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HERBIVORY, NOT SOIL NITROGEN, ALTERS GRASSLAND PLANT COMMUNITY STRUCTURE AND DECOMPOSITION

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HERBIVORY, NOT SOIL NITROGEN, ALTERS GRASSLAND PLANT COMMUNITY STRUCTURE AND DECOMPOSITION

A THESIS APPROVED FOR THE
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Table of Contents

List of Tables

List of Figures

Abstract

Human activity has shifted grazing and nitrogen (N) altering plant community structure worldwide. Plant community changes are expected to alter associated ecosystem functions (e.g., plant litter decomposition). Few studies address the concurrent effects of grazing and nitrogen inputs on community structure and ecosystem function. To better understand how herbivory and soil N availability can alter grassland structure and function, we first manipulated soil N (soil N addition vs. control) and invertebrate herbivory (present vs. reduced) within an established small mammal herbivore manipulation (small mammal present vs. reduced). We measured plant community structure (richness, evenness, diversity and composition) and productivity. Secondly, we collected plant litter and soil samples from grazing and soil N manipulated plots. We created microcosms similar to field plots by creating soil and litter laboratory incubations. From the microcosms, we measured soil CO_2 evolution as a proxy for plant litter decomposition. We found that small mammal and invertebrate herbivores, rather than soil nitrogen, altered grassland community structure and function. The presence of small mammal herbivores promoted plant species richness and diversity while decreasing productivity and altering compositional similarity. Invertebrate herbivores promoted plant dominance by reducing plant evenness without altering compositional similarity. Additionally, small mammals mediated the impacts of invertebrate herbivores such that invertebrates lowered plant diversity when small mammals were abundant, while promoting plant diversity when small mammals were reduced. Also, small mammal herbivory shifted species composition by promoting C_3 relative to C_4 plant species abundance. Further, shifts in plant community composition led to greater

plant litter decomposition rates. Microcosms representative of small mammal access plots had a 15% higher decomposition rate than small mammal reduction microcosms. Our findings provide further evidence that temperate grasslands can be strongly influenced by consumers rather than resources.

Chapter 1: Small Mammals Modify Invertebrate Herbivore Effects on Grassland Community Structure and Function

Introduction

Herbivore communities and nutrient availability are changing concurrently world-wide (Wilcove et al., 1998; Crain, Kroeker and Halpern, 2008). As such, it is important to understand the combined effects of consumers and resources on plant community biodiversity and productivity. Humans have decreased grazing intensity through management practices (Hughes, 1994; Welch and Scott, 1995). In grasslands specifically, natural disease and population control efforts have caused mammal populations to decrease (Knowles, 2002; Finch, 2005). Coupled to this decline in consumers has been an increase in nitrogen (N) inputs (Gruner et al., 2008). Nutrient inputs have increased more than two-fold over pre-industrial levels (Jefferies and Maron, 1997; Galloway et al., 2003) due to anthropogenic N deposition from ammonia production and fossil fuel combustion (Galloway et al., 2003), and, most significantly, fertilization (Liu et al., 2013; Nehring, 2016). As a result, these changes in herbivory (top-down) and soil nutrients (bottom-up) are altering the structure and function of ecosystems.

Recent studies find that herbivores can have positive or sometimes neutral effects on plant productivity (E. T. Borer et al., 2014; Borer, Seabloom, Mitchell, & Cronin, 2014; Gruner et al., 2008; Maron & Crone, 2006; Olofsson, de Mazancourt, & Crawley, 2007). Furthermore, the effects of herbivores on plant community diversity differ across productivity gradients, with herbivores promoting diversity under high productivity by limiting over yielding species and reducing overall resource limitation (Bakker, Ritchie,

Olff, Milchunas, & Knops, 2006; Hillebrand et al., 2007); while the opposite is true in low productivity environments. Additionally, differences in animal guilds based on feeding patterns, metabolic efficiency, spatial distribution, and size contribute to variation in plant community diversity in response to herbivores (Gruner et al., 2008; La Pierre, Joern, & Smith, 2015; Oduor, Gomez, & Strauss, 2010; Shurin & Seabloom, 2005). Even so, few studies have experimentally tested the relative and combined influence of vertebrate and invertebrate herbivore guilds on grassland productivity and diversity.

Although herbivores and soil N can concurrently alter an ecosystem, few studies have looked at their interactive effects on producer biodiversity and productivity. A meta-analysis by Gruner et al. (2008) showed inconclusive results for the interactive effects of nutrient fertilization and herbivory on producer productivity. A second meta-analysis, by Hillebrand et al. (2007), suggested that the effect of herbivory on diversity metrics differs based on the productivity of the system rather than soil N per se. Generally, in highly productive systems with high species dominance (low evenness), herbivores have a positive effect on diversity, whereas, in low productivity systems with lower dominance, herbivores have a negative effect on diversity (E. T. Borer et al., 2014; Hillebrand et al., 2007; Proulx & Mazumder, 1998). However, we lack a clear understanding of how herbivores of different guilds and soil N interact to alter plant community structure.

To better understand how herbivory and resource availability interact to alter grassland ecosystem structure and function, we manipulated invertebrate herbivory and soil N within an existing small mammal manipulation. We asked the following questions: (1) What are the main effects of small mammal herbivores on grassland diversity, composition, and productivity? (2) How do invertebrate herbivores and soil N affect plant community diversity and productivity in the presence vs. absence of small mammals? We predicted that: (1) Small mammals would decrease plant community productivity and diversity, and lead to shifts in plant community composition; (2) In the presence of small mammal herbivores, invertebrate herbivores and N addition would further contribute towards the decline in diversity. While we expected N addition would promote productivity, we predicted invertebrate herbivory would further reduce productivity when small mammal herbivores were reduced.

Materials and Methods

Study Site

We conducted our study at Kessler Atmospheric and Ecological Field Station (KAEFS, 34°59'N, 97°31'W), a mixed grass prairie in central Oklahoma, USA. The KAEFS landscape and management practices are representative of Oklahoma's vegetation physiognomy (mixed grassland, riparian, and woody habitats) and grazing regimes. Average annual precipitation is 930 mm and the mean annual temperature is 16°C, ranging from 3.5°C in January to 27.8°C in July (average value from 1971-2010, data from Oklahoma Climatological Survey). Soils have been characterized as a silt loam (35.3% sand, 55.0% silt, and 9.7% clay) (Zhou, Wan, & Luo, 2007). The most commonly occurring plant species at the study site include: *Tridens flavus*, *Bromus*

racemosus, *Commelina communis*, *Andropogon gerardii*, *Crouton glandulosus*, *Dicanthelium oligosanthes*, *Vicia americana*, and *Artemesia ludociviana*. We also identified over 75 other subordinate and transient species, both herbaceous and woody.

Experimental Design

We used a nested plot design to address how soil N addition and invertebrate herbivory effects on plant communities may be mediated by small mammal herbivores. We randomized invertebrate herbivory and soil N manipulations within existing small mammal reduction and access plots (Appendix 5). Four small mammal reduction plots (approximately 7m x 20m each) were previously established, spanning a total area of 15m x 40m. Reduction plots were composed of metal fencing buried 40cm below the soil surface and 82cm above the ground. Adjacent to this, an additional 15m × 40m area with no above or buried metal fencing was designated the small mammal access area. Welded wire fencing surrounding the entire site prevented access to all plots by grazing cattle, yet this fencing did not hinder the movement of small animals. Small mammals were trapped by Sam Nobel Natural History Museum mammalogists for three consecutive nights in 2014. They used Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) to estimate small mammal abundance following guidelines of the American Society of Mammalogists for animal care and use (Gannon, Sikes, & Comm, 2007). Total small mammal abundance was 20% higher in the small mammal access plots relative to the reduction plots; average biomass was more than 80% higher in mammal access plots, resulting from smaller-bodied species present in the reduction plots. The most common small mammals across access and reduction plots were the

white-footed mouse (*Peromyscus leucopus*), cotton rat (*Sigmondon spp.*), and woodland vole (*Microtus pinetorum*).

In the summer of 2013, we established invertebrate herbivore manipulation treatments nested within the existing small mammal herbivore removal experiment. We had two invertebrate removal treatments: (1) "invertebrate reduction", consisting of a mesh exclosure with no invertebrate access (a 1.1m in diameter × 1.5m tall metal cage enclosed in mesh (C18A mesh; Lumite Co.) + insecticide) and (2) "leaky mesh exclosure", with invertebrate access (a 1.1m × 1.5m metal cage enclosed in mesh with large holes cut out of the mesh). Invertebrate reduction plots were sprayed with a permethrin insecticide (Hi-Yield Kill-A-Bug; Voluntary Purchasing Group, Bonham, TX, USA) to further reduce invertebrate abundance; this method has been shown to reduce invertebrate abundance by 4-fold (Sanders et al., 2007). Insecticide was applied with a backpack sprayer at a rate of 0.23 L/m^2 every two weeks throughout the growing season. For six weeks, we sampled the invertebrate community within the immediate area of the plots using sweep nets and sticky traps (similar to Lane (2006) and Sanders et al. (2007)). Invertebrate abundances did not differ between small mammal access and reduction areas. The most common invertebrates found at our site were red-legged grasshoppers (*Melanoplus femurrubrum*), leafhoppers (*Cicadellidae* spp.), and ants (*Monomorium minimum*).

In a fully factorial design, we manipulated soil N by adding 10 g/m^2 of nitrogen in the form of urea pellets to half of the plots. Soil N manipulations began in July of 2013 and

again in May of 2014 and 2015 following NutNet protocol (http://www.nutnet.umn.edu/). This procedure mimics nitrogen deposition from agriculture and industrial sources in grasslands and old fields (E. T. Borer et al., 2014; Larson & Siemann, 1998; McLendon & Redente, 1992). We measured soil N by first deploying ion-exchange resin bags (H-OH form, number R231-500, Fisher Scientific International) approximately five cm below the soil surface in each plot in May of 2015. In August of 2015, we collected and air-dried the bags. Resin beads were mixed with 2 mol/L KCl to extract NO_3^- and NH_4^+ then later analyzed in solution with an autoanalyzer (Lachat Quikchem 8000, Hach) (Sanders et al., 2007). Analysis confirmed that across small mammal treatments, N values in the N addition plots were more than twice that of the control plots (NH_4: F-ratio 7.54, p-value 0.003; total N: 7.53, p-value 0.003) (Appendix 1).

