, woody browse, hispid cotton rats (*Sigmodon hispidus*), and white-tailed deer (*Odocoileus virginianus*) were sampled across 19 study sites. Liver copper was < 5 µg/g at 13 % of sites for rats and < 20 µg/g at 58 % of sites for deer, which indicated regional limitations for copper in herbivore populations. Leaves of woody browse were higher in copper, lower in iron, and similar in zinc when compared with grasses. Available mineral concentrations of soils were positively related to liver copper and zinc in rats, which was consistent with the short lives and high productivity of these small granivores that rely on grasses. Significant interactions between soil concentrations and plant growing conditions (i.e., summer precipitation and temperature) affected liver iron and zinc in deer, which reflected the greater complexity of trophic transfers in large, long-lived, browsing herbivores. Population density affected liver concentrations of copper, iron, and zinc in both rats and deer. Values for δC^{13} in heart muscle indicated a shift in diet with increasing density for deer but not for rats. My data indicate that supplies of essential trace minerals contribute to density dependence of herbivore populations. Local population density may therefore influence the prevalence of deficiency states and disease outbreak that exacerbate population cycles in wild mammalian herbivore populations.

DEDICATION

I dedicate this thesis to Eric, Laurie, and Michael Hollingsworth, who have always believed in me.

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CHAPTER I

INTRODUCTION*

Grasslands, such as prairies and savannahs, support one third of the world's human communities including those in North America that live and depend on the Great Plains grasslands (Havstad et al. 2007, Smith et al. 2009). The cover of grasses, forbs, shrubs, and trees that characterize these ecosystems is the result of a dynamic interplay among soil nutrients, water cycles, temperature and herbivores that is punctuated by drought, fire, and flood (Chapin III et al. 2011).

In the southern Great Plains, grasslands covered approximately two-thirds of the Texas landscape when the first European immigrants settled in the area in the mid 19th century (Diamond 2010). These native grassland ecosystems supported populations of free-ranging native herbivores, including species that are now locally extirpated, such as pronghorn (*Antilocapra americana*) and elk (*Cervus canadensis*) (Frank et al. 1998). Frequent fire maintained grasslands by removing plant litter, suppressing woody plants, destroying over-mature plants, and increasing nutrient recycling (Norton-Griffiths 1979). Prescribed burns are now used in an effort to improve palatable grass species by maintaining the grassland ecosystem in an increasingly immature, productive growth phase. Large expanses of these native grasslands have now been converted to agricultural systems for both domestic animal production and dryland crops with Texas serving as the nation's leader of exports in cotton, cottonseed, and beef

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in 2012 (Texas Department of Agriculture 2019). However, Texas grasslands still support wild herbivore populations. Additionally, generalist species such as white-tailed deer (*Odocoileus virginianus*) and hispid cotton rats (*Sigmodon hispidus*) are able to use a diversity of habitats, including both native grasslands and agricultural fields.

Texas is divided into 10 ecoregions (Gould et al. 1960) based on categories of climate, topography, and vegetation. Elevations across these ecoregions decline from 8,749 feet in the Guadalupe Mountains of the Trans-Pecos ecoregion to sea level on the coast of the Gulf of Mexico in the Gulf Prairies and Marshes ecoregion (United States Geological Survey 2015). Ten year average minimum daily temperatures range from 1 °C to 4 °C in winter, whereas summer maxima range from 32 °C to 38 °C (National Oceanic and Atmospheric Administration 2016). The coolest temperatures occur in the mountains of the Trans-Pecos and the warmest temperatures occur in the center of the state, in the Edwards Plateau and Post Oak Savannah. Precipitation includes winter snows in the Texas panhandle and summer monsoonal rains in southwest Texas that are combined with frequent floods in central Texas (Baker 1977, National Oceanic and Atmospheric Administration 2009). Plant productivity increases from northwest to southeast Texas as indicated by growing degree days (GDD) for crops such as corn, following trends of the thirty-year average temperature (PRISM Climate Group 2016) (Fig. I.1).

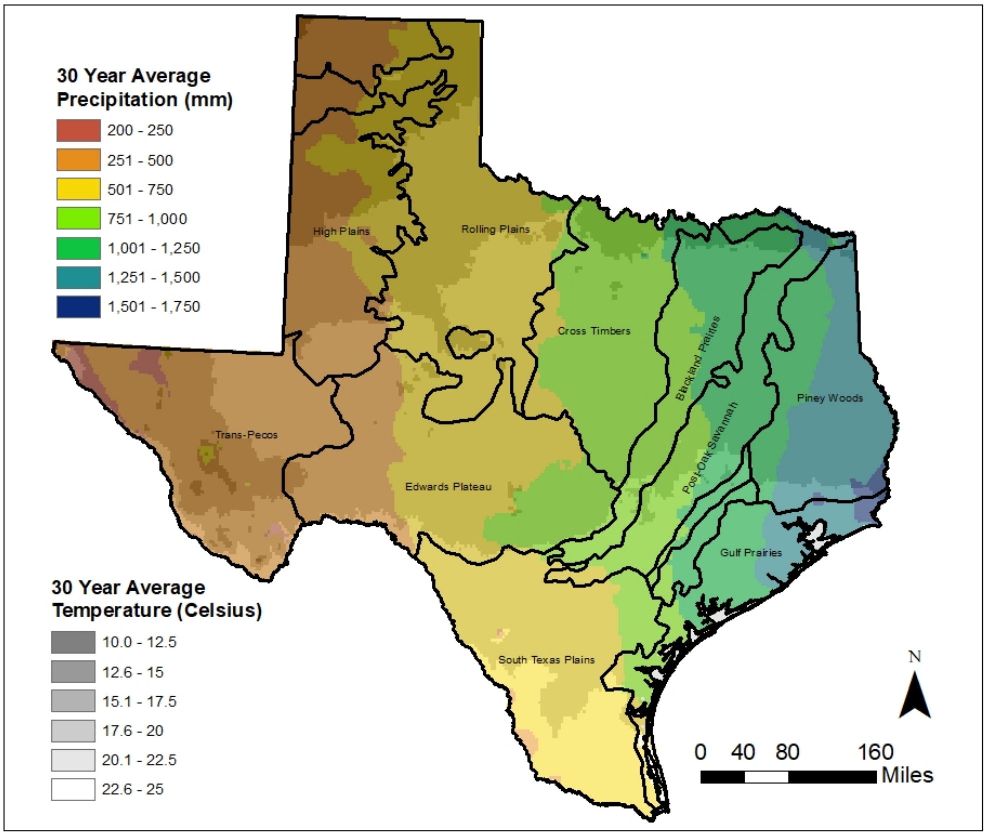

Figure I.1 Thirty-year annual average precipitation and temperature from 1981-2010 adapted from PRISM Climate Group (2016) across Gould Ecoregions of Texas, adapted from Gould et al. (1960) and Texas Parks and Wildlife Department (2010).

Three ecoregions span the grasslands of central Texas across a decreasing precipitation gradient from the humid Post Oak Savannah, through the Blackland Prairies, to the semi-arid Edwards Plateau (Fig. I.1). Annual average temperatures are similar across all three ecoregions, ranging from 17 ° to 21 °C (PRISM Climate Group 2016) with similar GDD for heat-tolerant plants such as corn. Soils vary widely across regions with geological substrate and topography through processes of weathering and organic action from plants and microbes (Franzmeier et al. 2016).

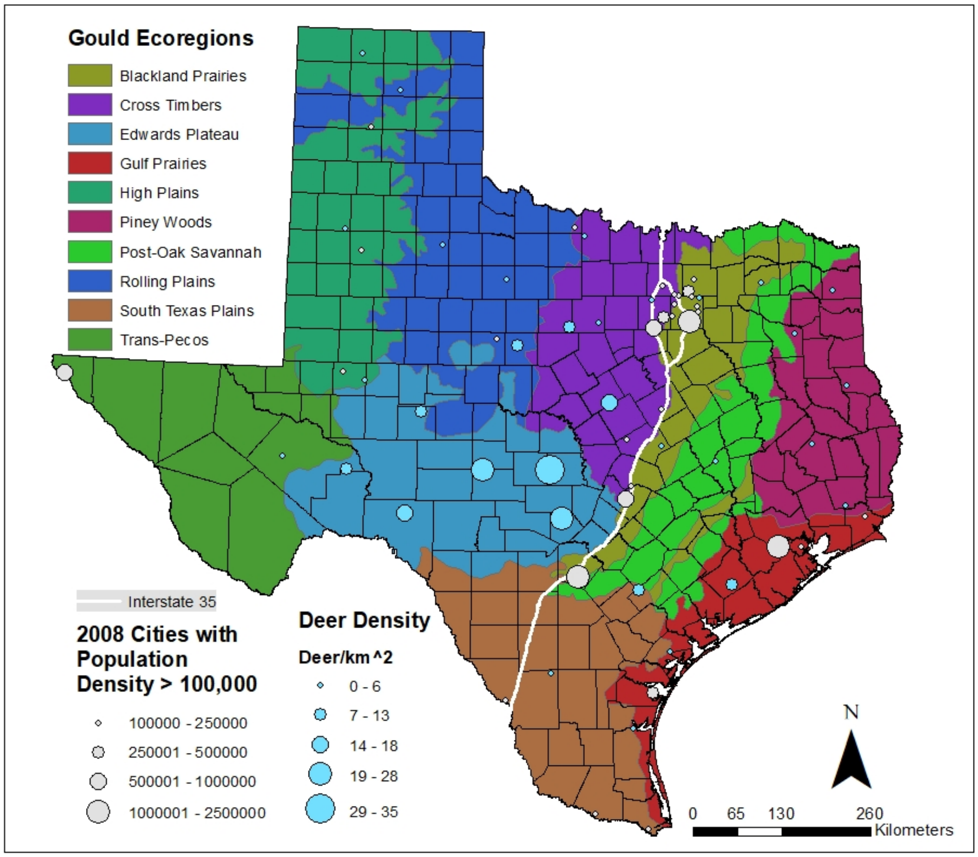

Figure I.2 Gould Ecoregions of Texas adapted from Gould et al. (1960) and Texas Parks and Wildlife Department (2010), 2008 population density of major Texas cities adapted from the United States Census Bureau (2016) and Texas Parks and Wildlife Department (2016), and average density of white-tailed deer (#/km^2) from 2005-2014 adapted from Cain (2015).

The Blackland Prairies ecoregion has a greater diversity of soils than the Post Oak Savannah and the Edwards Plateau. The “blacklands” are mainly comprised of vertisols that are rich in clay, which expands and contracts with changes in moisture. Soils in the savannah are dominated by alfisols with high levels of clay that may be more vulnerable to leaching of basic salts of calcium, potassium, and magnesium from the substratum. The Edwards Plateau consists of mainly mollisols, which are dark in color, rich in bases and minerals, and occur commonly between humid and arid climates at mid-latitudes (United States Department of Agriculture 1999). These soils are the principal source of trace minerals to plant communities (Kabata-Pendias 2004). The Blackland Prairies ecoregion includes highly productive agricultural land that is threatened by urban expansion from the largest cities in Texas, especially along the Interstate 35 (I-35) corridor (Fig. I.2). In their native state, Blackland Prairies are tall-grass ecosystems with fertile soils and a diverse community of plants and wildlife. Consequently, this ecoregion is categorized as likely “the most critically threatened in the state” and includes a large number of “Species of Greatest Conservation Need” (Texas Parks and Wildlife Department 2012c).

In its native state, the Post Oak Savannah is comprised of an understory of forbs and bunch grasses interspersed with post oaks (*Quercus stellata*), but much of the understory has been replaced with cultivated species such as bahia (*Paspalum notatum*) and bermuda grass (*Cynodon dactylon*) for livestock (Texas Parks and Wildlife Department 2012a).

The Edwards Plateau occurs at an intersection of grasslands, woodlands, and shrublands. The arid western portion historically consisted of grasslands and savannahs. Vegetation at the center of the plateau, on the Llano Uplift, mirrors that of the Cross Timbers ecoregion with small patches of forest separated by grassland. On the eastern edge, at the Balcones Escarpment, vegetation consists of hardwood-juniper old-growth forests. The majority of the Edwards Plateau is used for livestock production or orchards, but urban growth is replacing ranches as communities expand from the I-35 corridor (Texas Parks and Wildlife Department 2012b).

Plant communities differ widely across Texas and are dependent on many factors such as soil types, climate, and land management practices, such as grazing and disturbance. However, some plant species grow in all three ecoregions and are utilized by both small and large herbivores. Representative forages for large herbivores such as white-tailed deer, as well as other, smaller herbivores include forbs (e.g., *Euphorbia serpens*), woody browse (e.g., *Quercus stellata*) and native graminoids (e.g., *Nassella leucotricha*) in all three ecoregions (Dillard et al. 2006).

