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ABSTRACT

Global climate change—including increased temperature, altered precipitation patterns, and nutrient deposition—may reshape plant–arthropod relationships. Arthropods comprise the majority of animal biodiversity on Earth and provide critical ecosystem services. Thus, understanding arthropod responses to multiple climate-change stressors is an important step towards maintaining healthy ecosystems. This is especially true in natural grasslands, a habitat covering 37% of the Earth's land surface but rapidly shrinking due to anthropogenic impacts. In my dissertation I explore the effects of changing environments on plant communities and how those factors affect insect food choice and the long-term maintenance of grassland arthropod communities.

In chapter one, I explore how seasonal and diel temperature change shapes the foraging rate and demand for two resources, salt and sugar, in a grassland ant community. Across seven months, I found that recruitment to salt, but not sugar, accelerated with temperature. In ectotherms like ants, sugar is stored in cells and sodium is pumped out of cells proportional to temperature. A three-month follow up experiment verified that temperature-dependent recruitment to sugar concentrations of 20% (mimicking rich extrafloral nectaries), was half as temperature-dependent as recruitment to salt. Combined, I demonstrate how ecosystem warming accelerates the work done by a community of ectotherms, and how the demand and use of fundamental nutrients can be differentially temperature dependent.

In chapter two, I focus on how plants regulate grassland arthropod abundance and diversity via biotic and abiotic factors. I factorially combined three clipping treatments with NPK fertilization to manipulate plant biomass, plant quality, and habitat heterogeneity. Clipping raised surface temperature and simplified habitat structure. Together, this decreased arthropod

abundance and diversity while increasing arthropod activity. Fertilization mediated the reduction in arthropod abundance and diversity by increasing plant quality and plant biomass which indirectly decreased surface temperature. By itself, fertilization increased arthropod abundance, activity, and richness. Altogether, changing microclimate, plant quality, and plant biomass shifted arthropod community composition toward more diverse communities, demonstrating the importance of habitat heterogeneity and plant quality in structuring grassland arthropod community composition.

In chapter three, I explore how altered precipitation regimes and anthropogenic disturbances may change plant–arthropod relationships in grasslands. I used an experimental precipitation gradient combined with human management to examine: (1) how altered precipitation and biomass removal synergistically affect abiotic factors and plant communities and (2) how these effects cascade upward, impacting the arthropod food web. Both drought and hay harvest increased soil surface temperature while drought, but not hay harvest decreased soil moisture. Arthropod abundance decreased with low soil moisture and, contrary to my predictions, decreased with increased plant biomass. Arthropod diversity tracked arthropod abundance but was unaffected by plant diversity or quality. Combined, I show arthropod abundance is directly controlled by abiotic factors and plant biomass, in turn constraining local arthropod diversity. If robust, this result suggests climate change in the southern Great Plains may directly reduce arthropod diversity.

In chapter four, I investigate the effects of anthropogenic nutrient deposition on grassland food webs. I used a fertilization gradient to track nutrient addition through a food web, measuring changes to soil and plant fertility, plant and arthropod communities, and ultimately, herbivory. Using a multi-year experiment, I tested the mechanisms driving herbivory within and

across fertilizer quantities and durations. Fertilization increased soil fertility 100-fold and generated a 1.3-fold increase in herbivory. This herbivory increase weakened over time—from a 1-year pulse experiment to a 2-year press experiment—as herbivory damage shifted from sucking herbivores (e.g., aphids) to chewing herbivores (e.g., grasshoppers). Overall, I found the rather paradoxical result that fertilization increased herbivore abundance but decreased herbivory. Combined, I demonstrate the rippling effects of changing soil fertility on the abundance and function of a prairie food web, predicting herbivore abundance and herbivory.

CHAPTER ONE

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USING METABOLIC AND THERMAL ECOLOGY TO PREDICT TEMPERATURE DEPENDENT ECOSYSTEM ACTIVITY: A TEST WITH PRAIRIE ANTS

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Abstract

As ecosystems warm, ectotherm consumer activity should also change. Here we use principles from metabolic and thermal ecology to explore how seasonal and diel temperature change shapes a prairie ant community's foraging rate and its demand for two fundamental resources: salt and sugar. From April through October 2016 we ran transects of vials filled with solutions of 0.5% NaCl and 1% sucrose. We first confirm a basic prediction rarely tested: the discovery rate of both food resources accelerated with soil temperature, but this increase was typically capped at midday due to extreme surface temperatures. We then tested the novel prediction that sodium demand accelerates with temperature, premised on a key thermal difference between sugar and sodium: sugar is stored in cells, while salt is pumped out of cells proportional to metabolic rate, and hence temperature. We found strong support for the resulting prediction that recruitment to NaCl baits accelerates with temperature more steeply than recruitment to 1% sucrose baits. A follow up experiment in 2017 verified that temperature dependent recruitment to sucrose concentrations of 20% (mimicking rich extrafloral nectaries), while noisy, was still only half as temperature dependent as recruitment recorded for 0.5% NaCl. These results demonstrate how ecosystem warming accelerates then curtails the work done by a community of ectotherms, and how the demand and use of fundamental nutrients can be differentially temperature dependent.

Keywords: thermal ecology, grasslands, ectotherms, ants, metabolic theory of ecology, stoichiometry, foraging

Introduction

A key goal of ecology is to predict when and where resource shortfalls are likely to constrain consumer activity, and hence the work consumers perform in ecosystems (Sterner and Elser 2002, Kaspari et al. 2008, Anderson-Teixeira and Vitousek 2012). Consumer performance is often limited by shortfalls of sodium and sucrose (Galef 1996, Mayntz et al. 2005, Raubenheimer et al. 2009, Snell-Rood et al. 2014). Here, we explore the premise that temperature drives ectotherm foraging for salt and sugar based on temperature's effects on physiological demand and perceived risk of environmental shortfall (Danger et al. 2008, Geerling and Loewy 2008, Kaspari et al. 2010). We do so by exploiting seasonal and daily temperature variation in a sodium-limited prairie ant community (Kaspari et al. 2008). Ants are a model system for this question as they are ubiquitous in terrestrial ecosystems (Hölldobler and Wilson 1990, Kaspari et al. 2000), and most ant communities consist of herbivores that feed on sugary exudates, predators and scavengers that feed on animal tissue, and omnivores that feed on both (Blüthgen et al. 2003, Tillberg et al. 2006, Roeder and Kaspari 2017).

Temperature dependent foraging: insights from metabolic and thermal ecology

Foraging activity in ectotherms has long been linked to temperature via temperature's effect on metabolic rate (Gillooly et al. 2001, Andrew et al. 2013, Stuble et al. 2013, Baudier et al. 2015). Two links are relatively well explored. First, metabolic rate measures the ability of an organism to do work. In ectotherms, metabolic rate--a constraint on activity--increases exponentially over a range of environmental temperatures (Gillooly et al. 2001, Brown et al. 2004). Accelerating performance with temperature has been documented at the scale of organisms and their tissues (Dell et al. 2011) and for ecosystem processes like respiration and photosynthesis (Anderson-

Teixeira and Vitousek 2012). Second, beyond some critical temperature, the decline of metabolic rate and organismal performance is well documented, even as the mechanisms are not well-understood (Kingsolver and Huey 2008, Angilletta 2009). Combined, both the acceleration and abrupt decline of activity with temperature should apply to the collective action represented by an ecosystem's insect community (Losey and Vaughan 2006, Kaspari et al. 2015). This powerful prediction is rarely tested, despite the importance of arthropod actions such as pollination (Garibaldi et al. 2013), decomposition (Nichols et al. 2008), and seed dispersal (Hughes and Westoby 1990).

A third link between temperature and foraging activity is temperature's effect on nutrient demand relative to supply (Kay 2002). Two nutrients, sodium and sucrose, differ in their consumer demand based on their storage in the body. Sodium is constantly evacuated from cells by ATPases into an organism's intercellular fluid and has no organic storage form, so consumers must constantly harvest sodium to replace their losses through excretion (Maddrell 1972, Kaspari 2014). In contrast, sugars have numerous long chain forms (e.g. glycogen, starches) that can be stored in cells and released on demand. This leads to our third predicted connection between temperature and foraging: if an ectotherm's excretion rate (including its sodium losses) is proportional to its metabolic rate (Peters 1986) then as metabolic rate increases exponentially with temperature, demand for sodium should also increase exponentially with temperature to balance increasing losses. As sucrose is easily stored in the body, we predict demand for sodium will accelerate at a faster rate as temperature increases, relative to the demand for sucrose which will show weaker and noisier temperature sensitivity.

We test these three predictions across a seasonal and diel gradient of temperature in an Oklahoma prairie, surveyed with a grid of 300 salt and sugar baits multiple times during the day

over a seven-month period. Specifically, we predict that the overall number of baits that ants discover in an hour will accelerate with soil temperature (a proxy for colony temperature) since colony metabolic rate governs ant activity. Second, we predict that this relationship breaks down at high surface temperatures that impede individual forager performance outside the nest. Finally, since rising colony temperatures increase the loss of sodium relative to sucrose, we predict that the demand for sodium--measured by the number of ants recruited to baits--accelerates faster for NaCl than sucrose baits.