Field Measurements

Plant Community

During the growing season of 2015, we identified all plant species to determine richness (S) within the study plots and estimated species-specific foliar cover (N) in each experimental plot using modified Braun-Blanquet cover classes with seven foliar cover categories (0-2%, 2-5%, 5-15%, 15-25%, 25-50%, 50-75%, 75-100%) (Braun-Blanquet 1937). We then used each foliar cover class median to represent species-specific abundance and to calculate Shannon diversity index ($H' = -\sum_{i=1}^{S} J'_i ln J'_i$) (Shannon & Weaver, 1949). We also calculated evenness (J') as $H'/\ln(S)^2$. To determine the effects of herbivory and soil N on total community biomass (aboveground net primary

productivity: ANPP), we clipped all individuals rooted in a 0.25 m^2 area within each plot at ground level in fall of 2015. We oven-dried the plant material at 65°C for approximately 48 hours then weighed to estimate ANPP.

Microclimate

To determine how microclimate differed across herbivore and nutrient treatments, we measured light availability (photosynthetic photon flux density, PPFD), soil moisture (volumetric water content, %), and temperature. We measured light availability and soil moisture at the beginning and peak of the growing season (May and August). To estimate light availability, we first removed the plot's cage then used a light-integrating ceptometer (LP-80 AccuPAR; Decagon Device, Inc.) to record and then average two measurements per plot. We used a hand-held soil moisture probe (Hydro Sense II) to measure percent volumetric water content (%VWC) in two random spots in each plot and averaged within-plot values. We recorded soil temperature by deploying ibuttons (iButton® Temperature Logger; Maxim Integrated; San Jose, CA) at the soil surface, tracking seasonal temperature fluctuations (May-August).

Analyses

Both ANOVA (univariate) and PERMANOVA (multivariate) statistical tests were performed for each of the three data collection dates (May, July, August). In addition, we ran repeated measures ANOVA (univariate) and PERMANOVA analyses across all sampling dates.

Univariate Analyses

For each of the response variables (richness, evenness, diversity, and ANPP), we first performed a repeated measures analyses of variance (RM ANOVA) with nested factors. Small mammal access was included as the main factor and invertebrate access and nutrients were nested within small mammals (i.e., small mammals, invertebrates(small mammals), and N(small mammals)) (Appendix 2). Each RM ANOVA was performed to determine whether the impact of treatment differed across time. We used Shapiro-Wilk tests to determine the effect of time (i.e., month) on our focal response variables (richness, evenness, diversity, and ANPP) as a function of small mammals, invertebrates, and nutrients, (small mammal, invertebrate(small mammal) and N(small mammal)). We followed the RM ANOVAs with a series of one-way nested ANOVAs, run separately for each time period. We analyzed the response of the plant community (productivity, richness, evenness, and diversity) and microhabitat (light availability, soil moisture, temperature, and soil N) to the herbivore and nutrient treatments using nested ANOVAs. We analyzed data using JMP 11 to determine main effects of treatments and the variability among plots with nutrients and invertebrates nested within small mammals:

$$Y_{ijk} = \mu + \text{Small mammal}_{2i} + \text{N}_j(\text{Small mammal})_i + \text{Invertebrate}_j(\text{Small mammal})_i + \text{N}_j*\text{Invertebrate}_j(\text{Small mammal})_i + \epsilon_{ijk},$$

where μ is the overall mean, Small mammal is the treatment effect, N and Invertebrate are nested factors within Small mammals, N(Small mammal)*Invertebrate(Small

mammal) tests the interactive effects of N and invertebrates within Small mammals, and ϵ_{ijk} is the residual error associated with the measured dependent variable Y_{ijk}. Datasets were tested for normality and homoscedasticity with the Shapiro-Wilk *W*-test and Levene test, respectively. Data that did not meet normality assumptions were log (X+1) or 1/X transformed before analysis. We used Tukey's HSD as post-hoc tests to look at within-group variability of invertebrate herbivory.

Multivariate Analyses

We used a non-parametric, permutational multivariate analysis of variance (PERMANOVA) to determine the change in compositional similarity due to invertebrate herbivory and soil N in the context of small mammal herbivores (represented in our statistical model as the nested factors small mammals, invertebrates(small mammals), and N(small mammals)). We performed the PERMANOVA on a Bray-Curtis similarity matrix generated from the log transformed (log X+1) plant composition data (i.e., foliar cover (N) explained above). A significant pseudo F-ratio (the test static) for the PERMANOVA represents community composition dissimilarity either due to separation of communities by treatment in multivariate space (also known as location) or variation of communities within treatments in multivariate space (also known as dispersion) (Anderson, 2001; Bunn, Jenkins, Brown, & Sanders, 2010). To determine if compositional differences were due to location or dispersion differences, we followed up PERMANOVA analyses with PERMDISP (permutational multivariate analysis of dispersion) analyses (Bunn et al.,

2010). We used PRIMER version 6 (Plymouth Marine Laboratory, UK) for multivariate analyses.

To illustrate species composition in multivariate space, we performed a series of principal coordinate analyses (PCO) based on the Bray-Curtis similarity matrix. We used the first PCO axes, which accounted for a significant proportion of total variation in compositional similarities, to illustrate treatment differences in β diversity over time. We also performed a similarity percentage analysis (SIMPER) to determine which species contributed the most to overall differences in community composition dissimilarities between soil N and invertebrate herbivores in the context of small mammal herbivores.

Results

Impact of mammal herbivory on plant community

Small mammal herbivory decreased productivity, had mixed effects on diversity, and lowered compositional similarity between access vs. reduction areas (Tables 1 and 2). In August of 2015, total aboveground biomass in small mammal access plots was 70% lower than small mammal reduction plots (Fig. 1a; $p=0.0004$). Species richness was 19% higher in small mammal access plots than small mammal reduction plots ($p=0.004$ in August). Diversity was 10% higher in small mammal access plots relative to reduction plots late in July and August the growing season ($p=0.03$). Small mammals did not alter plant evenness in any month of 2015. Compositional similarity of the plant community was driven by small mammal herbivory, but not invertebrate herbivory or soil N (Table 2 and Fig. 2). Small mammal access plots had a higher C_3:C_4 species

abundance ratio, whereas small mammal reduction plots represented a lower C_3:C_4 species abundance ratio. Specifically, C_3 species were on average two-fold more abundant in small mammal access plots compared to reduction plots, whereas the abundance of C_4 species was two and a half-fold greater in small mammal reduction plots than small mammal access plots across seasons (Appendix 4).

Table 1 Nested ANOVA results. Bolded values represent statically significant values.

2015			May		July		August	
Response	Source	df	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Richness	Mammal	1	0.00	1.00	0.28	0.60	9.96	**<0.05**
	N(Mammal)	2	0.92	0.41	1.27	0.30	2.04	0.15
	Invertebrate(Mammal)	2	0.79	0.46	1.84	0.18	0.25	0.78
Evenness	Mammal	1	1.03	0.32	0.95	0.34	0.02	0.89
	N(Mammal)	2	0.16	0.85	3.19	0.06	1.37	0.27
	Invertebrate(Mammal)	2	3.93	**0.03**	2.34	0.12	0.40	0.67
Diversity	Small mammal	1	0.66	0.42	1.14	0.30	5.04	**0.03**
	N(Small mammal)	2	0.02	0.98	1.05	0.36	1.08	0.35
	Invertebrate(Small mammal)	2	1.25	0.30	3.53	**0.04**	0.57	0.57
ANPP	Mammal	1					14.47	**<0.001**
	N(Mammal)	2					0.74	0.49
	Invertebrate(Mammal)	2					0.29	0.75

Impact of invertebrate herbivory on plant community

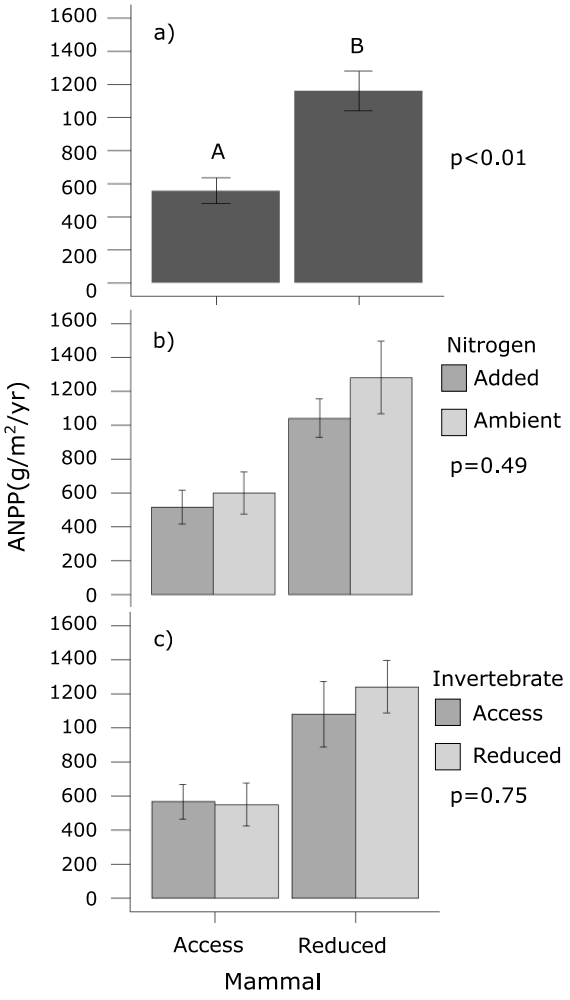

Figure 1 Small mammal herbivores decrease primary productivity. Mammals (a) decreased ANPP by more than half. Neither soil N (b) nor invertebrate herbivory (c) significantly altered ANPP. Bars with different letters denote significant differences. Error bars represent standard error.

Invertebrate herbivory had mixed effects on diversity, yet did not alter productivity or compositional similarity. The presence of invertebrate herbivores lowered plant evenness by approximately 9.5% in both small mammal presence and absence (p=0.03 in May; Table 1 and Appendix 6). Additionally, invertebrate herbivores had differing effects on plant diversity depending on the presence or absence of small mammals (p=0.04 in July Table 1 and Appendix 6). Within the mammal access plots, the presence of invertebrates lowered plant diversity by 9%. However, in the mammal reduction area invertebrates promoted plant diversity by 23%. While total species composition did not differ across invertebrate herbivore treatments, we detected differences of dispersion patterns within invertebrate treatments. These shifts in over-dispersion (increase in plant species turnover) were primarily within the small mammal access plots not reduction plots (Appendix 13). Within the small mammal access area, when invertebrate herbivores

were present, compositional variability was greater (July centroid average: Mammal Access-Invertebrate Access = 33.28; Mammal Access-Invertebrate Reduction=23.15; PERMDISP pairwise-p=0.03). However, when small mammals were reduced, dispersion patterns did not differ across invertebrate herbivore treatments (July centroid average: Mammal Reduction-Invertebrate Access = 30.56; Mammal Reduction-Invertebrate Reduction=30.34; PERMDISP pairwise-p>0.05). While invertebrate effects are relatively minimal, the influence of invertebrates tended to be contingent on small mammal herbivory.