The diversity of land mammals decreases from west to east across Texas with the highest diversity in the Guadalupe Mountains of the Trans-Pecos ecoregion and the lowest diversity in the Blackland Prairies ecoregion. This is similar to trends observed across North America, that increasing heterogeneity of landscape, including range of elevation, types and textures of soils, and diversity of vegetation types, yields greater species diversities (Davis and Schmidly 1994). Wildlife productivity varies across the state depending on species. Densities of white-tailed deer range from 0 to 35 deer/km^2 (Fig. I.2) with the greatest densities on rangelands in the Edwards Plateau where livestock densities are also high (Wilcox et al. 2012, Cain 2015). However, agricultural productivity is highest in the eastern ecoregions where the minimum area for tax assessment as “agricultural use” is 12.5 acres in the Blackland Prairies and Post Oak Savannah, but rises to 33 acres in the Trans-Pecos (Redmon and Cathey 2010). Hispid cotton rat populations exhibit wide fluctuations in densities on a year to year basis across the state, with many potential drivers, including precipitation, intraspecific competition, and intrinsic regulation (Haines 1963, Grant et al. 1985, Rodriguez et al. 2015).

A combined assessment of deer and rodent populations can provide indicators of wildlife productivity at large and small scales. Rodents are the most speciose mammals in Texas including six species that are ubiquitous throughout the state (Davis and Schmidly 1994). One of these species is the hispid cotton rat. This ubiquitous rodent species is able to reproduce year-round with up to five reproductive cycles per year and an average of six offspring per cycle

(Tekiela 2009). Hispid cotton rats are income breeders; that is, available food supports breeding with relatively small support from body stores of energy and nutrients. High fecundity and diverse use of habitat makes this animal ideal for studying the effects of habitat quality at small scales across ecoregions. Hispid cotton rats also forage on many of the same plants as larger ungulates and may thus reflect trophic constraints on mammalian herbivores (Schmidly 2004).

White-tailed deer in Texas are abundant (~4 million) large herbivores (Cain 2015) that can be used as indicators of habitat quality at large scales. White-tailed deer are highly fecund, capital breeders, using nutrient stores to breed once yearly, where one to two offspring are typically produced. White-tailed deer are also present in all ecoregions of Texas, which makes them well suited to measuring productivity across the state (Williams et al. 1995).

Estimates of population density of white-tailed deer are completed annually in Texas (Cain 2015). Population density of large ungulates is a useful tool to manage populations when combined with estimates of carrying capacity (K), determined by the number of animals an area can support over time (Bowyer et al. 2014). Animals at low population density relative to K are more likely to obtain adequate nutrient stores during the growing season due to decreased competition for forage. However, animals at high population density relative to K are likely to experience competition for forage, and may not obtain adequate nutrient stores to buffer against stressors such as harsh winters, human disturbance, or disease (Bowyer et al. 2000). Demographic measures of herbivores, such as density and the proportion of adults and young, can provide an index of ecosystem productivity, but those measures are best combined with metrics of constraints on production, such as limiting nutrients.

Trace minerals are essential nutrients for immune function and reproductive success in animals (Barboza et al. 2008). Wild herbivores obtain trace minerals directly from the landscape through forages and their underlying soils (McNaughton 1979). Mineral availability in ecosystems is affected by many variables, including environment (e.g., soil type and weather), as well as land management practices (e.g., fire, grazing, etc.) (Kabata-Pendias 2010). Large herbivores typically use large foraging radii, depending on season and food availability, to obtain energy and nutrients (Pennycuick 1979). In times of limited resources, animals allocate body stores to maintenance functions, such as defense against disease and infection. If maintenance costs are high, other traits, such as reproduction or immune function, may be constrained (Schmidt and Hood 2012, Wobeser 2013). Competition for food may further exacerbate this situation by reducing supplies of nutrients available on the landscape that could be utilized for animal storage. With lowered mineral stores, animals may be less able to defend against disease as population densities rise.

Copper (Cu), iron (Fe), and zinc (Zn) are trace minerals that are essential for multiple enzymatic pathways and normal immune function in mammals. Deficiency or imbalances in trace mineral concentrations can increase the spread and intensity of disease and exacerbate the adverse effects of other environmental stressors on populations (Barboza et al. 2008). Mineral limitations within wild herbivores have not been widely studied, but dietary threshold values for domestic herbivores are more readily available and are often used as proxies for wild herbivores. Indications of short-term mineral supply (weeks) in herbivores can be assessed in plasma samples while long-term mineral supply (months or years) can be assessed in mineral stores within the liver and bone (Hood 2012, Roug et al. 2015). Liver mineral concentrations give an indication of overall forage consumption, as well as the rate of use of liver stores, over a season. However, liver mineral concentrations do not lend insight into specific forage selection of herbivores.

Diet is the main determinant of isotope composition in animals; therefore, stable isotopes including ratios of carbon ($\delta^{13}C : \delta^{12}C$) and nitrogen ($\delta^{15}N : \delta^{14}N$) are assessed in herbivore tissue samples to identify forage selection of those herbivores. Muscle tissue (e.g., heart) can be used for long-term diet analysis while feces can be used as a short-term indicator (Barboza et al. 2008). Isotopic concentration varies with tissue, including a depletion of $\delta^{13}C$ by 2 to 8 ‰ in lipids of membranes and fats (Peterson and Fry 1987). Values of δC^{13} in tissues of herbivores provide an indication of diet selection because C3 plants range between -33 to -24 ‰ and C4 plants range between -14 to -11 ‰. Additionally, values of $\delta^{15}N$ in animals provide an indication of trophic position as well as metabolism (Ben-David and Flaherty 2012). Trophic shifts increase values of $\delta^{15}N$ by approximately 3 ‰ between consumed and consumer whereas recycling of body N pools tends to increase $\delta^{15}N$ values within the animal.

Soils and plant communities vary drastically across grasslands in Texas, providing a diversity of forage across ecoregions for herbivores (Gould et al. 1960). Wildlife species that inhabit multiple ecoregions (i.e., rats and deer) are subject to differences in available nutrients in the soils and plants as well as overall forage selection across space. With increases in population density of animals, there is likely to be competition for forage, as well as other effects, such as increased competition for mate selection, especially in areas with limited resources (Bowyer et al. 2014, Wolcott et al. 2014). If wild herbivores are unable to obtain the required nutrients from the landscape needed for basic life-history traits, it is likely that animals will be more vulnerable to external stressors, such as infection and disease (National Research Council 2007). As landscapes change over time due to human development, climate change, and woody encroachment, we have few ways to proactively monitor how wildlife populations may be affected.

I tracked trace minerals across trophic groups from soils to plants to animals across grassland systems in conservation areas and private lands of Texas. I combined measures of animal density (i.e., abundance indices and demographics) and mineral availability (e.g., soil minerals and plant minerals) with those of food supply (e.g., isotopic indices of diet), animal condition (e.g., liver mineral stores) and environmental stressors (e.g., summer maximum temperature and precipitation and winter minimum temperature). I studied grasslands across a precipitation and temperature gradient from the moist Post Oak Savannah in the east to the dry Edwards Plateau in the west. The objective of this study was to determine the drivers of three trace mineral concentrations (i.e., Cu, Fe, and Zn) across trophic levels, using a bottom-up approach from soils, to plants, to herbivores.

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CHAPTER II

LITTLE AND LARGE HERBIVORES AS INDICATORS OF TROPHIC TRANSFER: DO TRACE NUTRIENTS DRIVE POPULATIONS?*

Introduction

Trophic exchanges of nutrients are assumed to drive ecosystems, but the relationships are often masked by the number of trophic levels in the ecosystem, processes within each level, and variation in the rate of transfer between levels (Doak et al. 2008, Peterson et al. 2014, Ulanowicz et al. 2014, Boyce 2018). Minerals such as copper (Cu), iron (Fe), and zinc (Zn) are transferred from soils to plants to herbivores (Scholes et al. 2003). Stores of these trace nutrients are essential for reproduction, growth, and immunity that underlie fitness of individuals and growth of populations (Barboza et al. 2008). Trace nutrient stores vary among populations of animals and are widely documented for domestic herbivores; however, trace nutrient stores are poorly studied in wild herbivore populations (National Research Council 1995, 2007). Animals that are deficient in trace nutrients may be more susceptible to infection (poor immune response) and more vulnerable to environmental exposure and predation (anomalies of the skin, pelage, teeth, bone, muscle, and vasculature that impair thermoregulation and movement) (Chandra and Dayton 1982, Fraser 1991, Graham 1991). However, symptoms of deficiency appear and disappear on the landscape, making it difficult to detect and study deficiencies in populations of wildlife (Hnilicka et al. 2003). Conversely, toxicity is more easily detected for trace minerals

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especially where animals are exposed to high concentrations of the mineral in soils and plants (Garrott et al. 2008, Death et al. 2017). Trace mineral trophic transfers are affected by variation in concentrations of nutrients within each trophic level, variable connectivity between levels, and feedback mechanisms influencing nutrient transfer across levels (Clarkson 1985, Spears 2003, Budko et al. 2015). Consequently, the relationship between the quality of soils as supplies of minerals and the deposition of those minerals in the bodies of animals is rarely described for trace minerals that limit populations.

Availability of trace minerals in soils is influenced by inorganic and organic processes (Kabata-Pendias 2010). Trace minerals are weathered and leached from rocks in the formation of soils over multiple years, while temperature and hydrology can alter the availability of those soil nutrients during the annual cycle (Schulte 1992b, c, a). The physico-chemical structure of soils, including pH and soil mineral particle size, affect inorganic processes of mineral solubility and availability, which is further modified by organic processes of decomposition and microbial activity (Kabata-Pendias 2004). Trace mineral concentrations in plants depend upon microbial and fungal processes in the plant rhizosphere that drive uptake of minerals at the roots (Barea et al. 2002, López-Bucio et al. 2003). Plant species differ in biomass, tissue deposition, nutrient requirements, and storage of trace nutrients (Hemphill 1972, Kubota 1983). Consequently, dietary supplies of trace minerals for herbivores are affected by selective foraging among species of plants and on plant parts (i.e., leaves vs. stems) that are usually associated with avoidance of toxins (Laycock 1978, Dillard et al. 2006). Fermentative digestion may further influence the uptake of trace minerals that are ultimately stored in the liver of herbivores, and eliminated via hepatic or renal pathways (Ivan 1988, Barboza et al. 2008).

Macronutrients supply energy substrates and the carbon (C) and nitrogen (N) to build tissue in herbivores (Servello et al. 2005). Energy and nutrient supplies may be affected by shifts in diet due to changes in the growth and composition of the forage plants or competition for those foods among herbivores (Norton-Griffiths 1979, Belsky 1992). Density dependent declines in the size of herbivore populations are often assumed to be the outcome of competition for food that limits energy supplies (Mduma et al. 1999, Bowyer et al. 2014). However, supplies of N and phosphorus (P) for tissue growth and maintenance may ultimately drive populations of herbivores (Chapin and McNaughton 1989, McNaughton 1990, Johnson et al. 2018). Additionally, the integrity and function of tissue growth relies upon trace minerals (Soetan et al. 2010). Regional variation in trace mineral supplies may therefore intensify the decline in a population and slow its recovery by limiting reproduction and growth (Flueck 1994).

Wild herbivore populations integrate changes in the plant community at scales of space and time that increase with body size from the smallest rodents to the largest ungulates (Roth et al. 2009, Sensenig et al. 2010, Hempson et al. 2015, Raynor et al. 2015). Rodent populations exhibit greater variation in population densities at shorter time scales that reflect responses to landscape changes at finer scales than ungulates. This is commonly seen in the cyclical boom and busts in population size that are characteristic of hispid cotton rat (*Sigmodon hispidus*) populations in Texas (Schmidly 2004, Rodriguez et al. 2015). However, ungulates, such as white-tailed deer (*Odocoileus virginianus*) are often the principal focus of conservation planning and management of wildlife due to their economic and cultural importance. Consequently, populations of white-tailed deer are managed through harvest and habitat manipulation to avoid large perturbations in numbers within regions (Cain 2015).

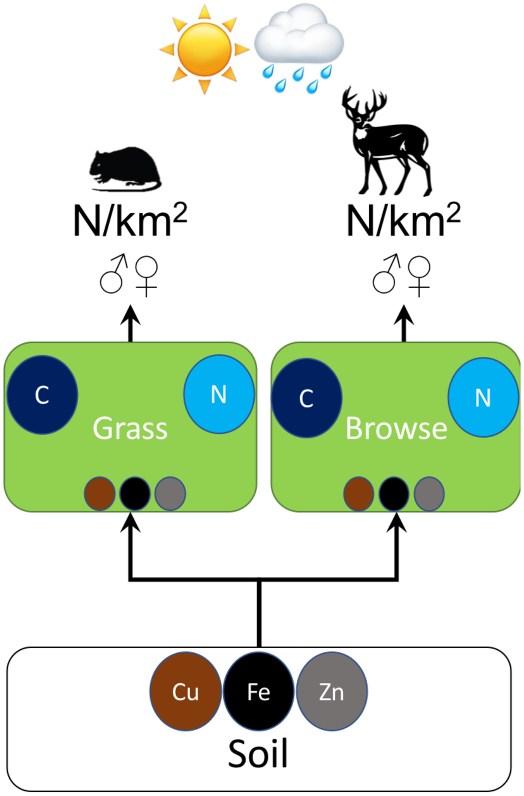


Figure II.1 Conceptual model of trace mineral (i.e., Cu, Fe, and Zn) and stable isotope (i.e., δN15 (N) and δC13 (C)) transfer across trophic levels. Animal stores of trace minerals as driven by fixed effects of soil trace mineral concentrations, diet through stable isotopes (C and N), animal effects (sex: ♂ and ♀), population effects (density: N/km^2), and weather effects (temperature and precipitation).