Methods

We studied two grassland ant communities: one at the University of Oklahoma (OU) Centennial Prairie (35.19° N, 97.45° W) in Cleveland County, Oklahoma, from April through October 2016, and the other at Pigtail Alley Prairie (33.89° N, 96.84° W) in Marshall County, Oklahoma, from May through July 2017. The OU Centennial Prairie is 7.7 ha and is mowed once a year in November, but is otherwise undisturbed. Pigtail Alley Prairie is 24.5 ha and was last farmed >20 yr ago but has been undisturbed since.

The OU Centennial Prairie has 16 ant species and is numerically dominated by *Crematogaster lineolata*, *Forelius pruinosus*, *Formica pallidefulva*, and *Monomorium minimum*. Pigtail Alley Prairie has 9 ant species and is numerically dominated by *C. lineolata*, *F. pruinosus*, and *Nylanderia terricola*. At both sites the dominant plant species are *Schizachyrium scoparium*, *Sorghastrum nutans*, and *Andropogon gerardii* (Kaspari et al. 2016b).

Sampling salt vs. sugar discovery

To measure how salt and sugar discovery changed with temperature we set out labeled 1.5-mL Eppendorf vials half-stuffed with cotton and saturated with either 1% sucrose or 0.5% NaCl

solution by mass at the OU Centennial Prairie (Kaspari et al. 2008). We conducted our sampling along three 100-m transects spaced 50 m apart. We flagged each transect every 1 m with white PVC surveyor flags. To run a transect, 50 closed vials of each solution were thoroughly mixed (100 vials total per transect). Walking along the transect, a vial was selected at random, opened, and placed next to a flag, every 1 m. To understand what temperatures were important to ant discovery and recruitment to baits, we recorded three measures of temperature every 20 m along each transect while the vials were out, for a total of six measurement locations per transect. We measured soil temperature at 10cm depth using a temperature probe (to 0.1 °C, Taylor Precision, Oak Brook, Illinois, USA); surface temperature with an infrared thermometer (to 0.1 °C, Nicety, Starmeter Instruments Co., Ltd., Shenzhen, Guangdong, China) next to the vial; and air temperature (to 1 °C, AcuRite Chaney Instrument Co., Lake Geneva, Wisconsin, USA) at 85 cm off the ground. After 1 h, we collected the vials by snapping the cap shut to capture all ants inside, and recorded the location of each vial containing ants. We used the above assay to sample ant foraging behavior three times a day (09:00, 13:00, and 17:00) from April through October 2016, four times a month, allowing us to explore ant response across a wide range of diel and seasonal temperature variation.

Recruitment at different sugar concentrations: a follow-up experiment

Ants obtain sugars from extra-floral nectaries and by tending hemiptera. These sources of sugar may have sucrose concentrations from 10% to 50% by mass (Josens et al. 1998, Paul and Roces 2003, Kim et al. 2011). Our first assay (OU Centennial Prairie, April through October 2016) may not have fully confirmed our hypotheses because it used a low sucrose concentration and thus did not adequately test absolute sucrose demand by the ant community. To remedy this problem,

and further explore the relationship between sugar demand and temperature, we performed a second assay at a different location, Pigtail Alley Prairie. Our goal was to see if ant recruitment to sucrose baits at higher temperatures would accelerate as sucrose concentration increased, and if so, whether that acceleration in recruitment was greater than the acceleration in recruitment to NaCl baits.

Similar to our 2016 experiment, we used labeled 1.5-mL Eppendorf vials half-stuffed with cotton and saturated with either a 1%, 5%, 10%, or 20% sucrose solution by mass (Kaspari et al. 2008). We conducted our sampling along two 80-m transects spaced 50 m apart. As before, we flagged both transects every 1 m with white PVC surveyor flags. To run each transect, we thoroughly mixed 20 closed vials of each solution (80 vials total per transect). As in our 2016 experiment, a vial was selected at random every 1 m, opened, and placed next to a flag. After 1 h we collected vials by snapping the lid shut to capture all ants inside. We performed this assay one time a week, twice a day (8:00 and 16:00) from May through July 2017, to observe ant foraging patterns at the hottest part of the day. In the hour while the vials were out, we measured soil, surface, and air temperature at five spots per transect (every 20 m) using the same equipment as in 2016.

Hypothesis Testing

We tested the prediction that increasing temperature accelerates discovery rates to both NaCl and sucrose baits but accelerates recruitment to NaCl baits at a higher rate relative to sucrose baits. To do this, we first compared the average discovery of NaCl and sucrose baits against the average temperature across the three transects for a given date and time of day. We found no consistent temperature difference among the three 100-bait transects, but consistent differences

in temperature with time of day (09:00, 13:00, and 17:00). Thus, the value reported for each nutrient is the response to 150 vials, averaged over three transects of 50 vials (expressed, for example, as the average number of NaCl baits (of 50) discovered at 09:00, 13:00, and 17:00). We generated 82 estimates of ant discovery and recruitment from April through October 2016.

We focus on two responses of the ant community to temperature. A vial was considered discovered if any ant was present in the vial after the 1-h sampling period. The number of salt and sugar vials containing ants per transect, averaged across transects, represented the average ant activity during a sampling period. While ants may have discovered a vial and abandoned it prior to pick-up, we observed vials while taking temperature measurements and vials containing ants after 10–30 minutes often still contained ants after 1 h. Next, we assumed the demand for a nutrient would be reflected by more worker ants being recruited, and hence accumulating in the vial. Therefore, we assume that recruitment, and hence colony demand, is the number of ants found in a vial (of those containing an ant) at the end of an hour (while acknowledging that factors such as worker size and colony size may also play a role). Moreover, while this method risks occasionally scoring two or more conspecific ants from different colonies as recruitment, we assume inter-colony competition makes this rare.

Statistical Analysis

To examine the seasonal change in discovery and recruitment to NaCl and sucrose vials we first performed four separate Kruskal-Wallis tests using month as the predictor and either average discovery to NaCl and sucrose baits or recruitment to NaCl and sucrose baits as the response. We tested Pearson correlation coefficients to check for a relationship between average soil and surface temperatures, average air and surface temperatures, and average air and soil

temperatures. We also checked for multicollinearity among the different temperature measures using a variance inflation factor cutoff of 3. Next, we examined the relationship between average soil and average surface temperature and average discovery and recruitment using multiple regression, leaving out air temperature because of multicollinearity. Because the multiple regression showed soil temperature had a stronger relationship to ant discovery and recruitment to vials relative to surface temperature, we used only soil temperature in our remaining analyses. To test the influence of temperature, nutrient, and temperature and nutrient interactions on ant discovery and recruitment to baits we performed an ANCOVA (using type III sum of squares) with either average discovery or recruitment as our response, nutrient (NaCl or sucrose) as our predictor, and average soil temperature as our covariate. To better partition the coupled effects of temperature promotion and inhibition on foraging activity, our analysis separated the 09:00, 13:00, and 17:00 trials, resulting in six separate ANCOVAs. We also used an ANCOVA to test the separate and interactive influence of temperature and sucrose concentration on average discovery and recruitment to four sucrose concentrations (1%, 5%, 10%, and 20%) at Pigtail Alley Prairie. Again, we used average discovery and recruitment as our responses, sucrose concentration as our predictor, and average soil temperature as our covariate. Our statistical analyses were conducted in R version 3.3.2 (R Development Core Team 2016).

To estimate the curve fit across the range of temperatures in the study linking temperature to discovery and recruitment of ants to baits, we used a non-linear iterative damped least squares algorithm initiated by a random number seed (Marquardt 1963, Press et al. 1986) and implemented by SigmaPlot version 14.0 (Systat Software, San Jose, CA). This algorithm fits a non-linear curve with parameter values that minimize the sum of squares differences between observed and predicted values of the response variable. We used the curves generated by

SigmaPlot to calculate the Q_{10} values for average discovery and recruitment to vials. Q_{10} is a descriptive statistic and is a standard measure of the temperature dependence of a process (Angilletta 2009). Q_{10} is calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where R_2 is the rate (i.e. discovery or recruitment) at a higher temperature (T_2) and R_1 is the rate at a lower temperature (T_1). Because we calculated Q_{10} values for a 10°C temperature change at each site, the exponent equals 1. Specifically, we calculated Q_{10} values for average discovery and recruitment rates to vials between 20° and 30°C at OU Centennial Prairie and between 24° and 34°C at Pigtail Alley Prairie because soil temperatures there did not drop to 20°C. Because Q_{10} maps on to the activation energy of metabolic theory (Dell et al. 2011) we use it as a descriptive statistic to compare the temperature dependence of the processes of ant discovery and recruitment to NaCl and sucrose vials. Specifically, we use Q_{10}s to corroborate the prediction that recruitment to NaCl baits has a higher Q_{10} than recruitment to sucrose baits.