Table 2 PERMANOVA results based on composition. Bolded values are statically significant.

PERMANOVA 2015		May		July		August	
Source	df	Pseudo-F	P-value	Pseudo-F	P-value	Pseudo-F	P-value
Mammal	1	5.78	**<0.001**	4.12	**<0.001**	5.68	**<0.001**
N(Mammal)	2	1.62	0.08	0.61	0.86	0.79	0.69
Invertebrate(Mammal)	2	1.12	0.33	0.42	0.96	0.96	0.49

Impact of nitrogen on plant community

Soil N addition had negligible effects on the plant community. Soil N addition marginally lowered species evenness by 10% in July (p=0.06) across small mammal treatments, but did not significantly alter any other community metric or composition (Table 1 and Appendix 6).

Microclimate responses to herbivores and nutrients

Herbivores and soil nutrients altered abiotic conditions and resources in our grassland ecosystem (Appendix 1). Small mammals increased light availability by 30% (p=0.007). Invertebrate herbivory increased soil temperature (p=0.02) by 2% across

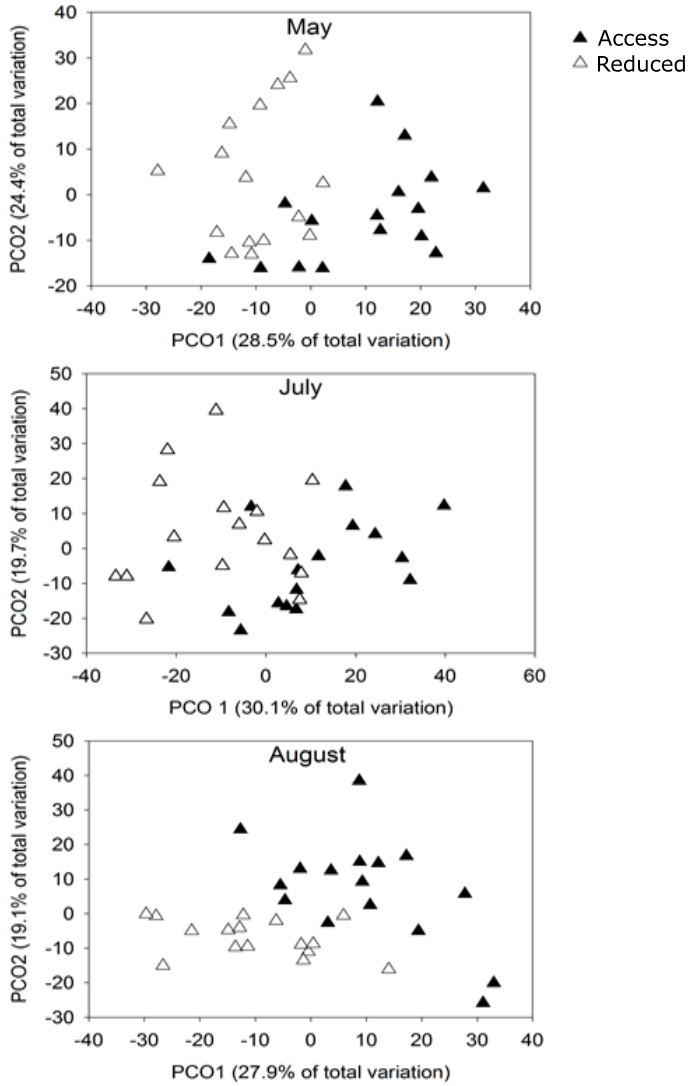

Figure 2 Small mammal herbivory leads to shifts in species composition. Axis coordinates represent variation in species present in each plot. Values for principal coordinates ordinate axes one for all two combinations (closed triangles= mammal access, open triangles=mammal reduction).

small mammal treatments. Nitrogen addition increased soil temperature by 2.6% within the small mammal access plots, yet decreased soil temperature by 2.5% in the small mammal reduction plots (p=0.03).

Discussion

Herbivores, especially small mammals, strongly altered the structure and composition of this grassland plant community and mediated the effects of invertebrate herbivory on shifts in plant dominance patterns. Overall, small mammal herbivory lowered plant productivity while increasing diversity. The presence of invertebrate herbivores further reduced plant evenness (a metric of diversity), but mostly in the presence of small mammals. N addition did not alter productivity regardless of small mammal presence, and marginally lowered evenness.

Vertebrate herbivores drive changes in plant community

Small mammal herbivores lowered plant productivity and increased richness leading to an increase in plant species diversity. Our findings are in agreement with other studies that showed small mammal herbivory lowered ANPP (Austrheim, Speed, Martinsen, Mulder, & Mysterud, 2014; Gruner et al., 2008; Olofsson, Tommervik, & Callaghan, 2012). Of the species that were present, small mammals may have selectively fed on particular species, altering species richness patterns of the plant community and leading to a reduction of grassland diversity (Henry F. Howe, Brown, Zorn-Arnold, & Sullivan, 2001; H.F. Howe, Zorn-Arnold, Sullivan, & Brown, 2006). Changes in the particular plant species present as a result of small mammal herbivory, not invertebrates or soil N, ultimately promoted compositional dissimilarity in our plant community. Overall, *A. gerardii* and *T. flavus* contributed most to the dissimilarly across small mammal treatments overtime. C_3 forb species, such as *A. ludoviciana* and *A. psilostachya*, were more abundant in small mammal access plots than reduction. These data suggest that as small mammal populations decline, grasslands will become more dominated by C_4 graminoids. This result conflicts with findings by Moorhead et al. (personal communication) in which small mammal herbivory promoted C_4 species rather than C_3. Additionally, Moorhead et al. characterize small mammal access plant material as being less palatable and having lower N concentrations. In our system, the presence of small mammal herbivory was associated with greater foliar N concentrations compared to small mammal reduction plots as a result of species compositional shifts (unpublished data). Shifts in species composition may lead to shifts in litter quality and, ultimately, the rate of soil carbon sequestration (Bardgett & Wardle, 2003; Gentile, Vanlauwe, &

Six, 2011). However, with little experimental data relating the effect of small vertebrate herbivory on plant litter quality, more studies manipulating multiple herbivore guilds are needed to fully understand this relationship.

Herbivore guild identity influences effects on plant community

Herbivores of different guilds have unique effects on plant community productivity and diversity (Bakker et al., 2006; Oduor et al., 2010; Shurin & Seabloom, 2005). Across guilds, differences in body size (Hopcraft, Olff, & Sinclair, 2010) and feeding preferences (Huntly, 1991) can lead to very different outcomes, primarily in plant diversity. For instance, it is suggested that herbivory by small mammals leads to a greater relative change in total biomass than invertebrate herbivory (Hulme, 1996). Furthermore, when these two guilds favor similar types of plants, they have similar effect patterns on diversity (Pusenius et al., 2002). La Pierre et al. (2015) provide one of the few studies, like ours, to examine the interaction of invertebrate and vertebrate herbivores on terrestrial ecosystems. Similar to our study, La Pierre et al. (2015) found an increase in plant evenness with a decline in invertebrate herbivore presence regardless of small mammal presence. A change in evenness suggests preferential feeding by the invertebrate herbivores. For instance, La Pierre et al (2015) explained that the shift in evenness in their system was driven by a change in the grass-to-forb ratio. In our system, unlike La Pierre and others (Borgstrom, Strengbom, Viketoft, & Bommarco, 2016; Throop & Lerdau, 2004; Tscharntke & Greiler, 1995), invertebrate herbivory did not lead to shifts in overall species composition. Additionally, invertebrate herbivory in our plots did not significantly reduce total productivity. While other studies have also failed to detect an effect of invertebrates on total productivity

(La Pierre et al., 2015), this could also be evidence of a lag effect (Gruner et al., 2008; H.F. Howe et al., 2006). It is possible that invertebrates must be reduced for longer than the two growing seasons in our study system to elicit plant productivity responses. Overall, our data show that different herbivore guilds can lead to unique independent and interactive effects on a plant community structure.

Vertebrate herbivory negates soil N addition

Soil N only marginally lowered species evenness. Consistent with a meta-analysis by Hillebrand et al. (2007), fertilization decreased evenness across small mammal treatments. Increased nutrient availability favors competitive dominance and exclusion of rare species (Hillebrand, Bennett, & Cadotte, 2008; Stevens, Dise, Mountford, & Gowing, 2004). Surprisingly, soil N addition had very little effect on the other plant community measurements regardless of vertebrate herbivore presence. However, other studies in similar systems have also shown that herbivores and fertilization do not have interactive effects on plant productivity and diversity (Blue, Souza, Classen, Schweitzer, & Sanders, 2011; Gruner et al., 2008; Souza, Zelikova, & Sanders, 2016). It is possible that N is not the limiting nutrient in our system; instead, another nutrient, such as phosphorus, may be the limiting productivity here (Blue et al., 2011).

Also, soil N addition did not significantly alter the microclimate. Herbivory and eutrophication have conflicting effects on plant community productivity and diversity. However, herbivory may mediate the effects of eutrophication by alleviating light limitation. E. T. Borer et al. (2014) suggest that an increase in ground-level light should correspond to a decrease in productivity and increase in diversity. In the context of

herbivores and nutrients, they propose that an increase in ground-level light by herbivory can counteract the effects of eutrophication. Our study shows soil N addition does not alter light availability in either the presence or the absence of small mammals. Without such an impact on the microclimate, soil N does not counteract the effects of the herbivores in our study.