We examined the relationship between the supplies of trace minerals in the soil and the stores of those minerals in two herbivores to assess the relative effects of weather, soil, plant, and animal processes on the transfer of nutrients from the soil to the animal (Fig. II.1). We hypothesized that trace mineral stores of two herbivores with different life history strategies (i.e., deer and rats) would differ in their drivers of liver mineral concentrations. Secondly, we hypothesized that these drivers would include a combination of soil mineral levels, plant mineral levels through diet (i.e., heart δN^{15} and δC^{13}), animal attributes (i.e., sex), and population attributes (i.e., population density) which are each driven by weather (i.e., summer maximum temperature and precipitation and winter minimum temperature) which affects growing conditions. We lastly hypothesized that the concentrations of each trace mineral (i.e., Cu, Fe, and Zn) would have different drivers at each trophic level.

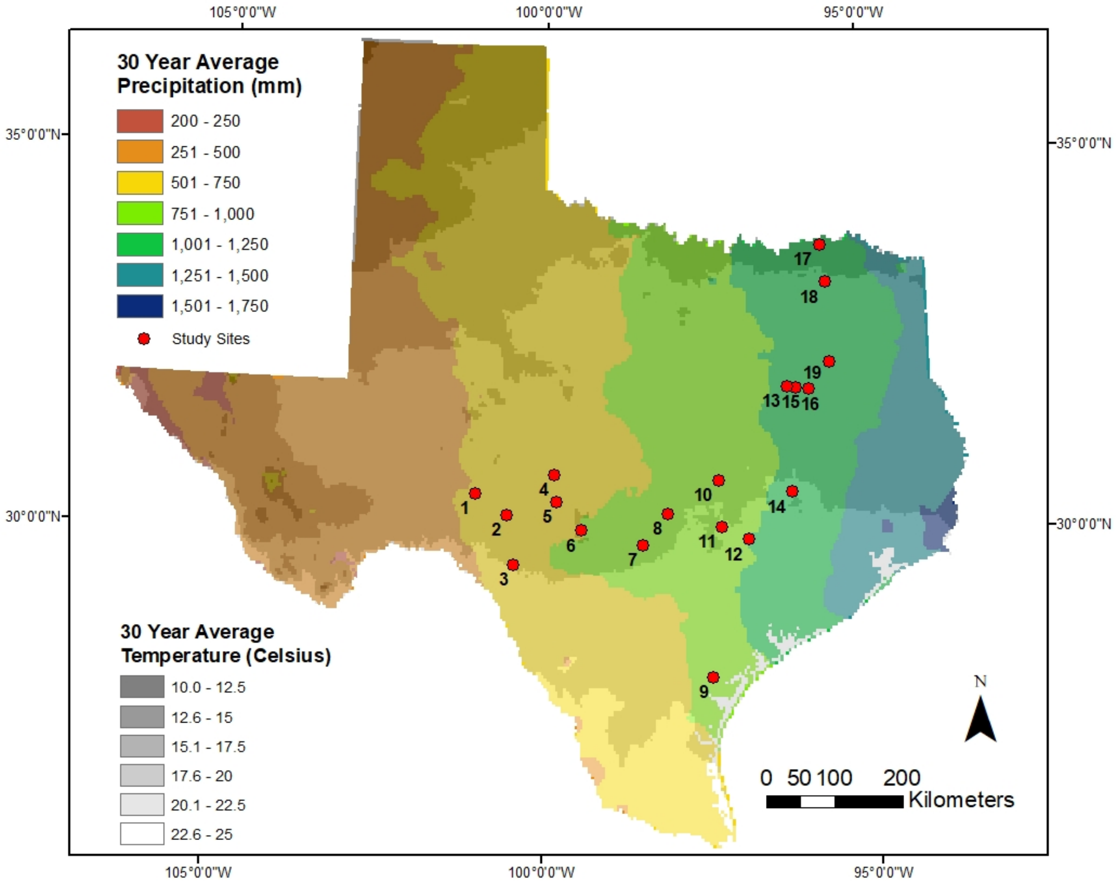


Figure II.2 Locations of grassland study sites (n = 19) in relation to long-term environmental conditions in Texas. Precipitation and temperature gradients are 30-year averages adapted from PRISM Climate Group (2016). Ecoregion, latitude, and longitude for each site are listed in A-1.

Methods

Study sites

We studied 19 grassland sites across four ecoregions (Gould et al. 1960) in central Texas (Fig. II.2; A-1): Edwards Plateau (n = 8), Blackland Prairies (n = 4), Post Oak Savannah (n = 6), and Gulf Prairies and Marshes (n = 1). We used weather records for the prior five years (2013 – 2017) to track environmental conditions across study sites (PRISM Climate Group 2018). The season of plant growth was characterized by monthly precipitation and maximum monthly temperature from May to September. Winter conditions were assessed by minimum monthly temperatures from October to February.

Soils, plants, and rats

Soils, grass species, woody browse species, and hispid cotton rats were sampled across 15 of the 19 study sites (A-2). Soils and plants were collected during peak growing season (May – June 2017) while rodents were trapped during late growing season (September – October 2017). At each site, we selected open grasslands to set three grids, containing four, 200 m transects spaced 10 m apart. Sherman live traps [large folding aluminum trap (7.62 x 8.89 x 22.86 cm), H. B. Sherman Traps, Tallahassee, Florida, USA] were placed every 10 m along each transect. Traps were baited with sunflower seeds and set for a total of 252 traps/night (Rodriguez et al. 2015). Sampling was conducted for two consecutive nights at each location for a total capture effort of 504 trap nights/site. Capture efficiency was calculated using the Effective Trap-Night metric (ETN), similar to Rodriguez et al. (2015) (A-2). Rodent density was calculated by dividing the total number of hispid cotton rats caught by the total area surveyed at each site (A-2). Traps were set at dusk and checked at daybreak to prevent rodent mortality due to overheating. All animals were weighed [Spring Scale #40300 (100 ± 1 g or 300 ± 1 g), Pesola, Schindellegi, Switzerland], identified to species, sex, and age class (i.e., adult or subadult). Non-target species were released after weighing and identification. Hispid cotton rats (n = 73) were collected and euthanized by inhalation overdose with chloroform in a sealed container (Sikes 2016). Carcasses were frozen for storage and thawed to remove heart and liver samples. Animals were humanely handled in accordance with the guidelines published by the American Society of Mammalogists (Sikes 2016) and were approved by the Texas A&M Animal Care and Use Committee (Permit # 2016-018A). Upon conclusion of this study, all specimens were deposited in the Biodiversity Research and Teaching Collections at Texas A&M University [Texas Cooperative Wildlife Collection (TCWC) # 66836 – 66908].

Soil samples were collected along each grid (n = 12), at the beginning (0 m), middle (100 m), and end (200 m) of each transect, for a total of 36 soil samples/site. We removed the duff soil layer and collected 0.5 L of soil to a depth of 15 mm. Soil depth was selected for consistency across sites in order to sample within the range of root depth of common annual grasses and perennial forbs (Schenk and Jackson 2002). Soil samples were stored on ice and frozen for later processing.

Common grass and woody browse species were selected across sites within and directly surrounding each grid. Grass species were collected (n = 11), including bermuda grass (*Cynodon dactylon*), king ranch bluestem (*Bothriochloa ischaemum* var. *songarica*), little barley (*Hordeum pusillum*), little bluestem (*Schizachyrium scoparium*), rescuegrass (*Bromus catharticus*), silver bluestem (*Bothriochloa saccharoides*), texas wintergrass (*Nassella leucotricha*), and four wildrye species [Canada (*Elymus canadensis*), southeastern (*Elymus glabriflorus*), squirreltail (*Elymus elymoides*), and Virginia (*Elymus virginicus*)]. Browse species (n = 9) were collected, including ashe juniper (*Juniperus ashei*), honey mesquite (*Prosopis glandulosa*), eastern red cedar (*Juniperus virginiana*), and six oak species [blackjack (*Quercus marilandica*), live (*Quercus virginiana*), plateau live (*Quercus fusiformis*), post (*Quercus stellata*), vasey (*Quercus vaseyana*), and water (*Quercus nigra*)]. We collected aboveground grasses and green leaves of woody browse in plastic bags that were stored on ice for future processing. Soil particles were removed by hand from roots of grass species to prevent contamination with soil minerals.

White-tailed deer

Adult white-tailed deer (n = 305) were hunter-harvested across 12 of the 19 sites during the 2015 – 2017 hunting seasons (October – February; A-3). We selected sites with no summer supplemental feeding (e.g., corn, protein pellets, etc.) of white-tailed deer. Liver, heart, kidney, and fecal samples were collected within three hours post-mortem and frozen for storage.

Distance sampling via spotlight surveys was conducted during late summer (July – August) 2018 to compare deer densities across 10 of the 12 sites where deer tissue samples were collected, with one additional site (i.e., Welder Wildlife Foundation). South Llano River State Park and Texas Parks and Wildlife Department (TPWD) Richland Creek Wildlife Management Area (WMA) were not surveyed due to high public use during summer months and inaccessibility, respectively. Surveys were completed after weaning young and before the mating season to minimize sex differences in habitat utilization (McCullough et al. 1994). We surveyed three road transects (5900 m/transect) on each site during each night to complete a total of nine surveys that included three repeats of each transect within each site over the course of four weeks. Spotlight surveys began 30 minutes before sundown to capture peak diel activity (Beier and McCullough 1990) and ended between 22:00 h and 04:00 h. Hand-held spotlights [ShowMe Series 08 (100,000 candlepower), Able2 Products Co., Cassville, Missouri, USA] were used by two observers (i.e., driver and passenger) from the cab of a truck to survey 180° on each side of the vehicle, which was driven under 15 km/h. Binoculars [Prostaff 3s (10 x 42), Nikon, Melville, New York, USA] were used to identify animals to sex and life stage (i.e., fawn or adult). We recorded location of the vehicle (GPS model: Oregon 650t; Garmin, Olathe, Kansas, USA) and the distance and bearing from the observer to the animal (laser rangefinder: RX-1200i TBR, Leupold, Beaverton, Oregon, USA). Animal clusters were defined as groups that moved as a unit in which the distance between animals was less than ~10 m (Buckland et al. 2001). We recorded distance to the middle of the cluster and the number of animals within the cluster. Perpendicular distances were calculated from the measured distance to animals and bearings in the Universal Transverse Mercator (UTM) coordinate system. We used the R (version 3.5.2) (R Core Team 2013) statistical package DISTANCE (version 0.9.7) (Miller et al. 2019) for conventional estimates of distance sampling. Detection functions were derived for each site with greater than 35 animal observations (A-3). We pooled observations of five sites to derive detection functions (Buckland et al. 2001) because those sites had few observations ($n < 35$).

Lab analysis

We used a convection oven at 80 °C to dry soil samples over 48 h. We randomly selected three samples of dried soil from each site for mineral analysis. Minerals were extracted by Mehlich III procedure with diethylenetriamine-pentaacetic acid (DTPA) (Mehlich 1978, Mehlich 1984) to determine available concentrations of Cu, Fe, and Zn (Lindsay and Norvell 1978) by inductively coupled plasma mass spectrometry at the Texas A&M Soil, Water, and Forage Testing laboratory in College Station, TX 77845 USA. All plant samples were freeze-dried and homogenized through a 1-mm screen in a centrifugal mill (Retsch ZM 200; Verder Scientific, Haan, Germany). Ground samples were analyzed for ash content by muffle furnace at 500 °C for five hours (Van Soest et al. 1991). Samples were weighed (0.25 – 0.30 g) and digested in 8 mL

HNO_3 with a microwave system (MARS 6; One Touch Plant Method; CEM, Mathews, North Carolina, USA) at 200 °C for 10 min. Duplicate standards of apple leaves (SRM 1515; NIST: National Institute Standards and Technology; US Department of Commerce, Gaithersburg, Maryland, USA) were included in each set of 40 digestions along with two method blanks (no sample). Sample digests and blanks were diluted with 60 mL of deionized water (-18 MΩ /cm; Thermo-Scientific Gen-CAD, Waltham, Massachusetts, USA) to produce a 10 % v/v HNO_3 solution. Diluted sample digests were analyzed for Cu, Fe, and Zn concentrations by atomic emission spectroscopy (MP-AES 4200; Agilent Technologies, Tokyo, Japan) (Li et al. 2013, Hermenean et al. 2015). Calibration curves were prepared from single element standards for atomic emission spectroscopy (1000 µg/mL; Specpure, Alfa Aesar, Ward Hill, Massachusetts, USA).