Finally, to examine consistency of temperature sensitivity among common species, we compared the temperature dependence of species discovery rates for the four numerically dominant species at the OU Centennial Prairie. To do this we first summed NaCl and sucrose discovery for a given species/sampling period then binned sampling events by integer temperature (e.g., 15°C = all observations from 14.5° to 15.49°C) and finally expressed the temperature-discovery curve of each species as a proportion of the highest discovery rate across all soil temperatures recorded (i.e., with a maximum of 1.0). We again used SigmaPlot to fit a curve on discovery rates for each species and used this curve to calculate the Q_{10} value for discovery.

Results

In the 2016 experiment, the average number of NaCl and sucrose baits discovered by ants varied monthly, although there was a larger seasonal change in the discovery of NaCl than sucrose baits (Kruskal-Wallis $X^2 = 44.8$, df = 6, $P < 0.001$ and K-W $X^2 = 29.0$, df = 6, $P < 0.001$ respectively; Appendix S1: Fig. S1a). The average recruitment of ants showed a similar pattern, varying 7-fold within a month for NaCl, while sucrose recruitment was lower and varied less (K-W $X^2 = 52.4$, df = 6, $P < 0.0001$ and K-W $X^2 = 13.7$, df = 6, $P = 0.03$; Appendix S1: Fig. S1b).

Prediction: rising soil temperatures enhance activity, extreme surface temperatures suppress it

Three measures of environmental temperature covaried with month and time of day in the 2016 experiment, but to varying degrees (Appendix S1: Fig. S2). Soil temperature averaged 26.2°C across June and July, and increased about 8°C from 09:00 to 17:00 every day. In contrast, surface temperatures were highly variable, fluctuating up to 30°C in a given day with a peak at 13:00. Dynamics of air temperature were intermediate. Across the 82 sampling events, all temperature measurements were correlated (average soil and surface temperature Pearson $r = 0.43$, $P < 0.001$; average air and surface temperature Pearson $r = 0.65$, $P < 0.001$; and average air and soil temperature Pearson $r = 0.81$, $P < 0.001$; Appendix S1: Fig. S3). As stated above, we tested for multicollinearity (average soil temperature VIF = 1.29, average surface temperature VIF = 1.35, average air temperature VIF = 4.82) and removed air temperature from analyses because of multicollinearity.

Soil, surface, and air temperatures differed in their association with discovery and recruitment (Appendix S1: Fig. S4). Multiple regression revealed that average discovery rates of NaCl roughly doubled with increasing soil temperature ($P < 0.001$; Table 1) and were suppressed

as surface temperatures rose ($P = 0.01$; Table 1), accounting for half the variation in NaCl discovery. Sucrose discovery rate showed a similar pattern of increasing with soil temperature ($P = 0.002$; Table 1), accounting for about one-third of the variation in discovery.

Prediction: discovery rates accelerate with soil temperature

We next test the prediction that activity of ant foragers should accelerate with the temperature they experience. Our ANCOVAs showed that average discovery rate of NaCl baits accelerated at all three times of day, with power law exponents >2.8 and Q_{10} values from 3.2 to nearly 14 (Fig. 1, Appendix S1: Table S1). Average discovery of sucrose baits showed a similar accelerating pattern at 09:00 and 17:00. At 13:00 and 17:00, average NaCl and sucrose discovery rates increased similarly (temperature effects $P = 0.04$ and $P < 0.001$ respectively, nutrient and interaction effects NS; Table 2). However, inconsistent with our implicit assumptions that discovery rates of both nutrients would increase with temperature, the average discovery rates were higher for NaCl than for sucrose at 09:00 ($P = 0.003$; Table 2), with almost four times the Q_{10} (Fig. 1). This inconsistency disappears in the other two time samples.

At the OU Centennial Prairie, four species made up 94% of the bait discoveries (Appendix S1: Fig. S5). Average discovery rates of three of the four species increased as a power law with temperature ($r^2 = 0.8$ to 0.93) with Q_{10}s of 4.4 to 5.0 (Appendix S1: Fig. S5). In contrast, average discovery rates of one species, *F. pallidefulva,* showed a relatively uniform, linear decrease with temperature (Appendix S1: Fig. S5c).

Prediction: demand for salt accelerates faster than demand for sugar

Across all three times of day, a significant interaction between temperature and nutrient reflected stronger temperature dependent recruitment to NaCl compared to sucrose ($P < 0.001$; Fig. 1,

Table 2). In each case, recruitment to NaCl increased exponentially with temperature (b = 4.4, 3.0, 3.7 and $P < 0.001$; Appendix S1: Table S1) and failed to vary for sucrose (b = 0, 0.1, 0.4 and $P > 0.05$; Appendix S1: Table S1). Q_{10} values for recruitment to NaCl baits ranged from 3.4 to 6.0 while Q_{10} values for recruitment to sucrose baits ranged from 1.0 to 1.2, further corroborating the prediction that recruitment to NaCl baits was more temperature dependent than recruitment to sucrose baits (Fig. 1).

As previously mentioned, a potential bias occurred by our use of 1% sucrose in the 2016 baits. This concentration may have been unattractive compared with other sugar sources such as extrafloral nectaries or exudates, resulting in low ant recruitment (Josens et al. 1998, Paul and Roces 2003, Kim et al. 2011). In a 2017 follow-up experiment, sucrose was offered at four concentrations (1%, 5%, 10%, and 20%). Our ANCOVAs showed average recruitment remained highly sensitive to temperature ($P < 0.001$; Appendix S1: Table S2) and sucrose concentration ($P < 0.001$; Fig. 2, Appendix S1: Table S2). However, given the noisy data, there was not a significant interaction between temperature and sucrose concentration for either discovery or recruitment to sucrose vials ($P > 0.05$; Appendix S1: Table S2), and the slopes of the power laws did not approach statistical significance (Appendix S1: Table S3).

Discussion

Temperature can drive the activity of ectotherm assemblages in at least three ways. Higher temperatures release a constraint on metabolism, allowing ectotherms to generate and use more ATP; higher temperatures can increase the rate that resources are used and depleted from the body and hence increase demand; and ultimately higher temperatures cause all metabolic activity to slow then shut down when thermal limits are exceeded (Gillooly et al. 2001, Clarke and Fraser

2004). We generated and tested three quantitative predictions and found that activity in a prairie ant community accelerated with soil temperature consistent with release of a basic metabolic constraint before shutting down at high temperatures. We found that demand for 0.5% sodium, a food resource more likely to be lost at higher temperatures, accelerated faster than the demand for 1% sucrose and had higher Q_{10} values than multiple sucrose concentrations (1–20%).

Our most novel discovery arises from the surmise that two vital foods have different temperature sensitivities. Herbivore and decomposer activity is frequently constrained by sodium shortfall given the need of these trophic groups to enhance their body tissue concentrations 100-fold over the plants they consume (Kaspari et al. 2008, Clay et al. 2014, Kaspari et al. 2014, Snell-Rood et al. 2014). However, ionic nutrients like K^+, Na^+, and Cl^- that are water soluble (as well as water itself) are all subject to excretion, with dynamics driven by metabolic rate (Peters 1986). As a consequence, higher temperatures create proportionately greater demand for these resources than for those that are more easily stored, like sugars. In this study, we found similar discovery rates for sodium and sucrose at 13:00 and 17:00. However, even when we provided an ant community with sugar akin to that found from rich extrafloral nectaries (Völkl et al. 1999, Kay 2002, Petry et al. 2012) the Q_{10} value for recruitment to 0.5% NaCl was at least double the one for sucrose (Fig. 2), suggesting stronger temperature sensitivity for sodium relative to sucrose.

A second novel element is our focus on community behavior. A basic prediction from both metabolic and thermal ecology is that constraints on ectotherm activity should ease as an accelerated function of the organism's temperature. This acceleration is often found when measuring the performance of individuals (Dell et al. 2011) and at the scale of ecosystem processes (Anderson-Teixeira and Vitousek 2012). In contrast, the study of communities

typically focuses not on the predicted similarities, but on the differences among species traits (Bennett and Lenski 1993, Cerdá et al. 1998, Feeley and Silman 2010). Here we show that the majority of individuals (and three of four most common species) in a prairie ant community accelerate their foraging activity with temperature (Appendix S1: Fig. S5).

At the same time, this larger trend highlights the behavior of an outlier species, *F. pallidefulva*, which by consistently decreasing its foraging activity with temperature clearly diverges from the foraging pattern predicted by a metabolic approach. This exploration of thermal space "around the edges" of the community points to the role that active competition or species filtering may play in driving this alternate thermal niche (Rosenzweig 1995, Cerdá and Retana 2000, Kaspari et al. 2016a). For example, subordinate species can alter their resource preference or the time of day they are active in the presence of a dominant species (Lynch et al. 1980, Savolainen and Vepsäläinen 1988, Andersen 1992, Cerdá et al. 1998, Sanders and Gordon 2003). In our prairies, the numerically dominant *C. lineolata* often filled salt vials, potentially reducing opportunities for *F. pallidefulva* and other species to use these vials when *C. lineolata* is active. If so, then studies of foraging time and bait preferences of *F. pallidefulva* and other subordinate species should converge on those of the dominant *C. lineolata* in the lab or in baits protected from *C. lineolata*.