Conclusion

We find that, while herbivores drive grassland diversity and productivity, soil nutrients have minimal impacts. We found small mammals to decrease productivity and alter community composition, ultimately increasing diversity. Further, we found no response of productivity to invertebrate herbivory or soil N. Invertebrates decreased evenness across small mammal treatments, yet had mixed effects on diversity; soil N did not significantly alter diversity metrics. These data contradict the paradigm assuming net primary productivity of terrestrial systems is strongly bottom-up controlled (Loreau et al., 2001), but provide strong evidence to show that grassland ecosystems can also be controlled by top-down factors (Schmitz, 2003; Schmitz, Hamback, & Beckerman, 2000). Further, we have shown that different herbivore guilds can both independently and interactively alter plant communities. In future studies, we suggest it is imperative take herbivore guild into consideration.

Chapter 2: Small mammal herbivores promote litter decomposition by altering litter, rather than soil, properties

Introduction

Biotic and abiotic factors affect the structure of a plant community and ultimately alter the quantity and quality of plant litter and ecosystem associated processes at the local scale. Furthermore, human activities have significantly altered biotic and abiotic factors causing an increase in nitrogen (abiotic) and decline of herbivores (biotic). Soil nitrogen (N) input, especially due to agricultural practices, has increased two-fold compared to pre-industrial levels (Doering, Galloway, & Theis, 2011; Galloway et al., 2003; Jefferies & Maron, 1997). Population control through management practices has also caused large and small herbivore populations to decline (Hughes, 1994; Li et al., 2016; Ripple et al., 2015; Welch & Scott, 1995). Disturbance events, such as herbivory and fertilization, can directly alter the plant community structure and consequently plant litter characteristics. Therefore, addressing the relative influences of concurrently altered biotic and abiotic controls is key in understanding changes in ecosystem processes such as litter decomposition.

Generally, increased herbivory promotes litter decomposition rates (Garibaldi, Semmartin, & Chaneton, 2007; Semmartin, Garibaldi, & Chaneton, 2008; S. W. Smith et al., 2015), whereas N addition has both positive and negative effects on decomposition (Fornara & Tilman, 2012; Henry & Moise, 2015). Aboveground net primary productivity (ANPP), plant community diversity and compositional similarity can be significantly altered by herbivory and soil N availability. Herbivory generally

reduces ANPP via consumption of plant material, while N addition has the opposing effect in nutrient limited ecosystems by promoting ANPP (Gruner et al 2008). However, changes in ANPP as a function of herbivore and nutrients are typically disproportional across plant species altering diversity and leading to shifts in compositional similarity (Harpole & Tilman, 2007; Lamb, Shore, & Cahill, 2007; Tilman, 1987). While herbivory typically promotes diversity, N enrichment decreases diversity (Bakker et al., 2006; Hillebrand et al., 2007). These changes in the plant community ultimately lead to shifts in decomposition rates by altering litter quality. For instance, herbivory by cattle was shown to promote litter decomposition rates by shifting a prairie plant community from graminoid dominated to forb dominated (Garibaldi et al 2007). This increased decomposition was associated with higher foliar N and lignin concentrations and lower cellulose concentrations in grazed litter compared to ungrazed litter. In a grassland system, N addition was shown to lower plant litter decomposition following a shift from C_4 to C_3 plant species (Fornara & Tilman, 2012). Fornara and Tilman (2012) documented litter from N addition plots to have a lower carbon-to-nitrogen ratio; however, a decline in leaf litter decomposition may be also related to microbial degrading enzymes rather than litter quality alone (Hobbie, 2008; Keeler, Hobbie, & Kellogg, 2009). With their conflicting effects on the plant community and resulting decomposition, it is unclear how herbivory and soil N addition will concurrently alter plant litter decomposition by changing litter quality or the soil microbes.

Studies have also addressed the role of soil community structure, microbial diversity and composition, in grassland ecosystems as a strong determinant of decomposition

rate. Therefore, in addition to changes in the plant community, it is important to understand how herbivores and soil N addition may influence the soil microbial community to alter plant litter decomposition. A growing volume of literature suggests the historical perception of the microbial community leads to specialization and promotes ecosystem processes such as litter decomposition and nutrient cycling (Ayers 2009; Milcu and Manning 2011; Wallenstein et al 2013). These studies show that the historical litter type deposited to the soil shapes that microbial community. The proposed mechanism which suggests that soil microbes decompose litter that is characteristic of plants that have been growing in that soil ('home') more quickly than they decompose a new or foreign type of litter ('away') is called home-field advantage (HFA) (Ayers 2009). It has also been shown that the structure of the microbial community may influence ecosystem functions (Balser and Firestone 2005; Reed and Martiny 2007; Strickland et al. 2008; Marschner et al 2003). Broadly speaking, fungi have longer life cycles and decompose material slowly, whereas bacterial-dominated soil promotes decomposition as a function of shorter of faster metabolism and shorter life span (Moore, McCann, & de Ruiter, 2005; Wardle et al., 2004). However, with possible functional redundancy within soil microbe communities (Cardinale et al. 2007; Jiang 2007; Verity et al. 2007) and opposition to HFA (Carrillo, Ball, Strickland, & Bradford, 2012; Giesselmann et al., 2011), it is difficult to understand the role of soil perception in leaf decomposition rates.

Our study addresses how concurrent changes in abiotic (soil N) and biotic (small mammal herbivory) factors shape ecosystem processes (litter decomposition) in a

prairie ecosystem. We specifically asked: (1) How does small mammal herbivory alter plant litter decomposition? (2) Do small mammal herbivores mediate soil N availability effects to alter plant litter decomposition? (3) What is the relative influence of litter quality vs. soil origin, as influenced by herbivores and soil N, determine the rate of litter decomposition? Specifically, we predicted: (1) small mammal herbivory would increase the rate of decomposition by promoting higher plant litter quality, (2) N addition would further increase decomposition rates in the presence rather than absence of small herbivores due to an increase in N content of the leaves, and (3) litter would decompose faster in soil found directly below it than in soil from a different origin.

Materials and Methods

Study Site

We collected soil and leaf litter samples from a field experiment located at Kessler Atmospheric and Ecological Field Station (KAEFS, 34°59'N, 97°31'W), a mixed grass prairie site in central Oklahoma, USA. The KAEFS landscape and management practices are representative of Oklahoma's vegetation physiognomy (mixed grassland, riparian, and woody habitats) and grazing regimes. Average annual precipitation is 930 mm and the mean annual temperature is 16°C, ranging from 3.5°C in January to 27.8°C in July (average value from 1971-2010, data from Oklahoma Climatological Survey). Soils have been characterized as a silt loam (35.3% sand, 55.0% silt, and 9.7% clay) (Zhou et al., 2007).

Field Plot Manipulations

Prior to the laboratory incubation experiment, we established a field experiment using a nested plot design where we completely randomized soil N manipulation treatments within existing small mammal reduction and access plots. Four small mammal reduction plots (approximately 7m × 20m each) were previously established, spanning a total area of 15m × 40m (for further details on experimental design see Poe et al. In Prep). We manipulated soil N by adding 10 g/m^2 of nitrogen in the form of urea pellets to half of the plots 1-m^2 diameter circular plots in both access and reduction areas. Soil N manipulations began in July of 2013 and again in springs of 2014 and 2015 following NutNet protocol (http://www.nutnet.umn.edu/).

Laboratory Incubation Experiment

Material Preparation

Between October and November 2014, we collected senescing leaves that had lost their green color but had not yet fallen off the stem. We collected leaves from the following species: *Ambrosia psilostachya, Andropogon geradii, Artemesia ludoviciana, Dichanthelium oligosanthes*, and *Tridens flavus.* These species differ in their abundance across small mammal access and reduction plots and in functional group identification. *A. psilostachya* and *A. ludoviciana D. oligosanthes* are C_3 species, whereas *A. geradii* and *T. flavus* are C_4 species. Next, we homogenized litter from inside and outside small mammal exclosures, but material from each plant species was stored separately. Litter was stored in paper bags to air-dry. After they were dried, leaves were ground to 0.4 mm using a Mini Wiley Mill.

Also in late 2014, we used a soil corer (13cm in depth and 4cm diameter) to collect two sets of soil samples: inoculum soil and bulk soil. Inoculum soil was collected by taking two cores per plot and homogenizing within a plot. Bulk soil was collected outside yet near the plots. All soil was sieved (2 mm) to remove debris (de Graaff, Classen, Castro, & Schadt, 2010). Inoculum soil was kept in a refrigerator until needed. Bulk soil was first autoclaved for 50 minutes to remove biotic contamination then kept in a refrigerator until needed.

Laboratory Incubation Assembly

To determine the relative influence of herbivores and nutrients on plant litter decomposition, we established a laboratory incubation experiment where we constructed microcosms in falcon tubes containing plant litter and soil combinations representative of our field plots. Each microcosm consisted of a mason jar with 90 g of soil and 0.9 g of leaf litter. The 90 g of soil was divided among three 50 mL falcon tubes (30 g per tube) and was composed of 27 g (+/-0.1 g) of sterilized bulk soil + 3 g of inocula from a single plot. The 0.9 g of leaf litter was divided among the three tubes (0.3 g per tube) and mimicked the plant community of its soil inoculum. For example, if the soil inoculum were collected from small mammal access plots, the leaf litter proportions would mimic those of the small mammal access plots. Mammal access treatments contained: 0.02 g of *A. psilostachya*, 0.01 g of *A. geradii*, 0.05 g of *A. ludoviciana*, 0.01 g of *D. oligosanthes*, and 0.21 g of *T. flavus*; mammal reduction treatments contained: 0.00 g of *A. psilostachya*, 0.07 g of *A. geradii*, 0.00 g of *A.*

ludoviciana, 0.03 g of *D. oligosanthes*, and 0.20 g of *T. flavus*. In addition, we had two control treatments: litter only and soil only. Litter only treatments consisted of 30 g (+/- 0.1 g) of sterile, bulk soil per falcon tube with 0.3 g of leaf litter and no soil inoculum. Soil only treatments consisted of soil inoculum without leaf litter. Before incubation, we brought soil in each tube to 60% water holding capacity. At the same time, we added approximately 10 mL of deionized water to maintain within-jar humidity during incubation.

Following the community assemblage experiment (all five species per jar), we created microcosm assemblages to test for species-specific effects on litter decomposition. Each microcosm consisted of a mason jar with 30 g of soil and 0.3 g of leaf litter. The 30 g of soil was composed of 27 g (+/-0.1 g) of sterilized bulk soil + 3 g of inoculum from a single plot. The 0.03 g of leaf litter comprised one of the five litter species: *A. psilostachya*, *A. geradii*, *A. ludoviciana*, *D. oligosanthes*, or *T. flavus*.