Two grass species (i.e., little bluestem and silver bluestem) and two browse species (i.e., eastern red cedar and honey mesquite) that occurred most commonly across sites were selected for stable isotope analysis (i.e., $\delta^{13}C$ and $\delta^{15}N$) at the Stable Isotopes for Biosphere Science (SIBS) Laboratory in College Station, TX 77843. Samples were weighed (1.075 – 2.025 mg) with a microbalance into 4 x 6 mm tin capsules (Costech Analytical Technologies, Valencia, California, USA), and analyzed for $\delta^{13}C$: $\delta^{12}C$ and $\delta^{15}N$: $\delta^{14}N$ ratios using an elemental combustion system (Costech Analytical Technologies, Valencia, California, USA) coupled to an isotope ratio mass spectrometer in continuous flow (He) mode (Thermo Fisher Scientific, Delta V advance, Waltham, Massachusetts, USA).

Liver and heart samples from rats were oven dried to constant mass at 80 °C for > 48 h. Tissues from white-tailed deer (liver, kidney, heart, and fecal samples) were lyophilized (FreeZone 18, Labconco Corporation, Kansas City, Missouri, USA) and ground using a commercial kitchen chopper (Pro Prep Chopper-Grinder, Waring Commercial, Stamford, Connecticut, USA).

Liver samples from rats and deer were digested by microwave in 8 mL nitric acid (MARS 6; One Touch Animal Tissue Method; CEM, Mathews, North Carolina, USA) to assay minerals by atomic emission spectroscopy against an internal standard of beef liver (486 ± 74 ppm Cu, 165 ± 29 ppm Fe, and 93 ± 22 ppm Zn). Lipids were removed in petroleum ether (E-812, Buchi, Flawil, Switzerland) prior to isotope analysis of heart muscle from white-tailed deer, however samples size of rodent hearts were not sufficient for lipid extraction (< 0.4 g). Heart samples were reground in an oscillating mixer mill (Retsch GmbH, Haan, Germany) for analysis at the Stable Isotopes for Biosphere Science (SIBS) Laboratory in College Station, TX 77843 USA. Samples were weighed (0.575 – 0.625 mg) with a microbalance into 4 x 6 mm tin capsules (Costech Analytical Technologies, Valencia, California, USA), and analyzed for $\delta^{13}C$: $\delta^{12}C$ and $\delta^{15}N$: $\delta^{14}N$ ratios using an elemental combustion system (Costech Analytical Technologies, Valencia, California, USA) coupled to a stable isotope mass spectrometer in continuous flow (He) mode (Thermo Fisher Scientific, Delta V advance, Waltham, Massachusetts, USA).

Statistical analysis

We used linear mixed model regression to track trace minerals across trophic levels (STATA 14.2; StataCorp, College Station, Texas, USA). Site was included as a random effect in each model to control for variation within each location (ε). Models for Cu, Fe, and Zn in soil (Y) included summer precipitation (PREC), summer maximum temperature (STEM), and winter minimum temperature (WTEM) as fixed effects: $Y = PREC + STEM + PREC * STEM + WTEM + \varepsilon$. Models for minerals in grass and browse included the respective soil concentrations (i.e., soil Cu, Fe, and Zn) (SOIL) and plant species (SPEC) and the same environmental effects:

Y = SOIL + SPEC + PREC + STEM + PREC * STEM + WTEM + ε. Models for Cu, Fe, and Zn in the liver of rats and deer included the respective mineral concentrations in soil, the same environmental variables, with attributes of animals including sex (SEX) and density of the respective population (DENS): Y = SOIL + PREC + STEM + PREC * STEM + SEX + DENS + ε. Models for δC^{13} and δN^{15} values in grasses and browse included plant species and the same fixed effects of environment: Y = SPEC + PREC + STEM + PREC * STEM + WTEM + ε. Models for δC^{13} and δN^{15} values in herbivores (i.e., heart tissues of rats and deer) included the same animal attributes and fixed effects of environment: Y = SEX + DENS + PREC + STEM + PREC * STEM + WTEM + ε. We used step-wise selection to exclude interactions and fixed effects with beta coefficients that were not significantly different from zero ($P > 0.05$). Margins ($\bar{X} \pm$ standard error) were calculated for the observed range of each fixed effect in the final model.

Results

Study sites and animals

Average monthly precipitation and temperature ranged from 43 to 99 mm and 17 to 22 °C, respectively over a 30-year period (1981 – 2010) across sites (PRISM Climate Group 2018; Fig. II.2). During 2017, summer and winter temperature and summer rainfall were similar to the 30-year average (A-1). Little bluestem and silver bluestem were the two most common grass species across sites (n = 10 sites and n = 11 sites, respectively), and eastern red cedar and honey mesquite were the two most common woody browse species (n = 8 sites for both species). Hispid cotton rat densities ranged from 0 to 2,028 animals/km^2 while capture efficiencies varied from 0 to 16 % (A-2). White-tailed deer densities ranged from 0.5 to 27 animals/km^2 (A-3).

Soil minerals

Concentrations of available Cu and Zn in soil were not affected by environmental conditions (A-4). Mean concentrations of available Fe in soil (32.5 ppm ± 31.7 ppm) were greater and more variable than those of Cu (0.5 ppm ± 0.3 ppm) and Zn (1.4 ppm ± 3.2 ppm) across sites. Soil Fe was affected by summer precipitation and temperature. Predicted Fe concentration increased from 11 to 64 ppm with increasing precipitation (72 – 125 mm) whereas increasing temperature (31 – 34 °C) decreased available Fe from 69 ppm to zero availability (i.e., unavailable; A-4).

Table II.1 Mixed model regression results for grass mineral concentration with standardized beta coefficients of fixed effects. Asterisks denote significant effects ($P < 0.05$), dashes represent tested, non-significant effects that were subsequently removed from the model ($P > 0.05$).

		Dependent Variable (Y)		
Parameters and main effects	Level	Grass Cu	Grass Fe	Grass Zn
Observations		153	153	153
χ^2 [df]		149.60 [10]	29.20 [11]	47.71 [10]
Intercept	Bermuda Grass	5.92	379.64	41.83
Species	Canada Wildrye	-4.22*	-166.29	-15.31
	King Ranch Bluestem	-1.84*	-259.21*	-14.35*
	Little Barley	-1.75*	-63.32	-6.68
	Little Bluestem	-1.46*	-93.06	-23.14*
	Rescuegrass	-0.60	-247.97*	12.96
	Silver Bluestem	-1.87*	-220.30*	-10.11
	Southeastern Wildrye	-4.04*	-142.90	-24.84*
	Squirreltail Wildrye	-2.86*	-224.19	-5.04
	Texas Wintergrass	-3.18*	-199.13*	-22.46*
	Virginia Wildrye	-3.12*	-175.49	-11.07
Soil Mineral		—	58.69*	—
Summer Precip.		—	—	—
Summer Max. Temp.		—	—	—
Summer Precip. * Summer Max. Temp.		—	—	—
Winter Min. Temp.		—	—	—
Random: Site		0.45	6,507.25	17.89

Plant minerals

Although concentrations of Fe in grasses increased (167 – 342 ppm) with available Fe in soils (5 – 75 ppm), available Cu and Zn in soil did not affect the concentrations of those minerals in grasses (Table II.1). Similarly, available Cu, Fe, and Zn in soils did not affect concentrations of those minerals in browse. Grasses were not affected by weather, whereas browse minerals were affected by interactions among environmental conditions as follows: summer maximum temperature and precipitation had a significant negative interaction on browse Cu. Predicted browse Cu was greater in warm, dry summers (14.3 ppm at 34 °C and 72 mm precipitation) than in cool, dry summers (0.75 ppm at 31 °C and 72 mm precipitation) and greater in cool, wet summers (20.9 ppm at 31 °C and 125 mm) than in warm, wet summers (< 0 ppm at 34 °C and 125 mm precipitation; Table II.2). Conversely, an increase in summer precipitation (72 – 125 mm) was associated with an increase in browse Fe from 72 to 114 ppm. Warming winter temperatures (3 – 11°C) were associated with increasing concentrations of Cu (1 – 16 ppm) and Zn (17 – 34 ppm) in browse (Table II.2). Concentrations of Fe in browse were lower and less variable than in grasses, however concentrations of Cu in browse were higher and more variable than in grasses. Concentrations of Zn did not differ among plant species (Fig. II.3).

Table II.2 Mixed model regression results for browse mineral concentration with standardized beta coefficients of fixed effects. Asterisks denote significant effects ($P < 0.05$), dashes represent tested, non-significant effects that were subsequently removed from the model ($P > 0.05$).

		Dependent Variable (Y)		
Parameters and main effects	Level	Browse Cu	Browse Fe	Browse Zn
Observations		64	64	64
χ^2 [df]		87.00 [12]	32.64 [9]	268.38 [9]
Intercept	Ashe Juniper	4.38	127.08	17.58
Species	Blackjack Oak	-0.08	-79.37*	1.70
	Eastern Red Cedar	-3.09	-38.17*	-9.31*
	Honey Mesquite	6.20*	-44.88*	26.16*
	Live Oak	-1.16*	-76.53*	-6.88
	Plateau Live Oak	4.80*	-50.56*	-3.25
	Post Oak	0.09	-61.31*	-3.00
	Vasey Oak	5.59	-45.95	-3.18
	Water Oak	0.05	-9.76	17.43*
Soil Mineral		—	—	—
Summer Precip.		0.07*	10.24*	—
Summer Max. Temp.		0.04*	—	—
Summer Precip. * Summer Max. Temp.		-1.54*	—	—
Winter Min. Temp.		3.43*	—	3.85*
Random: Site		1.12	1.89E-19	7.41E-21

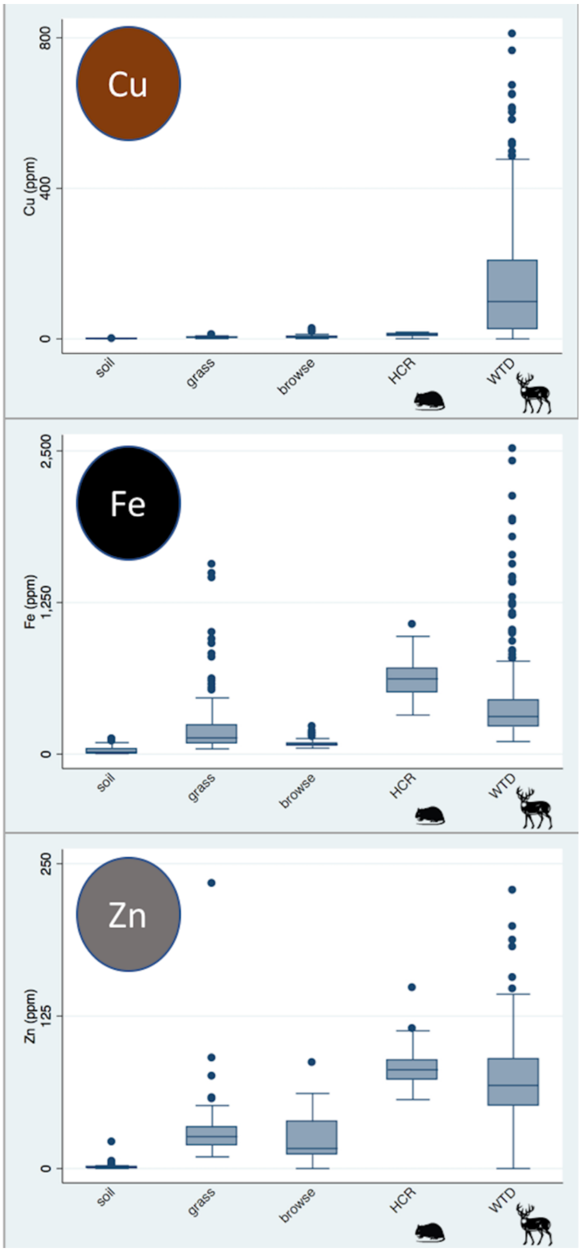


Figure II.3 Box-plot of copper (Cu), iron (Fe), and zinc (Zn) concentrations for soils, grasses, woody browse, hispid cotton rats (HCR) and white-tailed deer (WTD) across Texas grasslands (A-1).

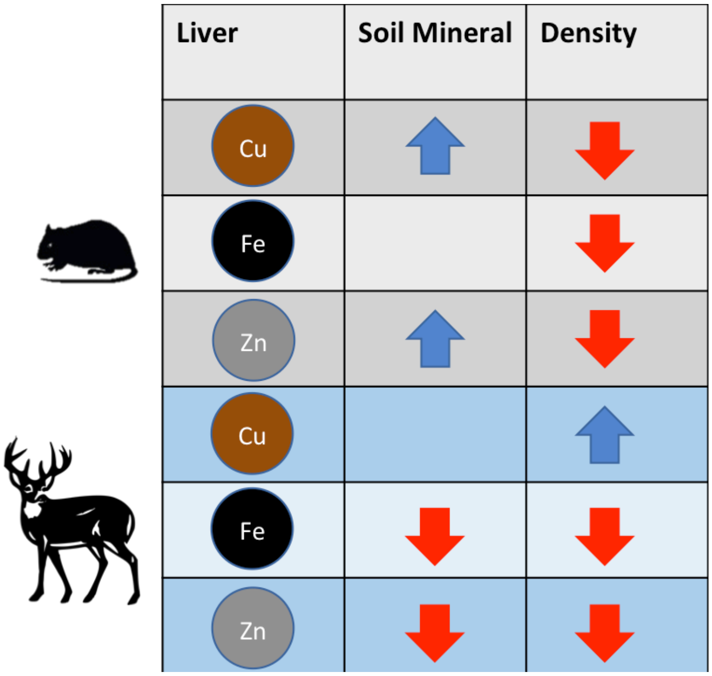
Animal minerals

Similarly, concentrations of Zn did not differ among animal species (Fig. II.3). However, concentrations of Fe in liver were lower in deer than rats, whereas concentrations of Cu in liver were greater in deer than in rats. Liver Cu, Fe, and Zn were more variable among deer than among rats (Fig. II.3).