Performance integrates over different measures of environmental temperature

During this study, we matched the ants in this prairie to temperatures they actually experience (Kearney and Porter 2009, Kaspari et al. 2015). Colonies that live in the soil experience a thermal environment that is predictable at a seasonal and daily timescale (Andrew et al. 2013, Stuble et al. 2013, Baudier et al. 2015). Specifically, soil temperature had a unimodal seasonal

and daily distribution (Appendix S1: Fig. S2). Soil temperature also appeared to be the most important factor driving ant discovery of, and recruitment to, food (see also Dunn et al. 2007). One confounding factor, however, that may shape the foraging behavior of ants is colony size as both the number of workers produced (Markin 1970, Tschinkel 1993) and the speed at which brood develop (Porter 1988, Penick et al. 2017) changes with temperature throughout the year. In future studies, disentangling how colony size contributes to the nutritional demands of ant colonies across temporal changes in temperature will undoubtedly result in new and exciting insights.

Surface temperature can represent the microclimate worker ants are exposed to while foraging (O'Neill and Kemp 1990) and frequently correlates with the number of ant species foraging (Cerdá et al. 1998, Bestelmeyer 2000, Lessard et al. 2009, Wittman et al. 2010, Stuble et al. 2013). At the OU Centennial Prairie, for example, high surface temperatures were useful in predicting when the ant community began its midday shutdown (i.e.16 species were recorded at 17:00, only eight at 13:00). Yet surface temperature can often be quite variable (Appendix S1: Fig. S2) and we posit that the resulting noisy foraging data at 13:00 likely arose in two ways, both based on insolation (Kearney and Porter 2009, Kaspari et al. 2015). First, early in the growing season, when soil was most exposed, high surface temperatures were common even when the air was still cool. Second, throughout the year, cloud cover could cool the soil surface and allow for higher midday foraging.

Future Directions

Our results have two implications for the ecology of a warming world. First, if the performance of ectotherm consumers accelerates with temperature as predicted by metabolic and thermal

ecology, then both the magnitude and starting point of any temperature change is key to predicting magnitude of the response (i.e., the Q_{10} from 10° to 20°C will be lower than that between 20° to 30°C). Put another way, in a warming world, the first response of ecosystem functions driven by consumers in ecosystems far from their thermal maximum (Deutsch et al. 2008) will be an acceleration, not a crash. One conclusion is somewhat paradoxical: that warm ecosystems, when warmed further, may show greater magnitudes of increase in herbivory, decomposition, seed dispersal and other ecosystem services driven by ectotherms than cooler ecosystems (or, perhaps, more variable responses as their thermal performance curves straddle the thermal optima).

Second, if the demand for ionic resources like sodium have strong thermal dependencies (Figs. 1 and 2), this reduces one opportunity for conservation in stressful environments. Consumers can choose to cease foraging to conserve storable resources like sugars; such a tactic is less viable for sodium, which is constantly pumped out of cells and excreted from the body, driving individuals closer to their minimum sodium set point. Thus, higher temperatures may release a community of consumers from a thermal constraint while increasing a sodium deficit in sodium poor environments. The size of an ectotherm community's activity Q_{10} (e.g., rate of herbivory or pollination) should thus, all else equal, be lower in inland ecosystems and higher in Na-rich coastal ecosystems (Kaspari et al. 2008).

In sum, evidence accumulates for the role of sodium shortfall as a constraint on terrestrial ectotherm assemblages (Kaspari et al. 2008, Clay et al. 2014, Kaspari et al. 2014, Snell-Rood et al. 2014). This study suggests an orthogonal factor, temperature, that can exacerbate or ameliorate the effects of low sodium supply. Increases in atmospheric CO_2 may enhance both temperature and carbohydrate production (Ainsworth and Long 2005). Soil nesting ants are

likely to be buffered from the direct effects of increasing temperatures and may benefit from increasing production of exudates. However, the accelerating effects of temperature on sodium demands may constrain ability of ant colonies to exploit these carbohydrates.

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Table 1. Results of multiple regression quantifying whether average soil or average surface temperature better account for average discovery (number of vials discovered per 50) and average recruitment (number of ants per vial) to NaCl and sucrose vials at the OU Centennial Prairie.

Nutrient and parameter	Estimate	Error	*t*	Pr > |*t*|
Discovery				
NaCl (r^2 = 0.47)				
Intercept	-28.78	5.84	-4.93	<0.001
Soil Temperature	2.30	0.27	8.53	<0.001
Surface Temperature	-0.32	0.12	-2.66	0.01
Sucrose (r^2 = 0.26)				
Intercept	-6.30	3.08	-2.04	0.04
Soil Temperature	0.56	0.14	3.94	<0.001
Surface Temperature	0.10	0.06	1.62	0.11
Recruitment				
NaCl (r^2 = 0.49)				
Intercept	-7.34	1.42	-5.16	<0.001
Soil Temperature	0.56	0.07	8.47	<0.001
Surface Temperature	-0.05	0.03	-1.56	0.124
Sucrose (r^2 = 0.02)				
Intercept	1.22	0.25	4.90	<0.001
Soil Temperature	0.003	0.01	0.26	0.80
Surface Temperature	0.003	0.01	0.63	0.53

Table 2. ANCOVA tests of temperature sensitivity on average discovery (log_{10} number of vials with at least one ant) and average recruitment (log_{10} number of ants per vial) of ants.

	Discovery		Recruitment	
Parameter	***F***	**Pr > *F***	***F***	**Pr > *F***
09:00 (df = 1,49)				
log_{10}(mean soil temperature)	48.68	<0.001	25.08	<0.001
Nutrient (NaCl vs. Sucrose)	9.58	0.003	21.91	<0.001
Interaction	9.97	0.003	27.02	<0.001
13:00 (df = 1,50)				
log_{10}(mean soil temperature)	4.65	0.036	13.95	<0.001
Nutrient (NaCl vs. Sucrose)	2.85	0.098	10.32	0.002
Interaction	2.99	0.09	12.71	<0.001
17:00 (df = 1,52)				
log_{10}(mean soil temperature)	68.94	<0.001	43.16	<0.001
Nutrient (NaCl vs. Sucrose)	1.80	0.19	19.95	<0.001
Interaction	2.07	0.16	25.15	<0.001

Note: Separate ANCOVAs were conducted for each time of day (09:00, 13:00, or 17:00). We test the predictions of (1) more overall bait discovery at higher temperatures (2) higher overall discovery of salt versus sugar baits, and (3) a steeper increase in discovery and recruitment to salt baits relative to sugar baits at higher temperatures.

Figure Legends

Figure 1. Responses of an Oklahoma prairie ant community to vials filled with 0.5% NaCl solution and 1% sucrose solution by mass as a function of mean soil temperature in trials from April through October 2016. Each point is the mean response to 150 vials per nutrient, split into three transects of 50 vials run during each time period. Number of vials discovered is a measure of discovery; ants per vial a measure of subsequent recruitment. Trials were run at 09:00, 13:00, and 17:00, representing gradually increasing soil temperature. Q_{10} values (see *Materials and Methods: Statistical analysis*) listed were calculated as the ratio of the rate of discovery or recruitment at 30°C vs. the rate at 20°C. Curves are best-fit power laws using the Marquardt-Levenberg algorithm and white asterisks represent a significant interaction (i.e., different slopes) using an ANCOVA analyzing $\log(Y) = \log(X)$.

Figure 2. Response of ant recruitment to different sugar concentrations over a range of soil temperatures. Curves are best fit power laws as in Fig 1. Q_{10} values are listed next to the line they represent, calculated as the ratio of the rate of discovery or recruitment at 34°C vs. the rate at 24°C. (A) Study of the ant community at Pigtail Alley Prairie, Oklahoma, USA, an ant community composed of species similar to those at the OU Centennial Prairie, Oklahoma, USA, offered baits with four different sucrose concentrations (1%, 5%, 10%, or 20%). (B) Temperature recruitment curves of ants to NaCl baits at 09:00 and 17:00 at the OU Centennial Prairie and the entire-day recruitment curve to 20% sucrose baits at Pigtail Alley Prairie.

Figure 1.