To determine the role of home-field advantage, we designed a reciprocal soil and plant litter experiment to compare plant litter and soil resembling 'home' assemblages to plant and litter resembling 'away' assemblages. Leaf litter and soil proportions were similar to those described above. However, instead of soil and litter from the same small mammal plot, soil and litter originated from different plots, i.e., mammal access soil was paired with litter proportions similar to those of small mammal reduced plots (Appendix 8)

CO_2 evolution measurements

Microcosm jars were incubated for 120 days in dark conditions at ambient temperature. We measured CO_2 evolution on the initial day and on days 1, 2, 3, 5, 8, 15, 30, 60, 90, and 120 using a Li-COR 6400 infra-red gas analyzer (LiCOR, Lincoln, Nebraska). Each mason jar lid had an embedded rubber septum. Through this septum, we extracted a 15 mL gas sample from each jar with an insulin needle. The gas sample was injected into the Li-COR 6400 tubing for 45 seconds per sample. After the sample was taken and measured, we removed the lid to air out each jar.

We used the measurements from the Li-COR and calculated area under the curve using KaleidaGraph. We also corrected our measurements by the volume of soil and the number of days elapsed since the last measurement to calculate CO_2 per gram of soil per day. We then used a series of conversions to calculate CO_2 evolution as µg of CO_2 per gram of carbon as a proxy for microbial decomposition activity.

Statistical Analyses

To determine the effect of herbivory and soil N addition on plant litter decomposition (CO_2 evolution in our microcosms), we first calculated Hedge's g effect size and 95% confidence intervals of herbivory and soil N on decomposition using the R package compute.es Borenstein 2008; Cohen J 1988; Furukawa and Leucht 2011; McGraw and Wong 1992; Valentine and Cooper 2003). This provides Hedges' g using the formula:

$$g = (1 - \frac{3}{4df - 1})(\frac{\bar{x}_1 - \bar{x}_2}{S_{within}})$$

Where, $\bar{x}_1$ is the mean response of CO_2 evolution in herbivory access or N addition, $\bar{x}_2$ is the mean CO_2 evolution response in herbivory reduction or ambient N, and S_{within} is the pooled standard deviation.

We followed this analysis with a nested analysis of variance (ANOVA) to distinguish the effect of N in the presence vs. absence of small mammals. For this analysis, the statistical model only included data from jars representative of our field plots. To estimate the presence of HFA, we also used a nested ANOVA. However, this model also included data from the reciprocal treatments. We analyzed data using JMP 11 to determine main effects of treatments and the variability among plots with nutrients nested within small mammals:

$$Y_{ijk} = \mu + \text{Mammal}_{2i} + \text{Nitrogen}_j(\text{Mammal})_i + \epsilon_{ijk} ,$$

where μ is the overall mean, Small mammal is the treatment effect, Nutrient is the nested factor within small mammals, and ϵ_{ijk} is the residual error associated with the measured dependent variable Y_{ijk}. Datasets were tested for normality and homoscedasticity with the Shapiro-Wilk *W*-test and Levene test, respectively. Data that did not meet normality assumptions were log (X+1) transformed before analysis. We used Tukey HSD post-hoc tests to look at within-group variability of N addition.

We performed model selection using all possible regressions to determine the best combination of variables that explained litter decomposition in the laboratory incubation. First, we identified violations of significant correlation among factors using Pearson's correlation coefficients. We did not include predictor variables with

significant correlation coefficients (-0.75>r>0.75) in the model (Kumar, Stohlgren, and Chong 2006). Using JMP 11, we generated CO_2 evolution slopes, correlation matrix, and multiple linear regressions with all possible combinations of the explanatory variables. We used the following five explanatory variables in our model: N addition (ambient or added), mammal (present or absent), average total available N 2014, average available NO_3 2014, estimated biomass 2014. We estimated average total N and NO_3 by deploying ion-exchange resin bags, extracting NO_3^- and NH_4^+ with a KCl solution, and analyzing the extract using an autoanalyzer (Lachat Quikchem 8000, Hach) (Sanders et al., 2007). We used species-specific abundance data collected from the experimental field plots to estimate biomass (g/m^2/year). We used the Akaike information criterion (AIC) to assess multiple regression models and determine the best predictor(s) of decomposition. All models

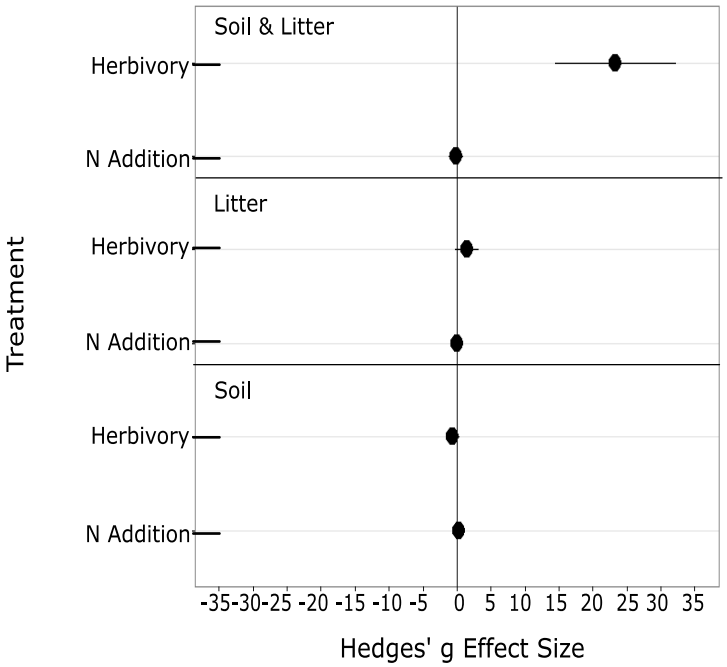

Figure 3 Small mammal herbivory had a positive effect while N addition had a neutral effect on plant litter decomposition. Points represent Hedges' g value. Error bars represent 95% confidence intervals. Within the soil and litter treatment, herbivory has a positive effect on decomposition (Hedges' g = 23.33, p-value <0.001). Within the litter only treatment, herbivory has a slightly positive effect on decomposition (Hedges' g =1.4, p-value =0.09).

and scores can be found in the appendix (Appendix 12).

Results

Main effects of herbivory and soil N addition on decomposition

Small mammal herbivores had a positive effect (Hedges' g = 23.33, p-value <0.001), while soil N addition had a neutral effect (Hedges' g = -0.12, p-value = 0.81), on leaf litter decomposition (Fig. 3, Appendix 10). In plots with small mammal access, decomposition rate was 15% higher than when small mammals were reduced (F-ratio=58.02, p-value<0.001, df=1). However, plots with added N did not significantly differ in litter decomposition rates from ambient N plots in either herbivory area (F-ratio=0.69, p-value=0.52, df=2).

Influence of other abiotic predictor variables

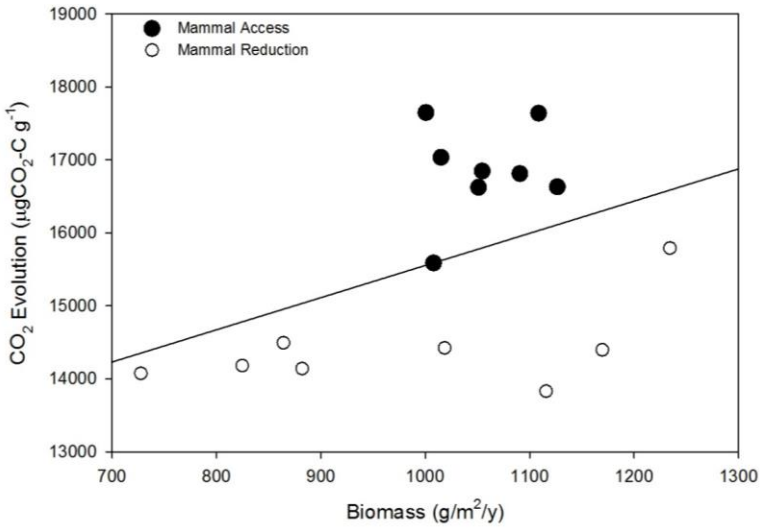

Figure 4 Biomass produced by the plants is positively correlated to CO_2 evolution (decomposition). This significant relationship (p<0.05) is shown by the regression line.

Of the seven predictor variables included in our multiple regressions model, biomass and small mammal presence were the most influential predictor variables (model R^2=0.84, adjusted R^2=0.81, sum of

error=4.68x10^6). Biomass was positively correlated with soil CO_2 evolution (Fig. 4, Partial R^2=0.80). Small mammal presence was positively correlated with soil CO_2 evolution (Fig. 4, Partial R^2=15%).

Reciprocal treatment

We did not find evidence of HFA. Instead, small mammal access litter decomposed more quickly regardless of soil origin (Fig. 5, p-value<0.0001). Small mammal access 'home' litter decomposed 22% more quickly than 'away' litter. However, within the small mammal reduced plots; 'away' litter decomposed 14% quickly than 'home' litter.

Herbivory presence effects on litter quality

Small herbivory access litter had a greater litter quality than small herbivore reduced litter (Appendix 9). Total N (t-ratio -2.82, p-value=0.10) and total carbon content (t=-2.28, p-value=0.15) did not significantly differ between treatments. However, the carbon-to-

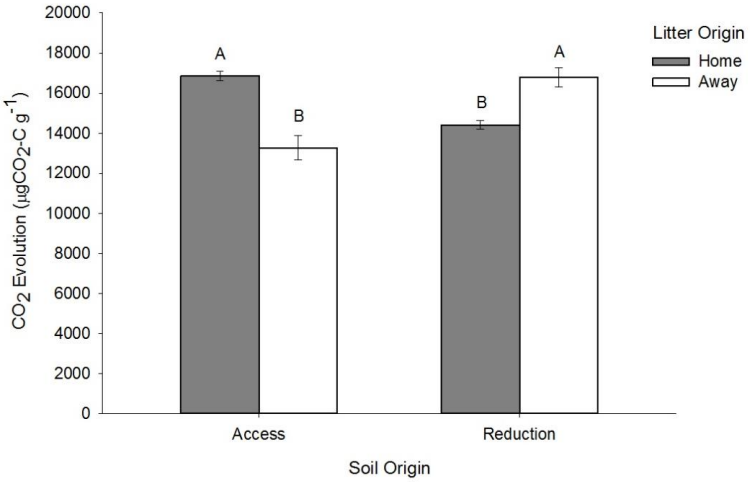

Figure 5 Reciprocal litter and soil origin experiment. We found significant differences with combined soil with a similar (home) or foreign (away) litter assemblage (p-value<0.0001). Within small mammal access treatment, 'home' litter decomposed 22% more quickly than 'away' litter. Within the small mammal reduction treatment, away' litter decomposed 14% quickly than 'home' litter. Error bars represent standard error. Different letters represent significant differences.

nitrogen ratio was significantly lower when small mammals were present rather than reduced ($t=4.15$, p-value=0.04).