Soil mineral concentrations were positively associated with rat liver concentrations for Cu and Zn but negatively associated with deer liver concentrations of Fe and Zn (Fig. II.4; Table II.3). Environmental variables affected liver mineral concentrations of both rats and deer. Summer maximum temperature and precipitation had a significant positive interaction on rat liver Fe, deer liver Fe, and deer liver Zn. The interaction caused predicted values of rat Fe, deer Fe, and deer Zn to be greater in cool, dry summers (2153 ppm, 1651 ppm, and 223 ppm, respectively at 31 °C and 72 mm precipitation) than in warm, dry summers (all minerals unavailable at 34 °C and 72 mm precipitation) and greater in warm, wet summers (4127 ppm, 3689 ppm, and 461 ppm, respectively at 34 °C and 125 mm) than in cool, wet summers (all minerals unavailable at 31 °C and 125 mm precipitation; Table II.3). Deer liver Cu also increased from 73 ppm to 448 ppm as summer precipitation increased, but with no interaction with summer temperature. Winter warming from 3 to 11 °C also affected rat liver Fe, deer liver Fe, and deer liver Zn levels, with a negative effect on rat liver concentrations (775 to 294 ppm), but a positive effect on deer liver concentrations (Fe: 76 to 1735 ppm, Zn: 43 to 181 ppm; Table II.3).

Table II.3 Mixed model regression results for hispid cotton rat and white-tailed deer liver mineral concentrations with standardized beta coefficients of fixed effects. Asterisks denote significant effects ($P < 0.05$), dashes represent tested, non-significant effects that were subsequently removed from the model ($P > 0.05$).

		Dependent Variable (Y)					
Parameters and main effects	Level	Rat Cu	Rat Fe	Rat Zn	Deer Cu	Deer Fe	Deer Zn
Observations		73	73	73	274	244	244
χ^2 [df]		53.83 [3]	15.42 [5]	13.82 [2]	30.71 [2]	84.11 [6]	98.88 [7]
Intercept	Female	8.56	521.96	82.50	180.26	478.18	-6.08
Sex	Male	2.27*	—	—	—	—	-14.13*
Density (#/km^2)		-1.87*	-85.95*	-4.83*	47.26*	-521.21*	-25.48*
Soil Mineral		1.15*	—	7.61*	—	-262.72*	-270.44*
Summer Precip.		—	191.24*	—	92.69*	-119.76*	-3.48
Summer Max. Temp.		—	-190.94*	—	—	26.73	-2.89
Summer Precip. * Summer Max. Temp.		—	339.91*	—	—	316.26*	36.71*
Winter Min. Temp.		—	-110.10*	—	—	379.81*	31.54*
Random: Site		3.48E-23	2.68E-14	4.56E-16	1,457.01	1.63E-14	3.13E-17



Liver	Soil Mineral	Density
Cu	↑	↓
Fe		↓
Zn	↑	↓
Cu		↑
Fe	↓	↓
Zn	↓	↓

Figure II.4 Direction of effect (i.e., increase (↑) vs. decrease (↓)) of liver stores of Cu, Fe, and Zn in hispid cotton rats (HCR) and white-tailed deer (WTD), with fixed effects of soil mineral concentrations of Cu, Fe, and Zn and population density of hispid cotton rats (HCR) and white-tailed deer (WTD).

Attributes of populations (i.e., density) and individual (i.e., sex) had an effect on liver minerals in both rats and deer. An increase in population density from 0 to 2,028 rats/km^2 negatively affected Cu (13 to 8 ppm), Fe (707 to 470 ppm), and Zn (91 to 77 ppm) in the liver of rats (Fig. II.4; Table II.3). Similarly, an increase in population density from 0.5 to 27 deer/km^2 negatively affected Fe (1558 to unavailable) and Zn (128 ppm to 30 ppm) in the liver of deer (Fig. II.4; Table II.3). However, population density was positively associated with Cu (40 to 224 ppm) in the liver of deer (Fig. II.4; Table II.3). Mineral stores were affected by sex in both species: females had less Cu than males among rats whereas males had less Zn than females among deer (Table II.3).

Plant isotopes

Grass δC^{13} values (-13.1 ± 0.5 ‰) were higher and less variable than browse δC^{13} values (-27.4 ± 1.0 ‰) across sites; however, grass and browse did not significantly differ in values of δN^{15} (Fig. II.5). Environmental variables affected stable isotope values in both grasses and browse. Summer precipitation and temperature positively affected δC^{13} values in browse, and δN^{15} values in both grasses and browse. The interaction caused predicted values of δC^{13} in browse and δN^{15} values in both grasses and browse to be greater in cool, dry summers (-25.8 ‰, 3.5 ‰, and 6.4 ‰, respectively, at 31 °C and 72 mm precipitation) than in warm, dry summers (-29.6 ‰, -6.1 ‰, -5.5 ‰ at 34 °C and 72 mm precipitation) and greater in warm, wet summers (-23.1 ‰, 6.5 ‰, and 3.6 ‰, respectively, at 34 °C and 125 mm) than in cool, wet summers (-32.2 ‰, -6.9 ‰, and -5.9 ‰ at 31 °C and 125 mm precipitation). Increasing winter temperatures (3 to 11 °C) were also associated with a decrease in browse δC^{13} values from -26.5 to -29.3 ‰.

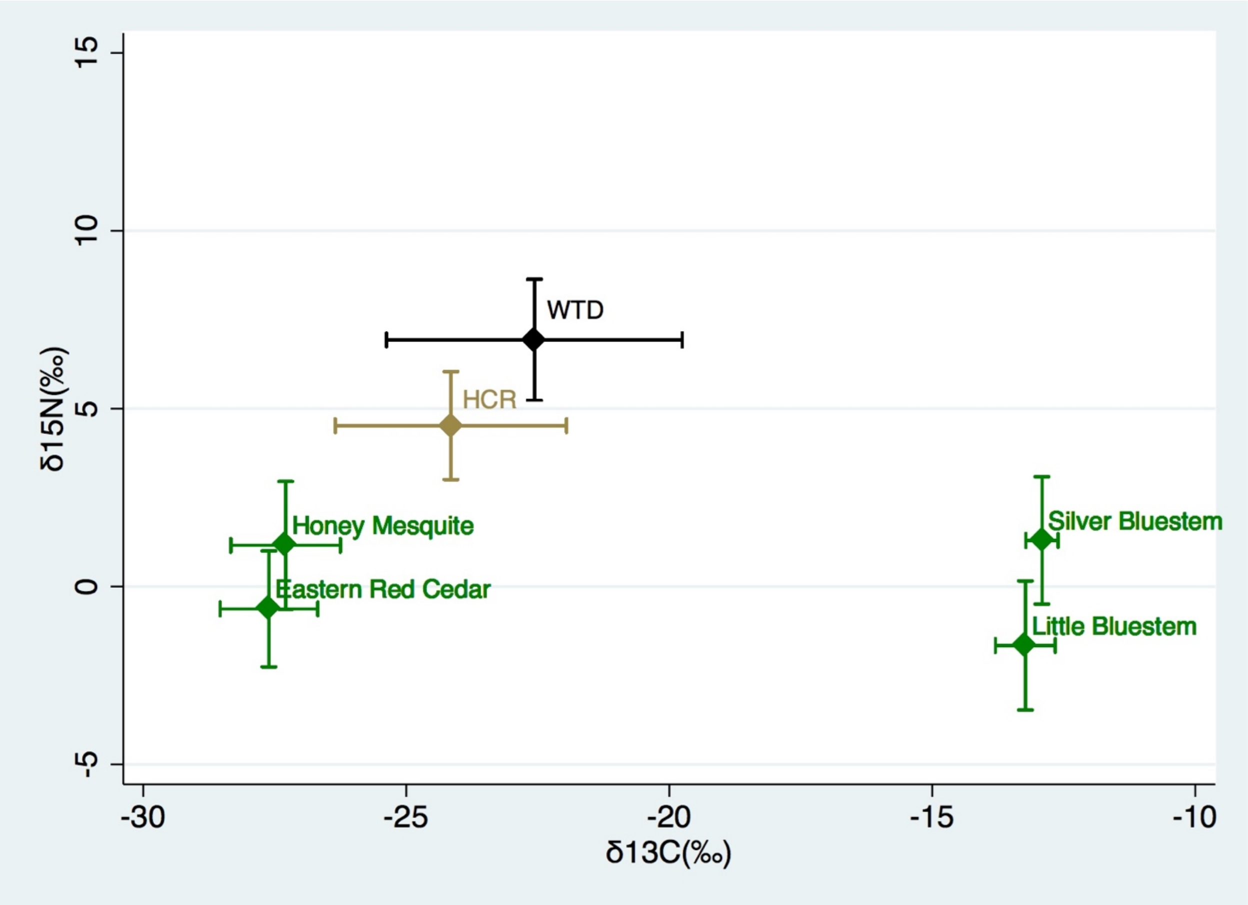


Figure II.5 Values for δN^{15} and δC^{13} in two species of grass [i.e., little bluestem (n = 23) and silver bluestem (n = 23)], two species of woody browse [i.e., eastern red cedar (n = 18) and honey mesquite (n = 20)], and two herbivores [i.e., hispid cotton rats (HCR; n = 73) and white-tailed deer (WTD; n = 283)] across grasslands in Texas (A-1). Symbols are average values with standard deviation bars.

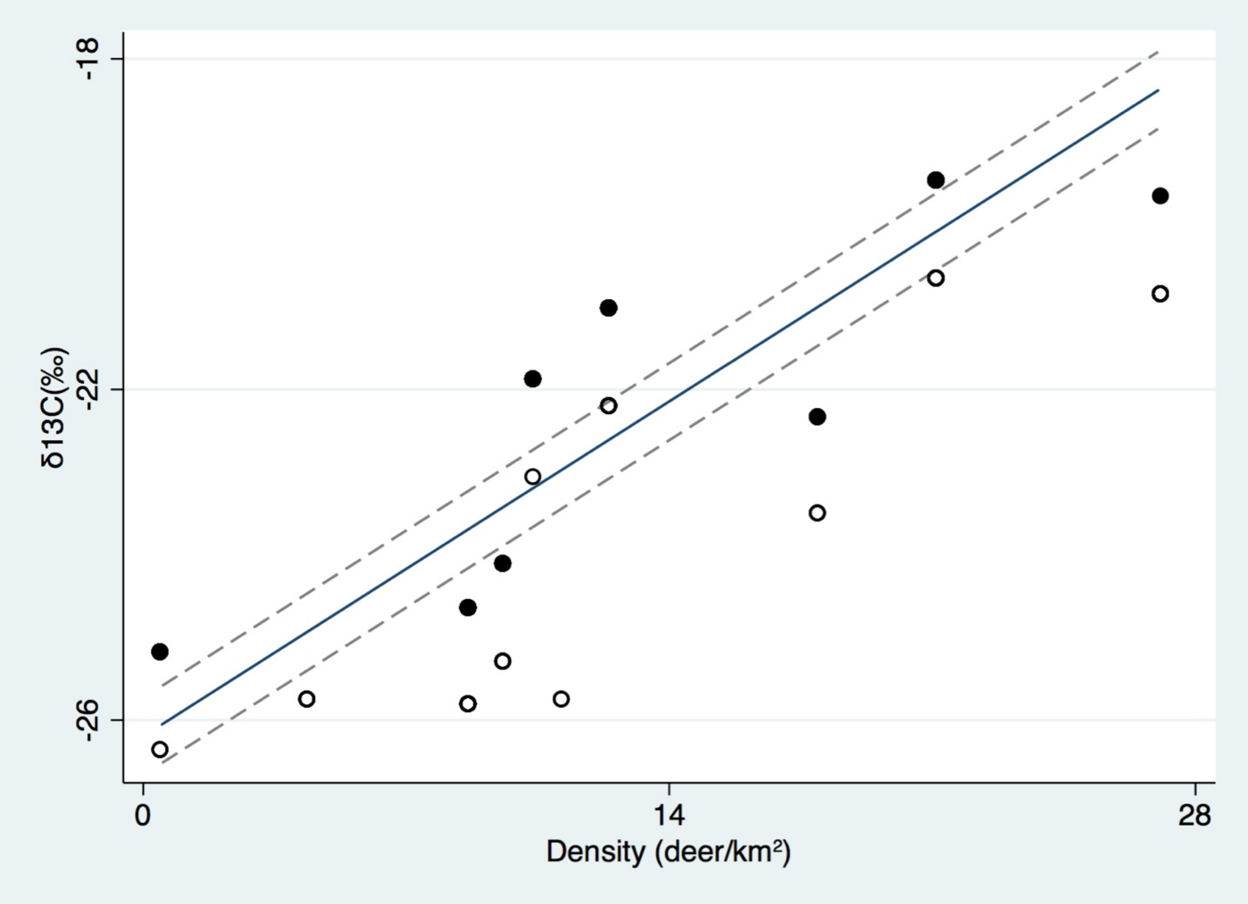


Figure II.6 Relationship between density of deer (#/km^2) and δC^{13} values of heart tissue (± 95 % CI) across 11 grassland sites in Texas (A-3). Symbols are values for males (solid) and females (hollow) at each site predicted by mixed model regression.