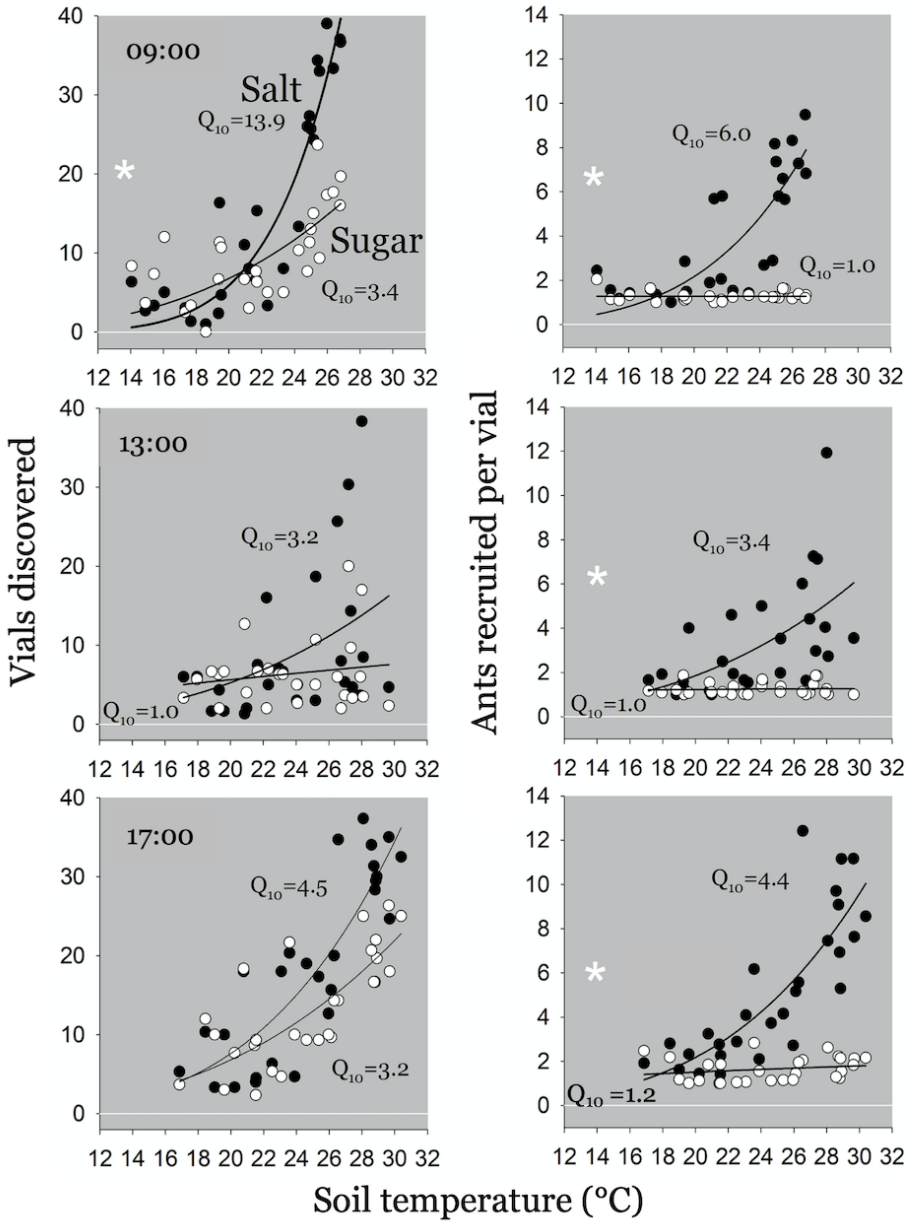
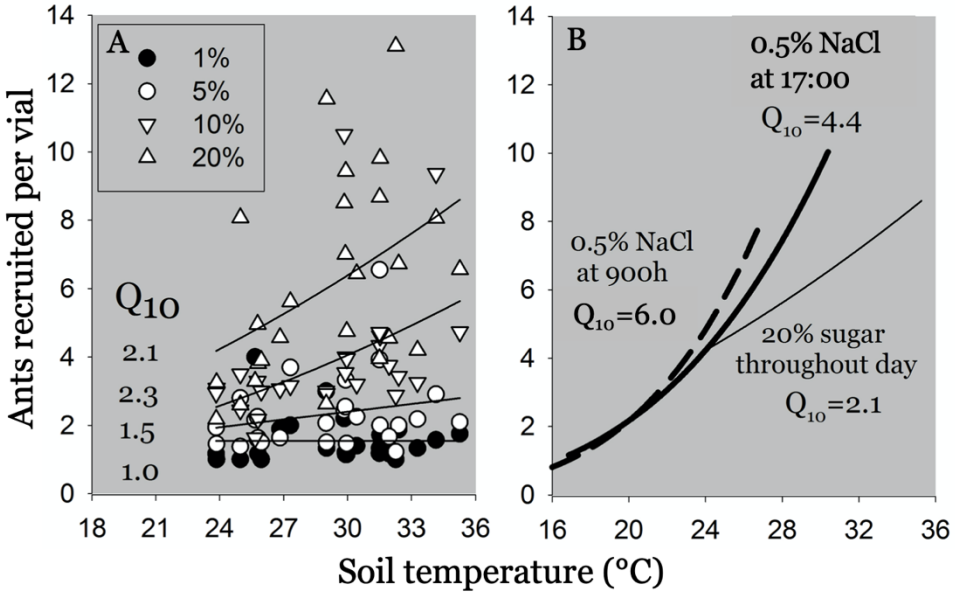

09:00
Salt
Q_{10}=13.9
Sugar
Q_{10}=3.4
Q_{10}=6.0
Q_{10}=1.0
13:00
Q_{10}=3.2
Q_{10}=1.0
Q_{10}=3.4
Q_{10}=1.0
17:00
Q_{10}=4.5
Q_{10}=3.2
Q_{10}=4.4
Q_{10} =1.2
Vials discovered
Ants recruited per vial
Soil temperature (°C)

Figure 2.


A
1%
5%
10%
20%
Q10
2.1
2.3
1.5
1.0
Ants recruited per vial
Soil temperature (°C)
B
0.5% NaCl
at 17:00
Q10=4.4
0.5% NaCl
at 900h
Q10=6.0
20% sugar
throughout day
Q10=2.1

Chapter 1: Appendix S1. Supplemental Data.

Table S1. Best-fit power law regressions ($y = aX^b$) for ant responses to NaCl and sucrose vials with soil temperature, broken down by time of day. Curves were fit with the Marquardt-Levenberg algorithm.

Parameter		Coefficient	Std. Error	*t*	Pr>*t*
09:00					
Discovery					
NaCl ($r^2 = 0.87$)	a	<0.001	<0.001	0.38	0.70
	b	6.50	0.81	8.07	<0.001
Sucrose ($r^2 = 0.44$)	a	0.001	0.002	0.42	0.68
	b	2.98	0.74	4.03	0.001
Recruitment					
NaCl ($r^2 = 0.66$)	a	<0.001	<0.001	0.37	0.72
	b	4.40	0.845	5.21	<0.001
Sucrose ($r^2 = 0$)	a	1.28	0.7254	1.76	0.09
	b	<0.001	0.1852	<0.001	1.00
13:00					
Discovery					
NaCl ($r^2 = 0.14$)	a	0.001	0.01	0.20	0.84
	b	2.86	1.52	1.88	0.07
Sucrose ($r^2 = 0$)	a	0.62	1.78	0.35	0.73
	b	0.74	0.90	0.82	0.42
Recruitment					
NaCl ($r^2 = 0.29$)	a	<0.001	0.001	0.29	0.77
	b	3.00	1.05	2.87	0.01
Sucrose ($r^2 = 0$)	a	0.97	0.87	1.11	0.28
	b	0.079	0.28	0.28	0.78
17:00					
Discovery					
NaCl ($r^2 = 0.74$)	a	<0.001	<0.001	0.55	0.59
	b	3.73	0.55	6.78	<0.001
Sucrose ($r^2 = 0.56$)	a	0.001	0.002	0.54	0.59
	b	2.89	0.56	5.17	<0.001
Recruitment					
NaCl ($r^2 = 0.0.66$)	a	<0.001	<0.001	0.47	0.64
	b	3.66	0.64	5.72	<0.001
Sucrose ($r^2 = 0$)	a	0.42	0.55	0.77	0.45
	b	0.42	0.40	1.05	0.30

Table S2. Results of an ANCOVA test of temperature sensitivity on average discovery (log_{10} number of vials with at least one ant) and average recruitment (log_{10} number of ants per vial) of ants to four sucrose concentrations at Pigtail Alley Prairie. We test the prediction of a positive effect of increased temperature on overall bait discovery and recruitment and whether discovery or recruitment changed with sucrose concentration.

	Discovery		**Recruitment**	
Parameter	**$F_{3, 96}$**	**Pr>F**	**$F_{3, 96}$**	**Pr>F**
Log_{10}(mean soil temperature)	1.51	0.22	16.96	<0.001
Sucrose Concentration (1%, 5%, 10%, 20%)	25.52	<0.001	64.39	<0.001
Interaction	0.54	0.66	1.99	0.12

Table S3. Best-fit power law regressions ($y = aX^b$) for ant recruitment with soil temperature for vials stocked with one of 4 concentrations of sucrose solution. Curves were fit with the Marquardt-Levenberg algorithm.

Parameter		Coefficient	Std. Error	*t*	Pr>*t*
Concentration					
1% ($r^2 = 0$)	a	1.54	1.94	0.80	0.43
	b	0.00	0.38	0.00	1.00
5% ($r^2 = 0.05$)	a	0.10	0.27	0.35	0.73
	b	0.95	0.84	1.12	0.27
10% ($r^2 = 0.17$)	a	0.004	0.01	0.34	0.73
	b	2.03	0.85	2.38	0.02
20% ($r^2 = 0.18$)	a	0.01	0.03	0.37	0.71
	b	1.84	0.79	2.33	0.03

Supplemental figure legends

Figure S1: Ant discovery of NaCl and sucrose vials by month. **(a)** Ant activity – the average number of vials in which we found an ant (of 50) across three transects. **(b)** An estimate of ant recruitment to a bait – the average number of ants in a discovered vial. In each month ant behavior at three transects was measured across four days at 09:00, 13:00, and 17:00.