Discussion

Our results show strong evidence of biotic control of plant litter decomposition in the studied prairie grassland ecosystem. Plots with small mammal herbivory had 15% greater litter decomposition rates compared to plots with reduced herbivory. Soil N enrichment, a field manipulated abiotic factor, did not significantly alter litter decomposition rates. In other words, changes in biotic rather than abiotic factors shaped a key ecosystem process. Also, biotic effects on litter decomposition were likely driven by litter composition (quality) rather than soil origin and associated properties.

Similar to previous studies, we found small mammal presence to increase decomposition (Garibaldi et al., 2007; Semmartin et al., 2008; S. W. Smith et al., 2015). For instance, Garibaldi et al. (2007) found grazing altered plant species composition. They then used graminoid and forb species collected from grazed and ungrazed plots to measure herbivory effects on decomposition. Litter from grazed plots, especially forbs, decomposed more quickly than litter from ungrazed plots. However, contrary to many studies, in our system, soil N addition had neutral effects on decomposition (Fog, 1988; Fornara & Tilman, 2012; Henry & Moise, 2015; Riggs, Hobbie, Bach, Hofmockel, & Kazanski, 2015). These studies have found that N addition leads to an increase in leaf N content (Henry & Moise, 2015) or increased cellulose due to increase in cellulase activity by soil microbes (Carreiro, Sinsabaugh, Repert, & Parkhurst, 2000) that promoted decomposition. A change in the aboveground plant community biodiversity

and productivity (Poe, et al. In Prep) and subsequent increase in litter quality (Appendix 9) due to herbivory, but no change due to soil N addition, suggests our system is not N limited.

The difference in litter decomposition between herbivore access vs. reduction areas, particularly due to HFA could be due to variability in the soil microbe community (Moore et al., 2005; M. S. Strickland, Lauber, Fierer, & Bradford, 2009) or leaf litter (Fornara & Tilman, 2012; Harrison & Bardgett, 2003; Semmartin et al., 2008). Our soil only treatments, in which plant litter was not added, did not decompose at different rates from one another when soils originated from herbivore access vs. herbivore reduction areas (Fig. 3, Appendix 10). However, litter only treatments, in sterilized soil, show that small mammal access litter decomposed faster than small mammal reduced litter (Fig. 3, Appendix 10). This suggests the differences in litter decomposition in herbivore access vs. reduction areas is likely driven by litter origin, rather than soil origin differences. Previous studies have shown that litter belonging to different functional groups or species decompose at different rates. Using our single-species treatments, we found species-specific variability in decomposition (Appendix 11). Specifically, *A. ludoviciana* and *A. psilostachya* plant species are likely contributing towards greater litter quality and decomposition rates in small access than small mammal reduction plots. These data provide evidence that short-term shifts in plant species composition due to small mammal herbivory can lead to alterations in ecosystem processes.

Unlike other studies, our work has not provided support towards the HFA concept. Previous studies that look at HFA are typically comparing drastically different systems (i.e., reciprocal forest and grassland treatments). Our study is unique in that we tested the presence of HFA within a single grassland ecosystem. However, we did not find strong evidence of HFA. Instead, litter quality rather than changes in microbial composition, influenced greater litter decomposition of litter originating from small mammal access communities regardless of soil origin. Several studies find no correlation between soil origin and decomposition and argue against the HFA (Freschet, Aerts, & Cornelissen, 2012; Giesselmann et al., 2011; Makkonen et al., 2012; Perez, Aubert, Decaens, Trap, & Chauvat, 2013; St John, Orwin, & Dickie, 2011). Others support HFA with some caveat. For example, Freschet et al. (2012) proposed the substrate-matrix interaction (SMI) hypothesis which extends HFA by stating suites of 'home' litter, rather than specific 'home' litter species, decompose more quickly than 'away' litter suites. Michael S. Strickland, Osburn, Lauber, Fierer, and Bradford (2009) suggest that soil previously exposed to low-quality litter (such as tree litter) can decompose either high or low quality litter similarly. Still others suggest that it is specialization by home microbes to their home litter which optimizes their ability to decompose litter from the same area. Therefore, a soil must receive the same litter for an extended period of time to have an increased decomposition rate. By measuring litter quality, we were able to dispute SMI or claims by Michael S. Strickland et al. (2009).

The litter assemblage representative of the small mammal access area had a higher quality litter and faster decomposition regardless of soil origin (Fig. 5, Appendix 9).

This finding agrees more with data that show litter of higher quality, meaning low carbon-to-nitrogen ratio, generally decomposes more quickly than low-quality litter (Moretto, Distel, & Didone, 2001; Semmartin, Di Bella, & de Salamone, 2010; Semmartin et al., 2008; V. C. Smith & Bradford, 2003). Lastly, our data do not seem to support the optimization hypothesis. The soil community between small mammal access and reduction do not seem to differ. Some studies have shown no change in the soil bacterial and fungal communities by herbivores (Hodel et al., 2014; Moorhead, Souza, Habeck, Lindroth, & Classen, In Revision). However, it is possible that our soil microbial community did not have adequate time to optimize their decomposition ability.

Several authors advocate a need to include soil dynamics in ecosystem modeling or predictions (A'Bear, Johnson, & Jones, 2014; Austin, Vivanco, Gonzalez-Arzac, & Perez, 2014; Wardle et al., 2004). This may be especially important when determining ecosystem responses to sudden changes in herbivory intensity (i.e., restoration or exclusion). Our findings provide evidence that historical norms need to be considered when predicting decomposition responses. The small mammal access plots historically received high-quality litter (‘home’). When we simulated a sudden shift in the plant community (‘away’ litter), the microcosm system decreased carbon release. The opposite is true for the small mammal reduction plots. Exposure to herbivore present litter assemblage by a historically herbivore absent soil increased carbon release by decomposition.

Conclusion

First, we found that small mammal herbivory promoted the rate of litter decomposition as predicted. Secondly, we predicted litter decomposition rate to increase with N addition when small mammals were reduced, but have no effect when small mammals were present; we found no significant differences in decomposition rates between N addition treatments in either the small mammal access or reduction treatments. Lastly, we predicted that plant litter would decompose faster in soils found directly below it "home soils" than in soils from a different origin "away soils", also known as HFA concept. However, we did not find evidence of HFA. Instead, small herbivore access litter decomposed faster than small mammal reduced litter regardless of soil origin. Overall, we have shown that herbivores alter a key ecosystem process. Additionally, litter quality may be a stronger predictor of decomposability than soil origin. We suggest future studies address the spatial-temporal component of HFA by investigating how both soil and litter inputs exert advantages within and across system types overtime.

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Appendix

Appendix 1 Microhabitat. Shown are mean values and p-values. Bolded p-values are significant ($p<0.05$). Letters represent values significantly different from one another.

		Microhabitat					
		Soil Moisture	Mean Temp	Light Below	NH_4^+	NO_3^-	Total N
Access		13.0	26.5	854.3 ^{A}	53.0	0.3	46.7
Reduction		12.2	26.5	626.0 ^{B}	74.5	0.3	65.4
	p-value	0.1	0.9	**0.0**	0.1	0.3	0.1
Access	N	13.7	26.2 ^{A}	832.2	31.9 ^{AB}	0.3	24.2 ^{AB}
	C	12.4	26.9 ^{B}	876.4	68.8 ^{B}	0.3	69.1 ^{B}
Reduction	N	12.5	26.8 ^{B}	567.9	35.8 ^{A}	0.3	31.6 ^{A}
	C	11.9	26.1 ^{AB}	684.0	113.2 ^{B}	0.3	99.3 ^{B}
	p-value	0.2	**0.0**	0.5	**0.0**	0.2	**0.0**
Access	L	13.3	26.6 ^{AB}	834.1	45.7	0.3	40.2
	F	12.8	26.4 ^{AB}	874.5	60.4	0.3	53.1
Reduction	L	12.3	27.0 ^{A}	629.9	63.1	0.3	55.5
	F	12.1	26.0 ^{B}	622.0	85.8	0.3	75.4
	p-value	0.7	**0.0**	0.9	0.7	0.6	0.7

Appendix 2 Repeated measures ANOVA results. Bolded values are statistically significant. Abbreviations: T: time (month), R: mammal herbivory, N: nitrogen addition, I: invertebrate herbivory. Richness (8.06 ± 0.24) and diversity (1.47 ± 0.04) were greatest in May, whereas evenness was greatest in July 0.79 ± 0.21). Overall, species richness ($p<0.0001$) and evenness ($p=0.01$), but not diversity, varied temporally. However, the effects of herbivores and soil N on diversity metrics did vary seasonally. First, the effects mammal herbivores interacted with time (time x mammal) to seasonally alter richness ($p=0.04$) and diversity ($p=0.002$). Secondly, invertebrate herbivore effects (time x invertebrate[mammal]) on evenness ($p=0.004$) and diversity ($p=0.003$) varied temporally. Lastly, the effects of soil N (time x N[mammal]) on evenness ($p=0.02$) and diversity ($p=0.05$) varied seasonally.