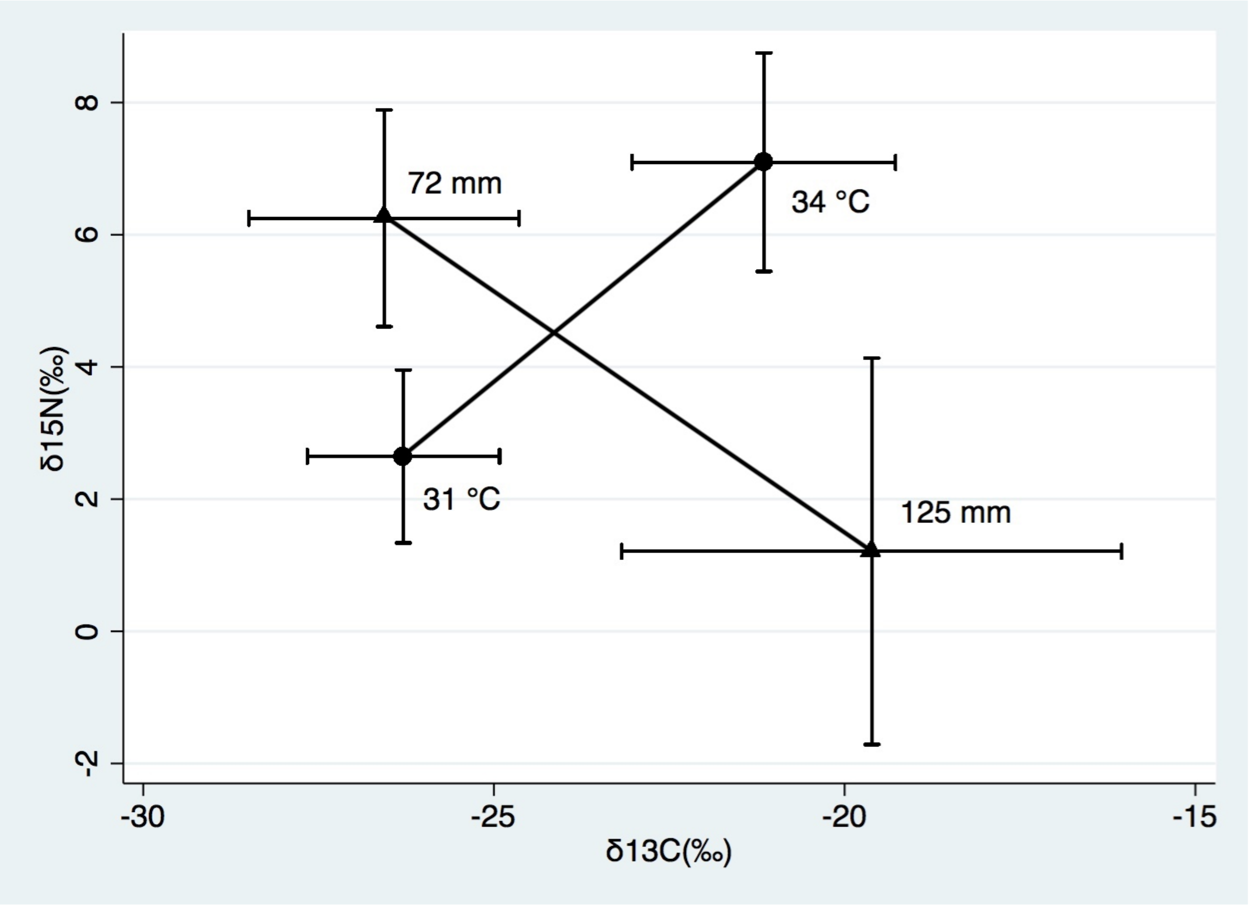


Figure II.7 Marginal effects of summer precipitation (72 to 125 mm) and summer maximum temperature (31 to 33 °C) on values for δN^{15} and δC^{13} in the heart muscle of hispid cotton rats across 15 grassland sites in Texas (A-2). Symbols are margin values from the mixed model regression with 95 % confidence interval bars.

Animal isotopes

Winter conditions similarly caused a decrease in δC^{13} values in deer heart muscle from -21 to -27 ‰, while increases in deer density (0.5 to 27 deer/km^2) caused an increase in deer δC^{13} values from -26 to -18 ‰ (Fig. II.6). Heart isotopes of hispid cotton rats were neither affected by winter conditions nor density, and were instead affected by growing season conditions (Fig. II.7). Values for δN^{15} (4.5 ± 1.5 ‰) and δC^{13} (-24.2 ± 2.2 ‰) of rats were lower and less variable than those of deer δN^{15} (6.9 ± 1.7 ‰) and δC^{13} values (-22.6 ± 2.8 ‰; Fig. II.5). Isotopic values were not affected by sex in rats, but male deer were more enriched in both δN^{15} and δC^{13} than female deer.

Discussion

We provide evidence that nutrient transfers contribute to density dependence of herbivore populations because population density affected stores of Cu, Fe, and Zn in hispid cotton rats and white-tailed deer. Body stores of those trace minerals were set by the context of soil availability and weather. Local population density may therefore influence the vulnerability of individuals to deficiencies of Cu, Fe, and Zn and increase their risk of impaired immunity or reproduction.

Our estimates of population density for rats and deer were similar to those in previous studies. Similarly, our capture efficiencies of rodents (0 to 16 % capture success) followed consistent trends of capture efficiencies seen in rodent populations in Texas (0 to 30 % capture success) (Rodriguez et al. 2015). Our estimates for white-tailed deer densities were likewise comparable to statewide annual surveys conducted by TPWD on white-tailed deer populations from 2005 to 2014 (Cain 2015) (A-3).

Liver mineral concentrations in both rats and deer followed the main food source of each species: grasses for rats and browse for deer (Fig. II.3). Requirements of trace minerals for hispid cotton rats and white-tailed deer have not been estimated. However, laboratory rats (*Rattus norvegicus*) and domestic sheep and goats (*Ovis aries* and *Capra aegagrus hircus*) may be used as the best available proxies for assessing liver mineral stores of hispid cotton rats and white-tailed deer, respectively (National Research Council 1995, 2007). Liver mineral levels were below deficiency thresholds of the domestic proxies for only 11 % (n = 8 of 73) of hispid cotton rats at 13% of sites and 23 % (n = 69 of 305) of white-tailed deer at 58% of sites (A-5). The site with the highest rodent population densities (i.e., TPWD Cooper WMA), had the largest number of rats with Cu concentrations in the liver below 5 µg/g dry weight (n = 7 of 8). Hispid cotton rats were not apparently limited by Fe or Zn for any of the locations because liver concentrations exceeded 35 µg Fe/g and 12 µg Zn/g dry weight (National Research Council 1995). Similarly, Fe was not apparently limiting for white-tailed deer at any location because liver concentrations exceeded 30 µg/g dry weight across all sites. However, concentrations of Zn may have been limiting for white-tailed deer because liver concentrations of five individuals were below the threshold of 20 µg/g dry weight (National Research Council 2007) on 17 % of sites (n = 2 of 12). Like hispid cotton rats, white-tailed deer were apparently limited by Cu; 21 % (n = 65 of 305) of both male and female white-tailed deer across seven of 12 sites had liver concentrations below the hepatic threshold of 20 µg/g dry weight (National Research Council 2007).

In the United States, Cu is the most commonly limiting trace mineral in deer and small domestic ruminants. Copper deficiency can eventually lead to clinical signs such as enzootic ataxia, uneven bone growth, weakness, and bone fractures due to osteochondrosis (Puls 1994, Wilson and Grace 2002, National Research Council 2007). Limitations of iron and zinc are rare in deer and small domestic ruminants, but excesses of Fe and Zn, especially in relation to Cu, are more common (National Research Council 2005). We did not observe any hispid cotton rats with elevated liver concentrations above toxic thresholds: 1,000 µg Cu/g; 5,000 µg Fe/g; 5,000 µg Zn/g, dry weight. However, 19 % (n = 59 of 305) of white-tailed deer, including both females and males, had liver Fe concentrations above thresholds of 500 µg/g dry weight, while no white-tailed deer contained liver concentrations over thresholds for Cu (1,000 µg/g) or Zn (300 µg/g), dry weight. Sequestration of Cu, Fe, and Zn in the liver can offset temporal and spatial variation in dietary supplies. The ability to sequester each trace mineral in the liver depends on the ability to prevent toxic consequences by incorporating the minerals into storage proteins (i.e., ceruloplasmin for Cu, metallothionein for Zn, and hemosiderin for Fe) (Cousins 1985, Reilly et al. 1998, Sheppard and Dierenfeld 2002, Bartoskewitz et al. 2007). In this study, white-tailed deer stored Cu at concentrations above 200 µg/g dry weight in liver tissue, which is significantly higher than reports of other cervids including red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus*) (Reid et al. 1980, Aastrup et al. 2000, Vikøren et al. 2005). Copper toxicity can result in hemolytic crises and sudden death (Fraser 1991). Consequently, toxicity may be more easily detected than deficiencies of trace minerals that manifest as decreased growth or reproduction (i.e., prolonged morbidity) before clinical signs are observed (Hurley and Doane 1989, Flueck 1994, National Research Council 2007). Differences in the uptake, storage, and use of trace minerals also make it unlikely for deficiencies to occur synchronously, even when animals are starving (Franzmann 1985). Furthermore, differences in chemical and structural properties of soils often cause asynchronous variation in the availability of minerals (Beeson and Matrone 1976, Kabata-Pendias 2010).

Although mineral availability in soils can indicate productivity for agricultural production of corn, alfalfa, and wheat, those mineral levels do not indicate productivity of wild plants and animals across landscapes (Kilmer 1979, Reid and Horvath 1980). Available concentrations of

Cu and Zn in soil were positively associated with liver stores of hispid cotton rats (Fig. II.4). Small, herbivorous, income breeders, such as hispid cotton rats may better indicate trophic transfers of minerals than larger, capital breeders, such as white-tailed deer because populations of hispid cotton rats turnover rapidly (Schmidly and Bradley 2016). High reproductive rates and short lives suggest a greater reliance on direct allocation of dietary nutrients to reproduction than in larger, long-lived species such as white-tailed deer. Hispid cotton rats also use smaller areas than larger mammals such as white-tailed deer; that is, they better indicate local feedbacks from soils and plants (Slade and Swihart 1983, Sargent and Labisky 1995, Nelson 2015). Furthermore, high fecundity in small home ranges intensifies density dependent effects such as competition for food (Cameron and Spencer 1985). Trace nutrient supplies may therefore exacerbate population cycles of rats especially where soil mineral availabilities are low.

White-tailed deer use larger foraging areas than hispid cotton rats and expand or shift their foraging areas when food is limited (Fleharty and Mares 1973, Sargent and Labisky 1995, Tekiela 2009, Webb et al. 2010). Deer therefore incorporate landscape availability of nutrients over a longer time period and a larger area than hispid cotton rats. Furthermore, storage of minerals in the liver attenuates the signals from lower trophic levels over a broad temporal and spatial scale. Consequently, variations in Cu, Fe, and Zn concentrations among deer were much greater than those among rats (Fig. II.3). Sex differences in foraging were also evident in white-tailed deer, but not in rodents. Male capital breeders reduce foraging time during short breeding windows (Mysterud et al. 2008), which can alter diet and movement (McCoy et al. 2005, Aiken et al. 2014, Foley et al. 2015). Heart tissues of male white-tailed deer had greater values of δC^{13} than those of females collected at the same locations (Fig. II.6). The isotopic difference between males and females probably reflects greater temporal variation in diet of males than females.