Figure S2: Distribution of three kinds of temperature soil **(a, d)**, surface **(b, e)**, and air **(c, f)** measured in °C over the duration of this study from April through October 2016. **(a, b, c)** Each line represents samples at one of three times of day: 09:00, 13:00, and 17:00. **(d, e, f)** Each point represents a sampling event (n = 82).

Figure S3: Correlations among the three measures of temperature (soil, surface, and air) associated with each sampling event.

Figure S4: Plot of discovery rates (vials discovered) **(a, b, c)** and recruitment (ants per vial) **(d, e, f**) against soil **(a, d)**, surface **(b, e)**, and air **(c, f)** temperatures measured in (°C).

Figure S5: The discovery rate of NaCl and sucrose vials versus soil temperature (°C), expressed as fraction of maximum for four ant species **(a)** *Crematogaster lineolata*, **(b)** *Forelius pruinosus*, **(c)** *Formica pallidefulva*, and **(d)** *Monomorium minimum* representing 94% of total bait discoveries in this prairie community. Linear and power Q_{10} curves were fitted to the data, and best fit curves are shown along with Q_{10} values.

Figure S1.

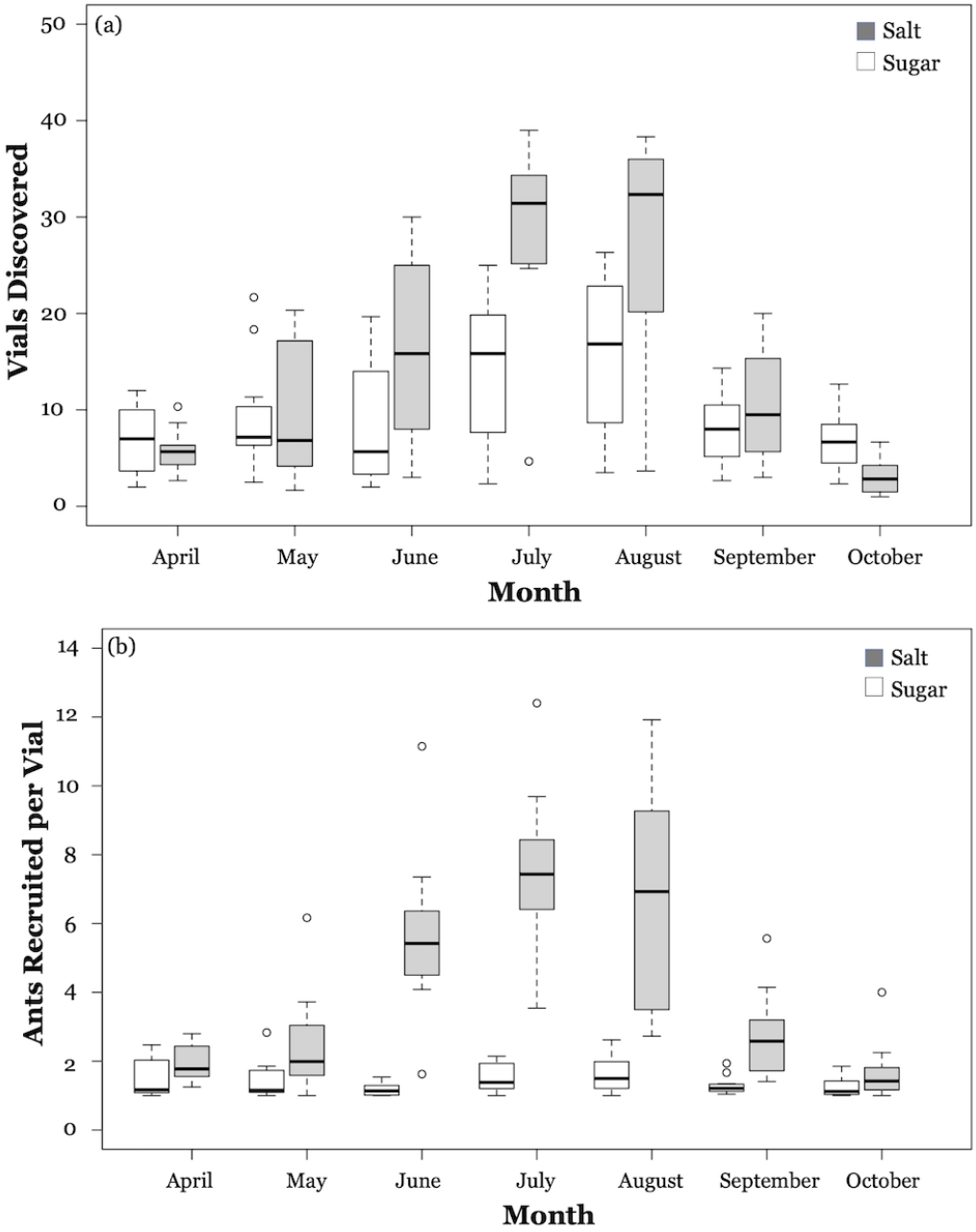

(a)
Salt
Sugar
Vials Discovered
0
10
20
30
40
50
April
May
June
July
August
September
October
Month
(b)
Salt
Sugar
Ants Recruited per Vial
0
2
4
6
8
10
12
14
April
May
June
July
August
September
October
Month

Figure S2.

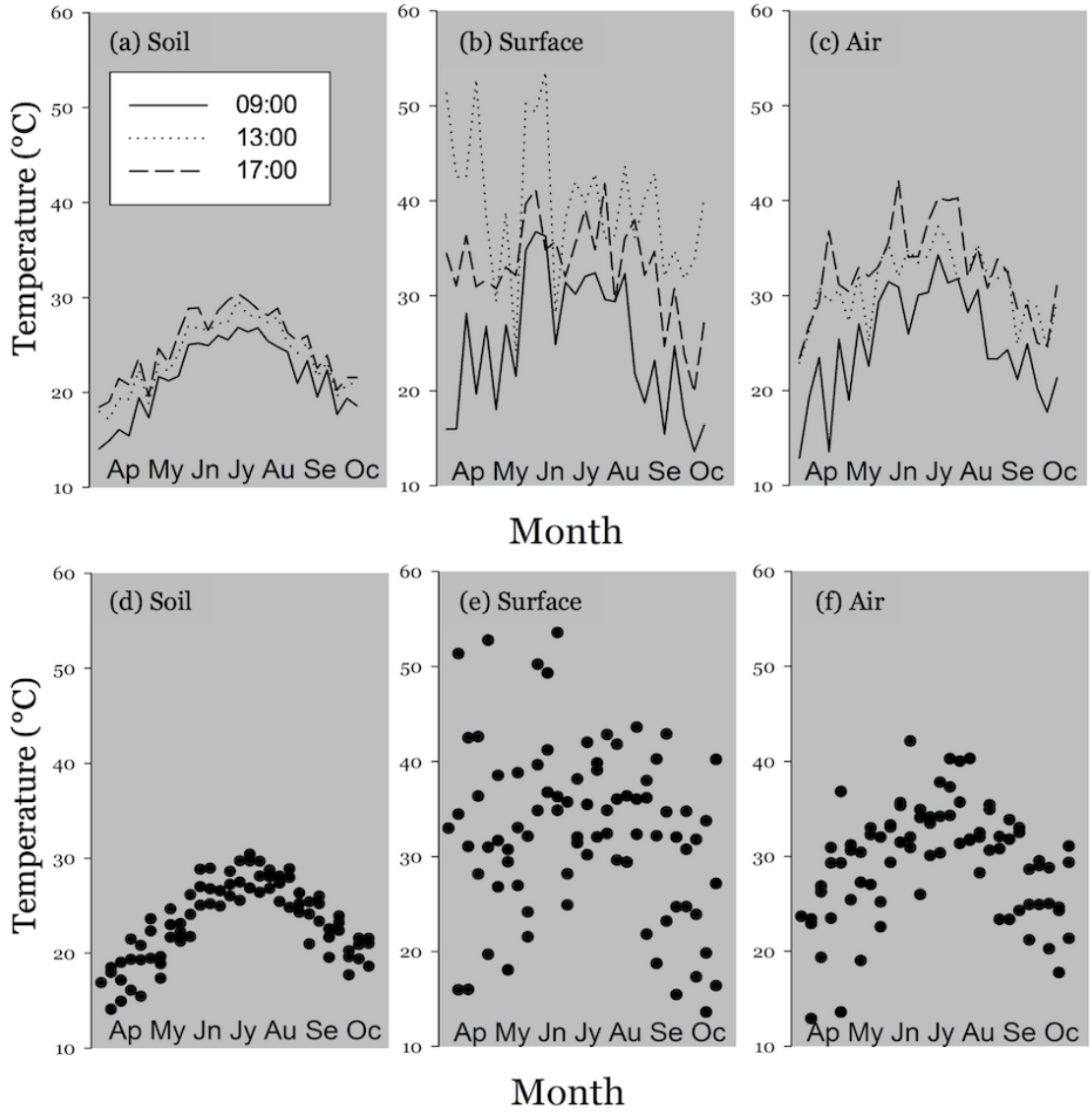

(a) Soil
09:00
13:00
17:00
(b) Surface
(c) Air
(d) Soil
(e) Surface
(f) Air
Temperature (°C)
Month
Ap My Jn Jy Au Se Oc
10
20
30
40
50
60

Figure S3.