Response	Source	Wilks' λ /F Test	Wilks' λ /F Test df	P-value
Richness	T	1.55	2,25	**<0.0001**
	T x R	0.28	2,25	**0.04**
	T x N(R)	0.94	4,50	0.82
	T x I(R)	0.88	4,50	0.52
Evenness	T	0.43	2,25	**0.01**
	T x R	0.10	2,25	0.31
	T x N(R)	0.63	4,50	**0.02**
	T x I(R)	0.55	4,50	**0.004**
Diversity	T	0.05	2,25	0.52
	T x R	0.66	2,25	**0.002**
	T x N(R)	0.69	4,50	**0.05**
	T x I(R)	0.53	4,50	**0.003**

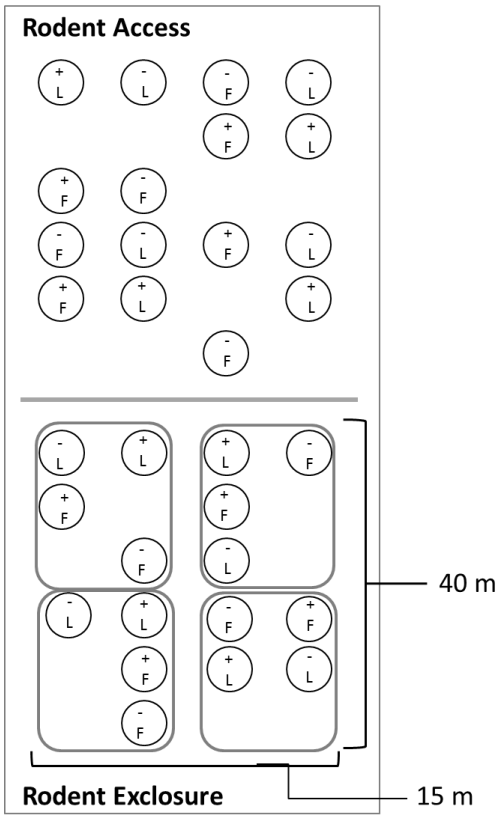
Appendix 3 PERMANOVA results showing shifts in composition during the year 2015. All analyses had more than 9900 unique permutations. Bolded values are statistically significant values. (Month: M, mammal herbivory: R, invertebrate herbivory: I, nitrogen addition: N). Unsurprisingly, species composition varied by season (p=0.0001). Also, the effect of mammal herbivores (p=0.0001) and soil N (N(mammal); p=0.004) on composition varied seasonally.

PERMANOVA 2015			
Source	df	Pseudo-F	P(perm)
M	2	27.94	**0.0001**
R	1	11.96	**0.0001**
N(R)	2	2.42	**0.0043**
I(R)	2	1.32	0.20
MxR	2	1.57	0.09
MxN(R)	4	0.16	1.00
MxI(R)	4	0.49	0.98
N(R)xI(R)	2	1.41	0.16
MxN(R)xI(R)	4	0.14	1.00

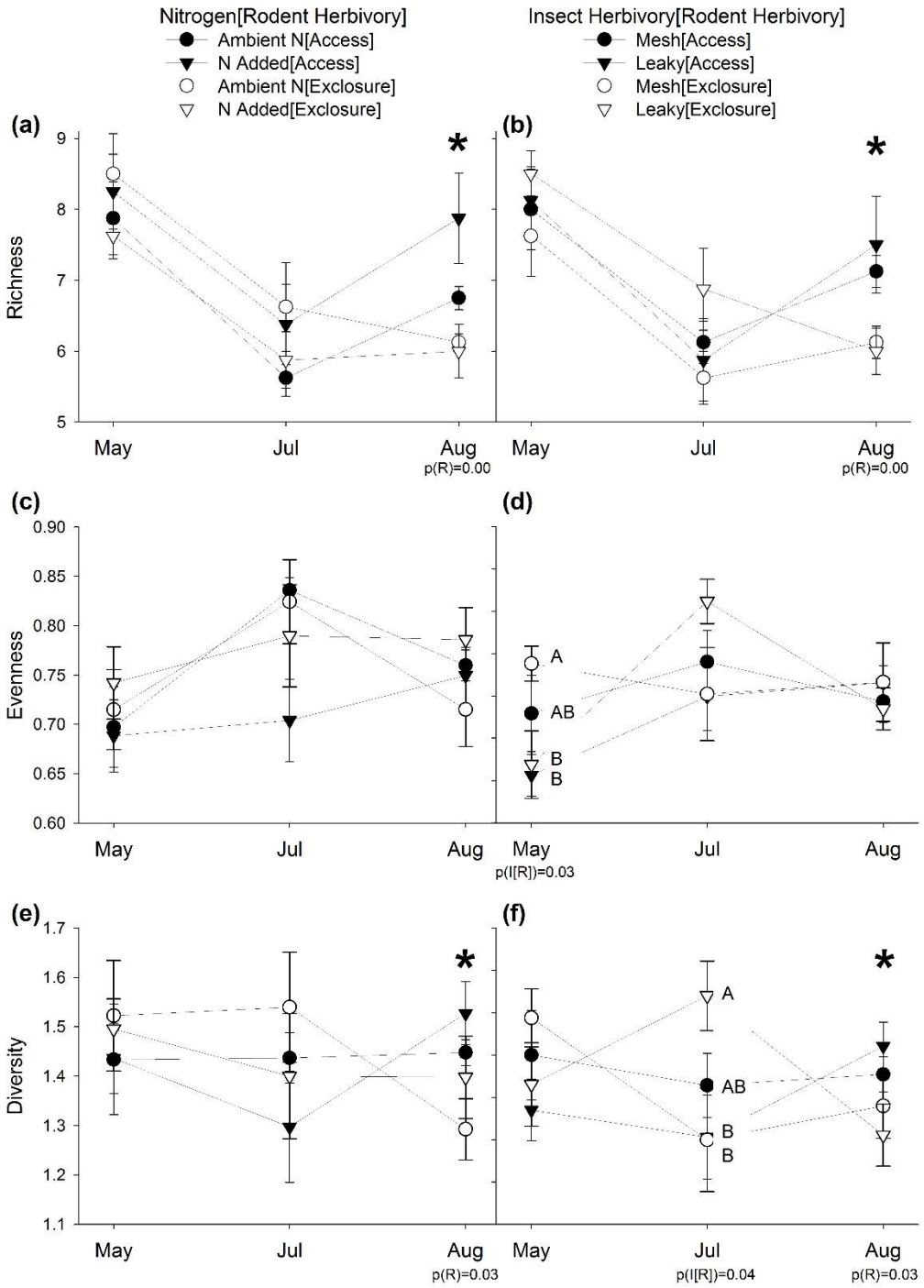
Appendix 4 SIMPER results for 2015. Contrib% shows how much each species contributes to dissimilarity of plot up to approximately 90% cumulative contribution (Cum.%). Mammal herbivory significantly altered plant community composition in 2015 (Table 2; for all months p=0.00). Early in the growing season, *A. gerardii*, *T. flavus*, and *Commelina* collectively contributed to about 35% dissimilarity between access and reduction plots. *A. gerardii* was approximately 177% and *T. flavus* was 90% more abundant in reduction plots than access; *Commelina* was more abundant in access plots by 45%. During this time, *Bromus* was the most abundant species across mammal treatments and 42% more abundant in mammal access plots compared to mammal reduction plots. In July of 2015, the same three species contributed to about 30% of the dissimilarity. *A. gerardii* was 125% and *T. flavus* was 85% more abundant in reduction plots than access; *Commelina* was 98% more abundant in access plots. *T. flavus* was the most abundant species across mammal treatments in July 2015. In August 2015, *T. flavus* and *A. gerardii* were still most influential, in addition to *Melothria*. These species contributed to 33% of the dissimilarity. *A. gerardii* (by 105%) and *T. flavus* (by 98%) were more abundant in reduction plots. *Melothria* was 200% greater in access plots. *T. flavus* was the most abundant species across mammal treatments in August 2015.

May 2015 Composition				
	Average Abundance			
Species	Access	Reduction	Contrib%	Cum.%
T. flavus	5.62	14.73	12.93	12.93
A. gerardii	0.99	15.97	12.69	25.62
Commelina spp.	5.84	3.69	9.61	35.23
Vicia spp.	10.96	5.22	7.11	42.34
Litter	13.47	15.46	6.64	48.98
A. ludoviciana	3.27	0.00	6.3	55.28
July 2015 Composition				
Commelina spp.	24.42	8.37	12.31	12.31
T. flavus	13.76	34.13	9.55	21.85
A. gerardii	2.18	9.41	8.68	30.53
C. glandulosus	10.61	8.77	8.50	39.04
S.vscoparium	2.64	6.17	7.49	46.52
Litter	33.39	15.47	6.45	52.97
August 2015 Composition				
T. flavus	11.04	32.34	12.73	12.73

M. pendula	8.23	0.00	10.60	23.33
A. gerardii	3.65	11.73	10.30	33.63
Commelina spp.	14.05	9.49	9.34	42.97
C. glandulosus	11.64	7.00	7.42	50.38
Dicanthelium spp.	1.54	2.26	6.51	56.89



Appendix 5 Arrangement of experimental plots in the field. Each circle is a plot with the symbol representative of the N treatment (+ = N added; - = ambient N) and the letter representative of the invertebrate treatment (F=full mesh, invertebrate reduction; L=leaky mesh, invertebrate access). Solid lines represent fences. N and invertebrate herbivore treatments were randomly assigned to plots within the mammal access and the mammal reduction areas.



Appendix 6 Herbivory alters diversity of this grassland. (a) Mammal herbivory, but not soil N, increased species richness by 17% in August. (b) Invertebrate herbivory did not alter species richness. (c) Mammal herbivory and N did not alter evenness. (d) Invertebrate herbivory decreased evenness by 8% on average across mammal treatments in May. (e) Mammal herbivory, but not soil N increased diversity in August by 10%. (f) Invertebrates decreased diversity by 17% across mammal treatments. (*) represent significant difference between mammal treatments. Different letters represent

significant difference among N treatments or among invertebrate treatments. Error bars represent standard error. For significance values not reported here, refer to Table 1.

Appendix 7 SIMPER results for invertebrate effects on composition during May 2015. Contrib% shows how much each species contributes to dissimilarity of plot up to approximately 40% cumulative contribution (Cum.%).