Population density also affected nutrient supplies of white-tailed deer. As density increased, deer shifted toward a diet more enriched in δC^{13} and with greater variation in δC^{13} (Fig. II.6). Grasses and other species that utilize the C4 or CAM photosynthetic pathway, contain more enriched values of δC^{13} in plant tissue. Conversely, C3 plants, including woody trees and many shrub species, contain plant tissue with depleted values of δC^{13} (Marshall et al. 2007). It is likely that deer included grasses in their diet as more preferred browse and forbs became less available as population density increased (Ben-David and Flaherty 2012). Increasing densities have been associated with a greater proportion of grasses in the diets of white-tailed deer in the Southern Plains ecoregion of Texas (DeYoung et al. 2019). Increasing density was associated with declines in liver Fe and Zn of deer but an increase in liver Cu concentration (Fig. II.4; Table II.3). Grasses were higher in Fe and lower in Cu than browse, that is, a shift to grasses would increase supplies of Fe and decrease supplies of Cu, a pattern opposite to those observed in the liver. Hepatic stores of deer could reflect the supply relative to the current store. Liver Cu stores were inadequate in some sites and thus greater storage may have been induced as dietary supplies decreased further with increasing density. Conversely, liver Fe stores were adequate across sites and declines in liver Fe stores may simply reflect a decline in intake as density increased.

Longer-lived species, such as white-tailed deer and woody browse, have the ability to incorporate effects of weather over several years, whereas shorter-lived species, such as hispid cotton rats and grasses are more subject to short-term weather events within a season or year. Weather trends did not have an effect on grass concentrations of trace minerals; however, browse minerals were affected by weather, most likely due to deeper and more extensive root systems than many grass species, allowing growth and incorporation of nutrients over several seasons to many years (Brown and Archer 1990, Mund et al. 2010). Grass and browse concentrations of trace minerals were also largely unaffected by soil concentrations, indicating an active uptake of trace minerals by most plants (Graham and Stangoulis 2003). Although grass and browse mineral concentrations were largely driven by species, taxa within the same Family varied widely in mineral content, which infers strong effects of life history and growing conditions on mineral storage. Juknevičius and Sabienė (2007) had similar results for agricultural plants, indicating that plant species and Family were largely associated with plant mineral content. Oster et al. (2018) demonstrated that even the same species of plant contained different concentrations of minerals over the course of the growing season, depending on location. This demonstrates the complexity of trophic transfer of nutrients beginning at the soil-plant interface within the rhizosphere, which is further complicated by differences among species and the functional responses of the animal to plant growth.

We hypothesized that trace mineral stores of rodents and deer would differ in their responses to environmental and population traits because animal responses are driven by life-history traits, such as reproduction, foraging strategies, and lifespan. We demonstrated that Cu, Fe, and Zn exhibit differences in drivers across each trophic level for rats and deer with a strong effect of population density. Trophic models that focus on energy (C) and protein (N) as drivers downplay the importance of macro and microminerals within ecological systems (Doak et al. 2008). We have demonstrated the importance of trace minerals on density dependence in little and large herbivore populations, which are ultimately driven by landscape processes, including soils, plants, and weather. Trace nutrient assessments may be a valuable addition to routine censuses because liver stores of Cu, Fe, and Zn may signal changes in population phase of rodents and vulnerability to disease within populations of white-tailed deer.

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CHAPTER III

CONCLUSIONS

My study identified several drivers of trace nutrient stores in animal populations over different landscapes. Although past studies have investigated energy, protein, and macronutrients as drivers of animal populations, none have demonstrated the role trace nutrients play in density dependent processes of wildlife populations.

Many wildlife diseases rely on density dependent transmission, where the rate of contact between susceptible and infected individuals depends on population density (Wobeser 2013). This causes an increase in the rate of disease transmission with increasing population densities. Bovine Tuberculosis, Epizootic Hemorrhagic Disease, and Bluetongue Virus are a few of the many diseases that are transmitted via density dependence in large wild herbivore populations (Menzies and Neill 2000, McCallum et al. 2001, Busch et al. 2014). Many of the diseases that affect wild herbivores also affect domestic livestock. Outbreaks of disease are more likely to occur in both wildlife populations and domestic livestock where there is large overlap in land use between domestic and wildlife species and where connectivity of animals is high. This is common in areas centered on beef production, such as the state of Texas, with abundant domestic livestock, exotic large ungulates, and sympatric populations of native wild herbivores (Traweek and Welch 1992, Cain 2015, Texas Department of Agriculture 2019).

In this study, I investigated the effects of intraspecific competition on mineral stores of native wild herbivores in Texas. However, with the large number of exotic ungulates and domestic livestock that coexist in areas of high population density of white-tailed deer (i.e., the Edwards Plateau ecoregion), it is possible that I underestimated the effect of competition on mineral stores of white-tailed deer in these areas. Axis deer (*Axis axis*), as well as domestic goats, overlap in diet with white-tailed deer (Bryant et al. 1979, Butts 1982, Hofmann 1985) and likely contributed to the effects of density that I observed on four of twelve sites. Therefore, white-tailed deer density may underestimate the competition for forage on sites where all three species coexist. Similarly, populations of other species of granivores may have increased competition for food and affected the mineral stores of hispid cotton rats in this study. Several species of rodents consume many of the same forages as hispid cotton rats (Schmidly and Bradley 2016). An alternative metric to account for interspecific competition would be to expand density to include all animals that overlap in diet with both hispid cotton rats and white-tailed deer, separately. However, to accurately account for the effect of competition of each additional species, I would need to establish the degree of overlap in diet among the herbivore community. I would then provide a weighted effect for each additional species on mineral content of liver stores in white-tailed deer and hispid cotton rats, separately. Increasing population densities may also contribute to competition for other resources, such as refugia from heat and predation, which would increase contact between individuals and the likelihood of disease transmission.

Liver Cu stores of wild herbivores may be a valuable indicator to track populations of animals that are at risk of disease, because Cu was limiting in both white-tailed deer and hispid cotton rats. I suggest monitoring Cu concentrations in tandem with population density and disease screening to identify nutrient limited, and therefore vulnerable, wildlife populations. For white-tailed deer populations, I propose that in accordance with sampling strategies already in place for Chronic Wasting Disease testing across Texas, to request a small liver sample to be taken for mineral analysis. Population density of white-tailed deer is assessed annually across most of Texas (Cain 2015), which would allow state biologists to identify populations of animals at risk of future disease transmission, with little additional effort. State wildlife biologists could then work with land managers to alter and minimize risk of disease outbreak by reducing population densities in those areas where liver Cu stores are low and high densities predispose the population to competition for resources and communication of disease.

This proactive approach to white-tailed deer population management has the potential to promote healthier, more resilient populations of wildlife, as well as economic stability in areas that depend on hunting as a source of revenue. Animals in good nutritional condition have a higher probability of successful breeding as well as a lower vulnerability to external stressors, such as severe winter weather (Bowyer et al. 2014). White-tailed deer rely on a combination of nutrition and genetics for antler and overall body growth each year (Foley et al. 2012). Deer populations with few nutrient limitations have the potential for growing larger antlers as well as larger body sizes, with increased muscle mass and fat stores. Multiple rural communities in Texas depend on the revenue made during hunting season to sustain their economy through the rest of the year. In 2006, all fish and wildlife related recreation brought $8.9 million for the Texas economy, while hunting alone was a $2.6 million industry (Allen and Southwick 2006). Disease outbreaks could have devastating economic effects for small businesses and real estate values in the area of the outbreak.

Understanding the population dynamics and disease prevalence of white-tailed deer and hispid cotton rats, along with trace nutrient limitations of the populations are important for wildlife, domestic animals, and humans. White-tailed deer serve as hosts of several diseases (e.g., Epizootic Hemorrhagic Disease, Texas Cattle Fever) that also affect domestic animals (Herms and James 1961, Graham and Hourrigan 1977, Gibbs and Greiner 1989), while hispid cotton rats are known to be hosts for several zoonotic diseases, including Chagas and Hantavirus

(Rollin et al. 1995, Kramm III et al. 2017) that affect human populations. Trace nutrients can provide the link between density dependence, population cycles, and disease prevalence in wildlife, with implications for livestock and human health, especially across grasslands where a large proportion of the human population, domestic livestock, and wildlife coexist.

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APPENDIX 1

Grassland study site locations [i.e., latitude (Lat.) and longitude (Long.)] from West to East across the Edwards Plateau (EP), Gulf Prairies and Marshes (GP), Blackland Prairies (BP), and Post Oak Savannah (PO) Ecoregions (Eco.) in Texas. Sites consisted of Texas Parks and Wildlife Department (TPWD) Wildlife Management Areas (WMA), TPWD State Parks (SP), Texas EcoLab (TX Eco) Private Properties, one private property (Texana Springs Ranch), the Rob and Bessie Welder Wildlife Foundation, and the Texas A&M University (TAMU) AgriLife Research Station and Ranches. Values are means ± standard deviation for precipitation (Precip.) and temperature (Temp.).

Site #	Site	Lat.	Long.	Eco.	2017 Monthly Precip. (mm)	2017 Average Temp. (℃)	30-Year Monthly Precip. (mm)	30-Year Average Temp. (℃)
1	TAMU AgriLife Read Ranch	30.5472	-101.0497	EP	52 ± 34	19 ± 7	43 ± 17	18 ± 8
2	TAMU AgriLife Sonora Research Station	30.2670	-100.5655	EP	44 ± 31	19 ± 6	48 ± 17	18 ± 7
3	Kickapoo Cavern SP	29.6100	-100.4525	EP	50 ± 33	20 ± 6	50 ± 20	20 ± 7
4	TAMU AgriLife Martin Ranch	30.8050	-99.8484	EP	47 ± 33	19 ± 6	55 ± 17	18 ± 7
5	South Llano River SP	30.4454	-99.8041	EP	43 ± 24	19 ± 7	50 ± 18	18 ± 7
6	Texana Springs Ranch	30.0672	-99.4347	EP	52 ± 31	19 ± 6	63 ± 21	18 ± 7
7	Guadalupe River SP	29.8739	-98.4863	EP	60 ± 39	21 ± 6	73 ± 21	20 ± 7
8	TX Eco Hays Co.	30.2862	-98.1082	EP	68 ± 50	20 ± 6	70 ± 21	19 ± 7
9	Welder Wildlife Foundation	28.1213	-97.4420	GP	76 ± 63	23 ± 5	74 ± 29	22 ± 6
10	TPWD Granger WMA	30.7219	-97.3214	BP	80 ± 59	21 ± 6	73 ± 23	20 ± 7
11	Bastrop SP	30.1102	-97.2872	PO	92 ± 126	21 ± 6	76 ± 22	20 ± 7
12	TX Eco Fayette Co.	29.9357	-96.8729	PO	108 ± 178	21 ± 6	85 ± 21	21 ± 7
13	TX Eco Navarro Co.	31.9485	-96.2396	BP	95 ± 44	20 ± 7	87 ± 21	19 ± 8
14	TX Eco Brazos Co.	30.5551	-96.2041	PO	115 ± 152	21 ± 6	89 ± 20	20 ± 7
15	TPWD Richland Creek WMA	31.9369	-96.1033	BP	96 ± 42	20 ± 6	89 ± 21	19 ± 7
16	TPWD Gus Engeling WMA	31.9071	-95.9029	PO	89 ± 36	20 ± 6	91 ± 21	19 ± 7
17	TPWD Pat Mayse WMA	33.8120	-95.6772	PO	103 ± 79	18 ± 7	99 ± 23	17 ± 8
18	TPWD Cooper WMA	33.3184	-95.6035	BP	105 ± 65	19 ± 7	96 ± 23	18 ± 8
19	TX Eco Henderson Co.	32.2652	-95.5848	PO	94 ± 50	20 ± 6	92 ± 22	19 ± 7

APPENDIX 2

Hispid cotton rat (HCR) abundance and density estimates ($\bar{X}$) across sites from west to east. Capture efficiency (Cap. Eff.) is expressed as a percentage of number of hispid cotton rats caught out of available Estimated Trap-Nights (ETN) per site. Site abbreviations are defined in A-1.