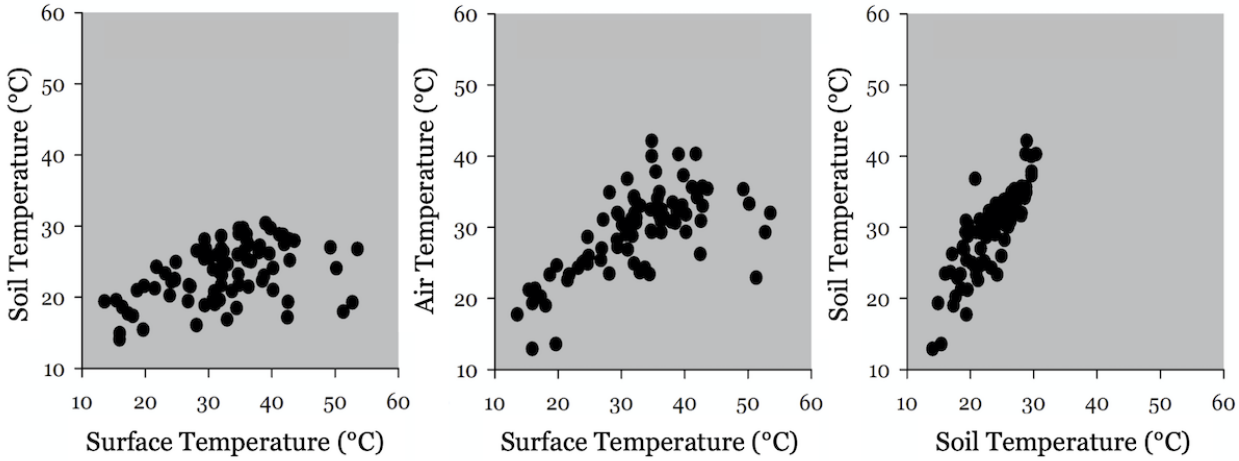

Soil Temperature (°C)
Surface Temperature (°C)
Air Temperature (°C)
Surface Temperature (°C)
Soil Temperature (°C)
Soil Temperature (°C)
10
20
30
40
50
60

Figure S4.

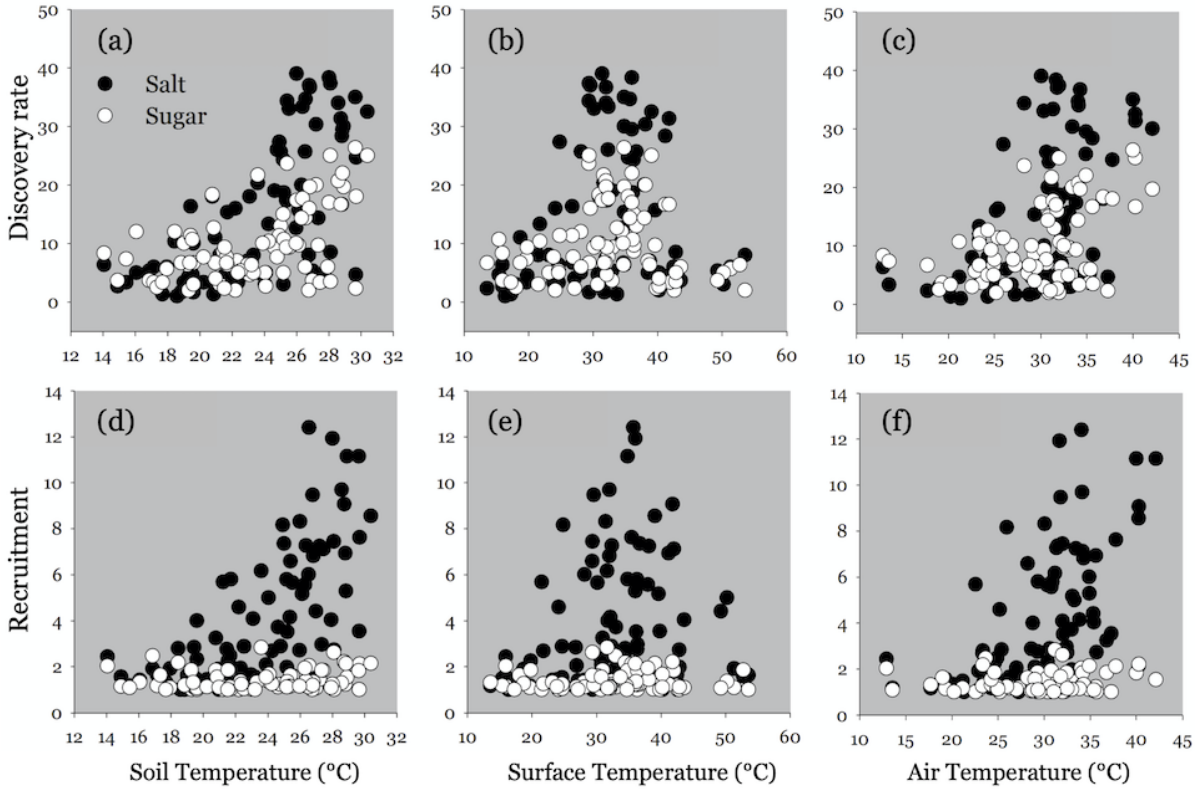
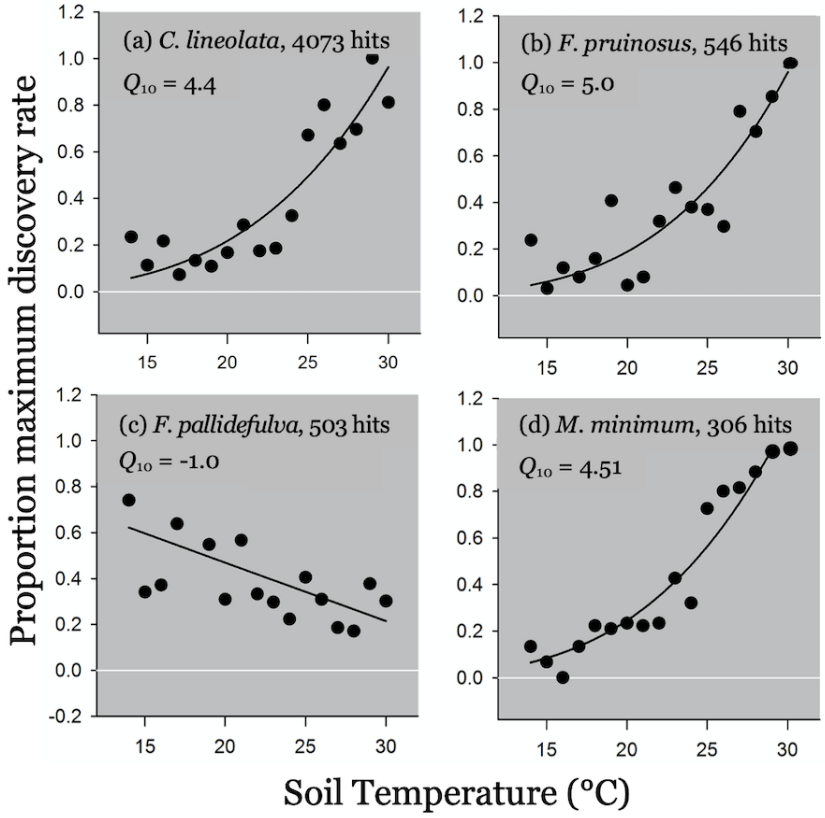

(a)
Salt
Sugar
(b)
(c)
(d)
(e)
(f)
Discovery rate
Recruitment
Soil Temperature (°C)
Surface Temperature (°C)
Air Temperature (°C)

Figure S5.