May 2015 Composition

Average Abundance Across Mammal Treatments

Species	Invertebrate Access	Invertebrate Reduction	Contrib%	Cum.%
T. flavus	10.63	12.47	12.09	12.09
A. gerardii	9.28	6.31	11.70	23.79
Commelina spp.	6.53	2.66	10.55	34.34
Vicia spp.	6.34	10.78	6.60	48.17
A. ludoviciana	1.25	2.09	5.69	53.85
Geranium spp.	1.53	0.63	5.01	58.86
C. glandulosus	2.09	1.91	4.87	63.73
Bromus spp.	43.44	43.44	3.99	72.22
Ulmus spp.	0.06	0.00	3.85	76.08
Dicanthelium spp.	0.72	0.28	3.40	79.48
Cyperus spp.	0.13	0.91	2.59	85.17
A. psilostachya	0.56	0.00	2.22	87.39
S. halepense	1.25	0.00	1.72	89.11
Vitis spp.	0.00	1.25	1.59	90.70

Average Abundance Within Small Mammal Access

	Invertebrate Access	Invertebrate Reduction		
T. flavus	5.88	6.25	11.72	11.72
Commelina spp.	8.19	3.50	11.56	23.29

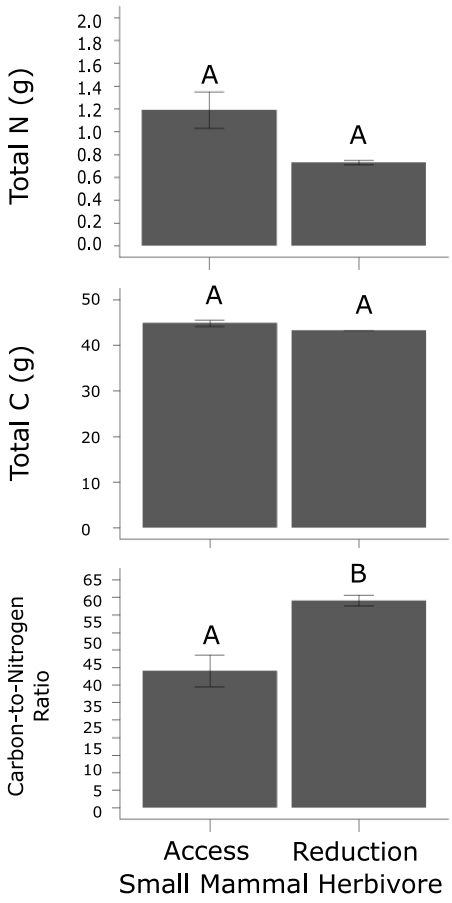
A. ludoviciana	2.50	4.19	9.71	33.00
Vicia spp.	8.38	14.69	7.22	49.15
C. glandulosus	2.25	1.69	5.50	61.13
A. gerardii	1.69	0.44	4.91	66.03
Cyperus spp.	0.13	1.69	4.09	70.12
S. halepense	2.50	0.00	3.57	77.41
A. psilostachya	0.88	0.00	3.18	80.60
Bromus spp.	56.25	48.75	2.75	83.35
Dicanthelium spp.	0.00	0.56	2.29	85.64
Geranium spp.	0.44	0.13	2.04	87.68
Smilax spp.	0.00	0.44	1.72	89.40

Average Abundance Within Small Mammal Reduction

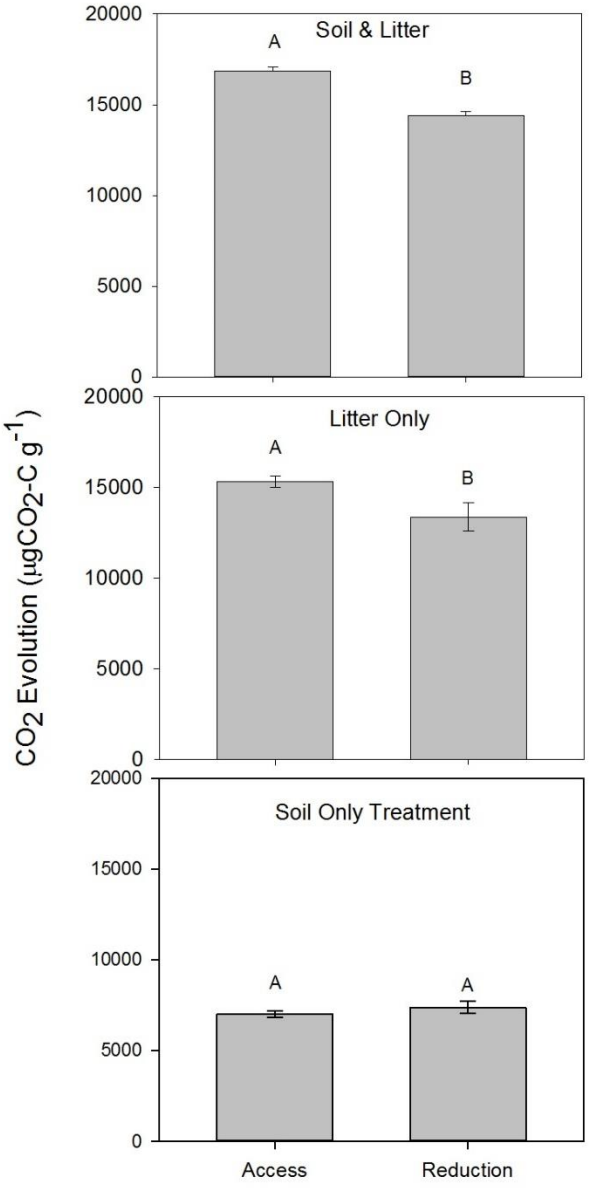
	Invertebrate Access	Invertebrate Reduction		
A. gerardii	16.88	12.19	17.37	17.37
Commelina spp.	4.88	1.81	10.81	28.18
T. flavus	15.38	18.69	10.5	38.68
Geranium spp.	2.63	1.13	8.04	46.71
Ulmus spp.	0.00	10.31	7.15	53.86
Dicanthelium spp.	1.44	0.00	6.08	66.27
C. glandulosus	1.94	2.13	4.85	76.01
Vicia spp.	4.31	6.88	4.49	80.5
Bromus spp.	30.63	38.13	4.34	84.84
Vitis spp.	0.00	2.50	3.47	88.31
Gallium spp.	0.00	2.50	2.63	90.93

Appendix 8 Treatment combinations for laboratory incubation.

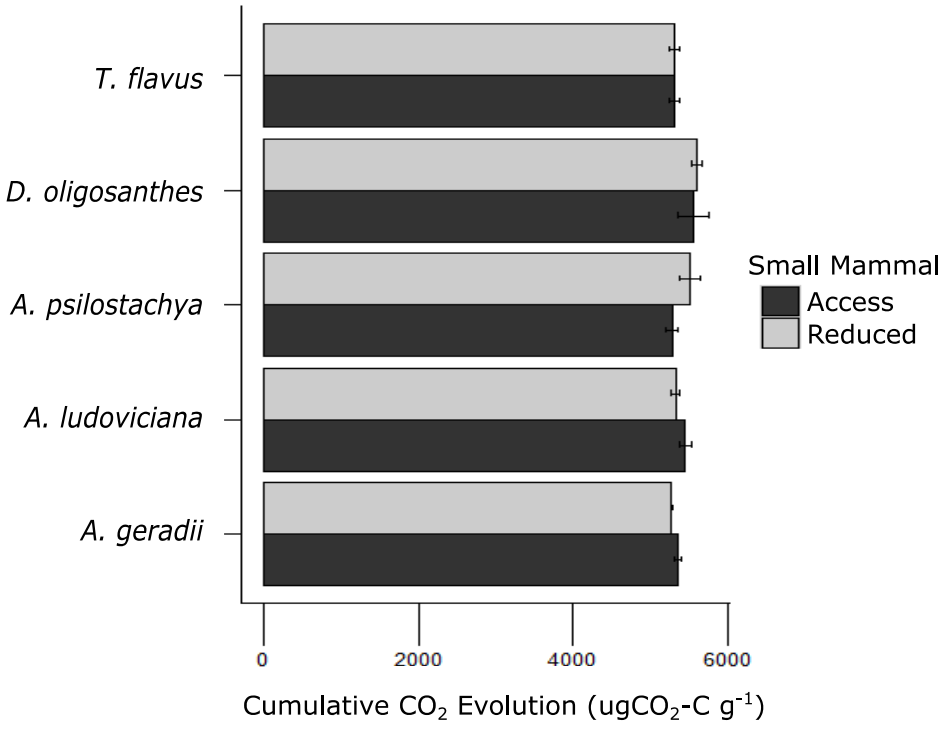
	Litter Origin		
Soil Origin	Herbivore Access	Herbivore Reduced	No Litter
Herbivore Access	Access Home	Access Away	Soil Only Access
Herbivore Reduced	Reduced Away	Reduced Home	Soil Only Reduced
Sterilized Soil	Litter Only Access	Litter Only Reduced	N/A



Appendix 9 Litter quality differences between small mammal access and reduced litter assemblages. Different letters represent significant differences according to a t-test assuming unequal variances. Total N (t-ratio -2.82, p-value=0.10) and total carbon (C) content (t=-2.28, p-value=0.15) did not significantly differ between treatments. However, the carbon-to-nitrogen ratio was significantly lower when small mammals were present rather than reduced (t=4.15, p-value=0.04). Error bars represent standard error.



Appendix 10 Differences in decomposition were most likely driven by litter differences. Decomposition rate was 15% higher when both litter and soils originated from small mammal access treatments in laboratory incubation studies (F-ratio 58.02, p-value<0.0001). Also, decomposition rate of plant litter alone, without the influence of soil properties), was 60% higher in small mammal access treatments than reduction (F-ratio 5.51, p=0.05). However, small mammal treatments did not significantly differ in decomposition rate in soil only microcosms (F-ratio=0.95, p-value 0.34). Error bars represent standard error.



Appendix 11 Some species differ in decomposition rate between small mammal treatments. Bars represent mean CO_2 evolution. Error bars represent standard error.

Appendix 12 AICc ranking for models with lowest AICc scores.

Model	Number	RSquare	AICc
Small Mammal	1	0.81	257.3
Total Aboveground Biomass, Small Mammal	2	0.84	258.5
Total Aboveground Biomass, Small Mammal, Diversity	3	0.87	259.3

Appendix 13 PERMDISP results for plant species composition in multivariate space for each data collection time. Letters represent different treatments: Acc = mammal access, Exc = rodent reduction, N = Nitrogen added, C = ambient N, F = full mesh (invertebrate reduction), L = leaky mesh (invertebrate access). Bolded values are statically significant.

PERMDISP 2015			May			July			August		
Source		df	t	P (perm)	Model P	t	P(perm)	Model P	t	P(perm)	Model P
Mammal	Acc,Exc	1,30	0.95	0.36	0.36	0.73	0.50	0.50	0.10	0.93	0.92
N	Acc[N,C]	3,28	1.03	0.32	0.20	1.21	0.28	0.63	2.59	0.04	**0.03**
(Mammal)	Exc[N,C]		1.21	0.29		0.15	0.89		2.19	0.06	
Invertebrate	Acc[F,L]	3,28	1.21	0.28	0.63	2.65	**0.03**	0.10	0.94	0.44	0.59
(Mammal)	Exc[F,L]		0.48	0.67		0.07	0.96		1.42	0.21	