Site #	Site	Property Area (km^2)	Area Surveyed (km^2)	# HCR Caught	ETN	Density (HCR/km^2)	Cap. Eff. (%)	Juvenile / Adult
1	TAMU AgriLife Read Ranch	21.63	0.036	0	492.5	0	0	0
2	TAMU AgriLife Sonora Research Station	14.01	0.036	0	501.5	0	0	0
4	TAMU AgriLife Martin Ranch	20.25	0.036	3	489	83	1	0
6	Texana Springs Ranch	7.98	0.036	0	497.5	0	0	0
8	TX Eco Hays Co.	0.42	0.036	0	484	0	0	0
9	Welder Wildlife Foundation	31.57	0.036	4	488	111	1	0.3
10	TPWD Granger WMA	27.62	0.024	16	326.5	667	5	1.0
12	TX Eco Fayette Co.	0.42	0.036	0	479.5	0	0	0
13	TX Eco Navarro Co.	0.25	0.024	4	328.5	167	1	3.0
14	TX Eco Brazos Co.	0.43	0.036	0	502.5	0	0	0
15	TPWD Richland Creek WMA	57.76	0.036	45	470.5	1250	10	0.9
16	TPWD Gus Engeling WMA	44.35	0.036	15	496	417	3	0.1
17	TPWD Pat Mayse WMA	36.12	0.036	2	502.5	56	0	0
18	TPWD Cooper WMA	57.30	0.036	73	467.5	2028	16	0.4
19	TX Eco Henderson Co.	0.40	0.036	0	500.5	0	0	0

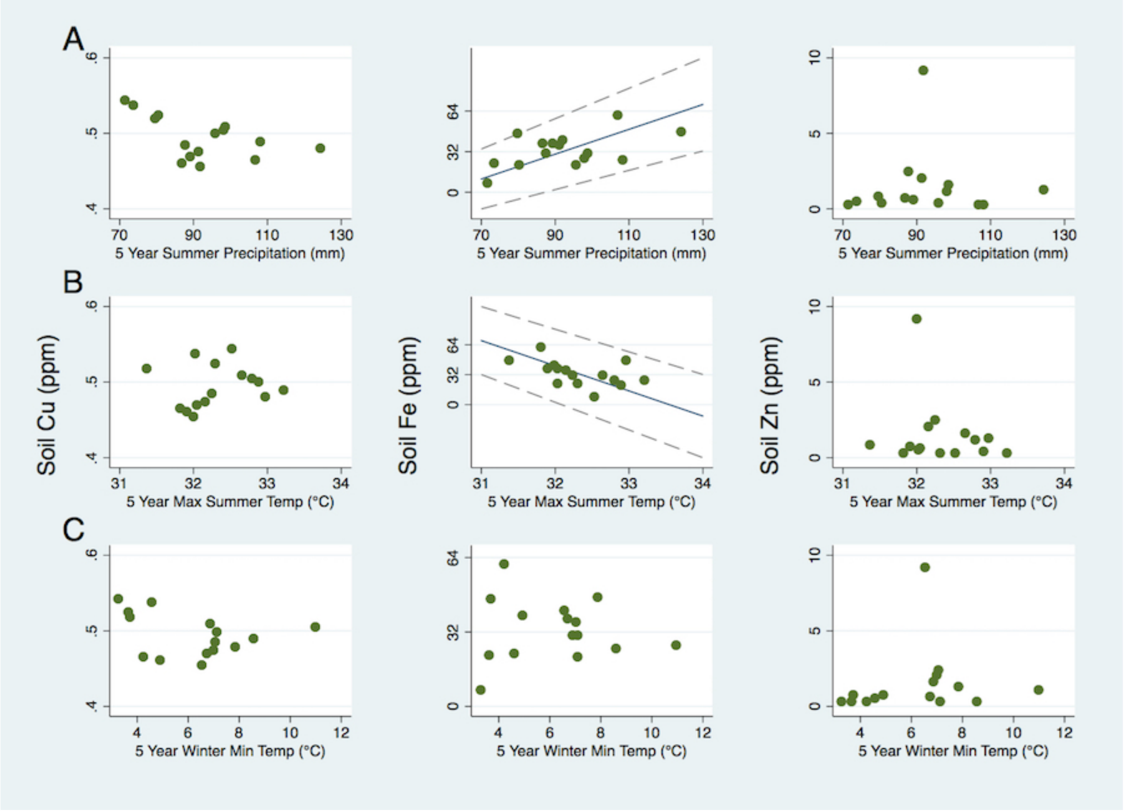
APPENDIX 3

Conventional distance sampling detection functions and white-tailed deer (WTD) estimated abundance ($\bar{X}$ ± SE) and densities across Texas grassland sites. Pooling indicates site observations (i.e., total number of detections at a site) pooled by ecoregion in order to create an accurate detection function for sites with few observations (< 35). Key function refers to the general shape, and adjustments refer to corrections to that shape, used to create the detection function that best fit the data distribution observed at each site, selected by lowest AIC value. Bin refers to the interval at which the data were grouped, post-survey, for fitting a detection function for each site. Site and ecoregion abbreviations are defined in A-1.

Site #	Site	Property Area (km^2)	Pool	Key Function	Adjustments	Bin	Observations	Abundance (# of WTD)	Density (WTD/km^2)
1	TAMU AgriLife Read Ranch	21.63	Null	Half-normal	Null	50 m	68	269 ± 59	12
2	TAMU AgriLife Sonora Research Station	14.01	Null	Half-normal	Null	50 m	108	296 ± 63	21
3	TPWD Kickapoo Cavern SP	25.78	Null	Half-normal	Cosine	50 m	59	248 ± 71	10
4	TAMU AgriLife Martin Ranch	20.25	EP	Half-normal	Null	50 m	31	166 ± 35	8
7	TPWD Guadalupe River SP	16.86	Null	Uniform	Cosine	20 m	61	303 ± 138	18
9	Welder Wildlife Foundation	31.57	Null	Hazard-rate	Null	Null	33	639 ± 241	20
10	TPWD Granger WMA	27.62	Null	Hazard-rate	Null	50 m	131	748 ± 117	27
11	TPWD Bastrop SP	26.62	PO	Half-normal	Cosine	50 m	33	297 ± 119	11
16	TPWD Gus Engeling WMA	44.35	PO	Half-normal	Cosine	50 m	24	386 ± 89	9
17	TPWD Pat Mayse WMA	36.12	PO	Half-normal	Cosine	50 m	3	18 ± 9	0.5
18	TPWD Cooper WMA	57.30	BP	Uniform	Cosine	Null	17	251 ± 79	4

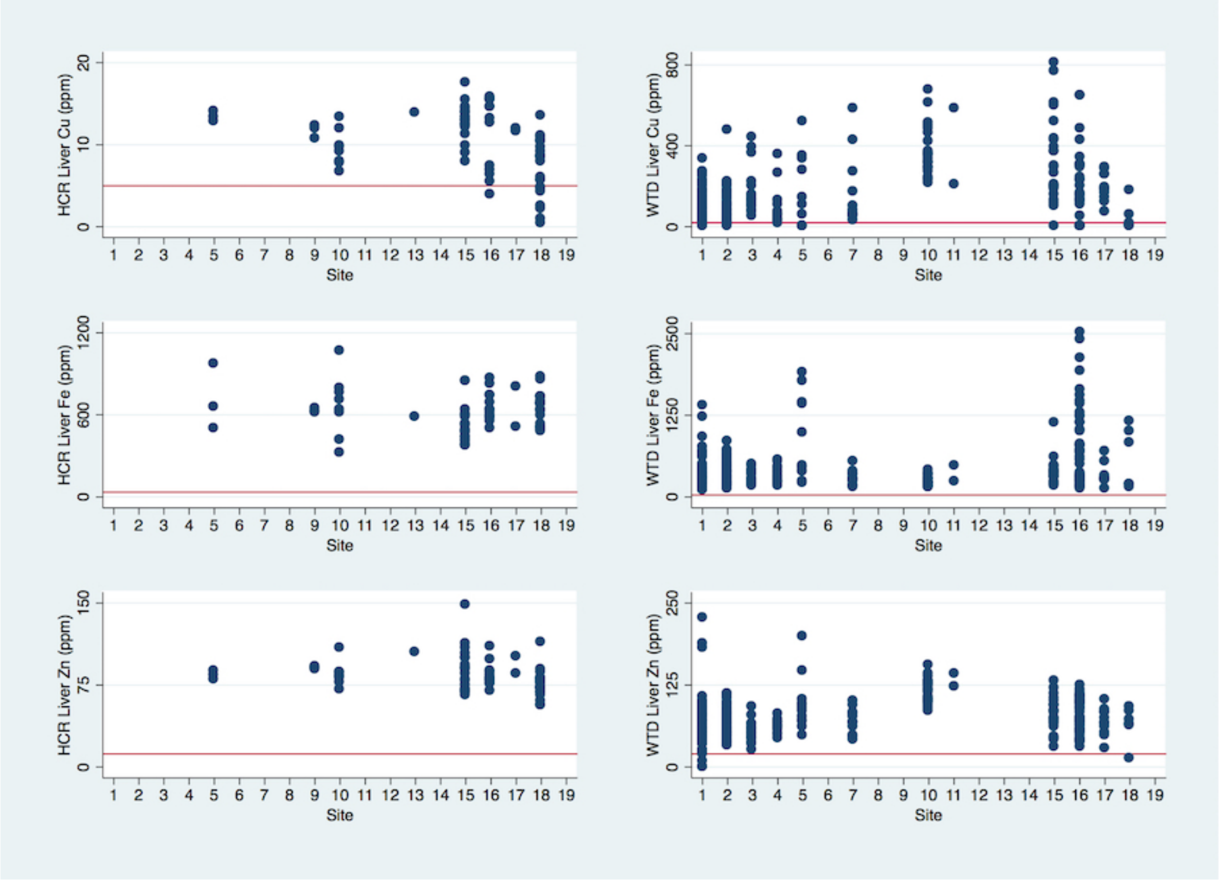
APPENDIX 4

Relationships between concentrations of available Cu, Fe, and Zn in soil and summer precipitation (A), summer maximum temperature (B) and winter minimum temperature (C) across Texas grasslands (A-1). Symbols are predicted values from mixed model regression for each site. Lines indicate the marginal effect with 95 % CI.



APPENDIX 5

Hispid cotton rat (HCR) and white-tailed deer (WTD) liver concentrations of copper (Cu), iron (Fe), and zinc (Zn) across Texas grassland study sites from West to East. Site information is defined in A-1. The red line indicates maintenance threshold of Cu, Fe, and Zn concentrations for hispid cotton rats and white-tailed deer. Points are raw data across sites.



APPENDIX 6

Title: Tracing the nutrients on the landscape for wildlife health

Kaylee Hollingsworth, Rachel Shively, Selma Glasscock, Jessica Light, Doug Tolleson, and Perry S. Barboza

Sickness during the hunting season can be a hunter's worst nightmare. During cold and flu season, people will often increase vitamin and mineral supplements in order to boost their immune systems. We know that vitamins and minerals are an important part of a well-balanced diet, and they play a role in fighting infections. However, unlike humans that have the ability to take a pill to obtain these additional nutrients, wild herbivores, such as white-tailed deer, get all their nutrients from the plants they eat. So, if deer can't eat the nutrients they need to maintain a fully functioning immune system, chances are, they are more likely to get sick. So, it's not just the hunter's health that matters, but also the deer's health.

Plant buffet

Although deer have a diverse diet, the available food varies widely based on where that deer is located. It's like being at a buffet, that in some areas has great quality food at low quantities, and in other areas has poor quality food at high quantities, and vice versa. The quality and quantity of the buffet depends on many factors, including how rich the soil is in nutrients, how hot and cold it gets, how much rain there is during the summer and winter, and even how many other deer share that buffet.

Buffets across Texas

Texas is a diverse state, with 10 distinct ecoregions that each have different soils, weather, plants, and animals. The Texas population of over 4 million white-tailed deer are found in every one of these ecoregions. So, white-tailed deer encounter and eat different plants all over the state from the dry Trans-Pecos all the way to the moist Pineywoods. That leads us to the question, are some areas better for deer? How do we determine a good quality location from a poor-quality location?

Why can’t we just measure the soils?

Wouldn’t it be convenient if we could measure nutrients in the soils and be able to predict how the plants and wildlife in that area were doing? However, it’s not that simple. Firstly, most plants do not passively uptake minerals. It is an active process, which involves soil pH and moisture, plant roots and microbes. Secondly, white-tailed deer are large bodied, long-lived animals that build up nutrients in their bodies from the plants they eat over the growing season. They also likely carry over nutrient stores from the previous fall and winter, or even the previous growing season.

We studied what was driving these nutrient stores in white-tailed deer across Texas; specifically copper, iron, and zinc, that are stored in the liver. We investigated if soils, weather, diet, sex, or population density of deer were affecting white-tailed deer liver stores. We also checked for mineral deficiencies in deer across sites, in order to identify locations where deer might be more at risk of a disease outbreak.

Soils, weather, and density

We found that nutrient stores in white-tailed deer were driven by a combination of soils and their interactions with weather during the growing season. Simply put, soil nutrients do affect what is getting into deer, but that depends on the weather. We also found that population density affected copper, iron, and zinc stores in deer. With increased deer densities, there was increased competition for food. With increased densities, deer shifted their diets away from preferred plants (woody plants) toward less preferred plants (grasses). Additionally, copper was the only mineral that fell to levels that would have been considered deficient for livestock. Of the total deer sampled, one-fifth had copper levels below the threshold for deficiency; that is, deer on over half of our sites were at risk of copper deficiency. A high risk of nutrient deficiency in a population at high density is the "perfect" condition for a disease outbreak. So, how do we prevent this situation from occurring?

What makes a good property for wildlife?

Since mineral stores are partially driven by soils through their interactions with weather, well managed soils are essential for healthy plants and healthy deer. The weather cannot be controlled; however, soil management may be beneficial. We do not know how the use of fire, grazing, and restoration of native forages affect the trace nutrient stores and the health of deer populations. Additionally, regular monitoring of mineral stores in deer populations would be useful in order to identify nutrient limited, and therefore vulnerable segments of the population. Land managers and wildlife biologists would be able to alter harvest to decrease population densities where liver copper stores are low and high densities predispose the population to competition for resources and transfer of disease. This proactive approach to white-tailed deer population management has the potential to promote healthier, more resilient populations of white-tailed deer that also have the potential for growing larger body sizes and larger antlers.