(a) C. lineolata, 4073 hits
Q10 = 4.4
(b) F. pruinosus, 546 hits
Q10 = 5.0
(c) F. pallidefulva, 503 hits
Q10 = -1.0
(d) M. minimum, 306 hits
Q10 = 4.51
Proportion maximum discovery rate
Soil Temperature (°C)

CHAPTER TWO

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PLANTS REGULATE GRASSLAND ARTHROPOD COMMUNITIES THROUGH BIOMASS, QUALITY, AND HABITAT HETEROGENEITY

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Abstract:

Habitat heterogeneity affects both biotic and abiotic factors important in determining arthropod community composition. In a sandy, mixed grass prairie in the southern Great Plains, we used clipping and NPK fertilization to manipulate plant biomass, habitat heterogeneity, and plant quality to quantify their relative effects on the abundance and diversity of its arthropod community. Both clipping and fertilization treatments affected plant biomass and microclimate, including light availability, temperature, and humidity. By decreasing plant biomass, clipping simplified habitat structure and resulted in reduced arthropod abundance and diversity and increased arthropod activity. This reduction appeared to be mediated by fertilizer addition which increased total plot carbon, plant biomass, and habitat volume, resulting in lower average surface temperature and higher average humidity. By itself, increasing plant biomass through fertilization increased arthropod abundance, activity, and richness. In addition, we show that changing microclimate and plant biomass promoted shifts in arthropod community composition. These results demonstrate the role of habitat heterogeneity and plant quality in structuring arthropod community composition, specifically by regulating microclimate and providing habitat space.

Key words: arthropod, clipping, fertilization, grassland, habitat heterogeneity, NPK.

Introduction

Plant biomass, plant quality, and habitat heterogeneity are three key factors shaping abundance, diversity, and species composition of grassland arthropods (Dennis et al. 1998; Lassau and Hochuli 2004; Arnan et al. 2007). For animals the size of arthropods, variation in plant biomass (high or low) and vegetation spacing (clumped or uniform) combine to generate habitat heterogeneity (Landis et al. 2000; Langellotto and Denno 2004). This heterogeneity in turn can shape arthropod abundance and diversity via its effects on microclimate (Wan et al. 2002), food availability and variety (Báldi 2008), and arthropod competitive interactions (Langellotto and Denno 2004; Janssen et al. 2007). Given the important role that plant diversity plays on insect diversity (Haddad et al. 2001) and that short-term fertilization changes plant biomass but not diversity (Haddad et al. 2000), one effective way of manipulating both plant quality and quantity but not plant diversity are short-term, or pulse, fertilization experiments. We explore how a one-year pulse experiment generated a cascading effect on a grassland arthropod community.

Fertilization can shape grassland arthropod abundance in at least two ways: via increasing plant biomass and/or plant nutrient quality (Siemann 1998; Haddad et al. 2000; Moran and Scheidler 2002; Kaspari et al. 2017). Increasing plant productivity can increase herbivore food availability, promoting larger herbivore populations (Siemann 1998; Haddad et al. 2000; Moran and Scheidler 2002; La Pierre and Smith 2016), which in turn support larger predator and parasitoid populations (Hairston et al. 1960; Fretwell 1987; Langellotto and Denno 2004). Fertilization can also increase food quality (Borer et al. 2015), decreasing plant carbon to nitrogen ratios (Tilman 1986; Siemann 1998; La Pierre and Smith 2016), so herbivores need to consume less plant matter to satisfy their nutritional requirements (La Pierre and Smith 2016). If arthropod abundance is limited by plant biomass and quality, then fertilization should result in

increased arthropod abundance and diversity because of sampling more species from the local pool (Srivastava and Lawton 1998; Kaspari et al. 2003), increased niche diversity (Rosenzweig 1995), and more predator-free space (Langellotto and Denno 2004; Janssen et al. 2007) in the resulting high-volume plots.

In grasslands, large ungulates are also major players shaping the arrangement and biomass of plants, with potential consequences for arthropods. Selective grazing opens up patches in a uniform sea of tall grass, increasing spatial heterogeneity (Adler et al. 2001), but more intensive grazing, by uniformly removing plant biomass, can reduce landscape heterogeneity (Debano 2006). Thus, beyond the direct effect of reducing food availability and habitat volume (Morris 2000; Post et al. 2000), grazers, by enhancing or reducing habitat heterogeneity, may shape arthropod densities and local competition (Savolainen and Vepsäläinen 1988; Finke and Denno 2002) while also altering microclimate (Greenslade 1983;Wan et al. 2002). In particular, by removing the shade of tall vegetation, grazers may enhance local temperatures, an important constraint on the activity of tiny ectotherms (Gillooly et al. 2001; Dell et al. 2011).

Combined, short-term grazing and fertilization can create a grassland patchwork while leaving local plant diversity unchanged, isolating the role of habitat heterogeneity, plant quality, and plant biomass in structuring arthropod community composition (Hutchinson 1961; Chase and Leibold 2003). Moreover, while many studies have examined how altered resource availability affects arthropod assemblages via increasing plant biomass (Siemann 1998; Haddad et al. 2000; Moran and Scheidler 2002; La Pierre and Smith 2016), few have looked at the separate and interacting effects of changing both habitat heterogeneity and resource availability

on the abundance, diversity, and richness of arthropod communities (but see Arnan et al. 2007; Cole et al. 2008).

Here, we set up a one-year factorial field experiment, manipulating habitat heterogeneity by clipping vegetation and manipulating plant nutrient quality and plant biomass through short-term fertilization. We test four predictions: **(1)** clipping treatments and fertilization will shape abundance of grassland arthropods via their effects on a prime variable, plant biomass, **(2)** increases in arthropod abundance will drive increases in local diversity, **(3)** for a given plant biomass, enhanced heterogeneity (the Coefficient of Variation in plant height per m^2, our measure of heterogeneity) will drive higher arthropod diversity, and, **(4)** these changes in abundance and diversity across clipping and fertilization treatments occur without changes in overall arthropod community composition.

Methods

Site Description

We studied the arthropod assemblage from May through August 2017 in Pigtail Alley Prairie, a 24.5-ha mixed-grass prairie last farmed >20 yr ago in southern Oklahoma, USA (33.89° N, 96.84° W). Pigtail Alley Prairie has sandy soil with *Andropogon virginicus* and *Vulpia octoflora* as the dominant grasses and *Croton glandulosus* and *Agalinis heterophylla* as the dominant forbs. Mean annual rainfall is 967.7 mm and average summer air temperature is 24.7°C (Oklahoma Climatological Survey).

Experimental Design

To test the combined and separate effects of habitat modification via plant biomass removal and resource addition on arthropod communities, we set up a factorial field experiment. Plant

biomass removal (i.e., clipping) had three levels crossed with two resource addition levels, resulting in six treatment combinations (Appendix S1: Fig. S1, S2). Each treatment combination was replicated 15 times, resulting in 90 plots, each 4 m^2 (2 × 2 m). Plots were randomly assigned a treatment using a random number generator, arranged in a grid, and separated from one another by 10 m on all sides to reduce neighbor effects (Haddad et al. 2000; Appendix S1: Fig. S1).

Plant biomass levels and habitat heterogeneity were manipulated using three clipping treatments: fully clipped, half-clipped, and unclipped. From April to August 2017, every three weeks we cut vegetation in clipped and half-clipped plots down to 3 cm using a weed-whacker (ECHO SRM-225 straight shaft string-trimmer) and removed the plant clippings from plots. Half-clipped plots were divided into four 1-m^2 quadrats. We clipped vegetation from two diagonal 1-m^2 quadrats, leaving the other two quadrats intact and forming a checker pattern to best mimic patchy grazing by ungulates (McNaughton 1984; Appendix S1: Fig. S2). Unclipped plots were disturbed by moving the weed-whacker through them (while off) on days we clipped in order to mimic disturbance similar to clipping.

We changed plant biomass and resource availability using two fertilization levels: fertilized or unfertilized. Plots were fertilized in March 2017. Amount and composition of fertilizer was based on protocols from the Nutrient Network experiment and designed to ensure no nutrient limitation (Borer et al. 2014). Fertilizer consisted of N, P, and K applied at a rate of 10 $g \cdot m^{-2} \cdot yr^{-1}$ by elemental mass and a micronutrient mixture applied at a rate of 100 $g \cdot m^{-2} \cdot yr^{-1}$. We added N as time-release humic coated urea, P as super triple phosphate, and K as potassium sulfate. For micronutrients, we used Scott's Micromax fertilizer containing calcium (6 g/m^2), magnesium (3 g/m^2), sulfur (12 g/m^2), boron (0.1 g/m^2), water-soluble copper

(1 g/m^2), water-soluble iron (17 g/m^2), water-soluble manganese (2.5 g/m^2), molybdenum (0.05 g/m^2), and water-soluble zinc (1 g/m^2).

Monitoring changes in plants and microclimate

To evaluate how our experimental treatments shaped the abiotic environment, we measured light, temperature, and humidity from May to August on a subset of our plots (n = 18; three randomly selected plots per treatment). We collected light incidence at the soil surface using a HOBO pendant (UA-002-08) and both temperature and humidity using a HOBO Pro v2 (U23-002). Loggers were left out all summer and recorded light, temperature, and humidity data every 5 min. One pendant and one Pro v2 were used on fully clipped and unclipped plots, while two were used on half-clipped plots—one in an unclipped quadrat and one in a clipped quadrat. For each treatment, we averaged data across month, taking the additional step in half-clipped plots to average the temperatures from the clipped and unclipped portions of the plot.

Plant biomass was a key variable in translating our experimental treatments into a biotic variable shaping arthropod abundance and diversity. We measured plant biomass every month on plots using a pasture disk meter (Bransby and Tainton 1977). We took four measurements per plot each time we measured plant biomass, starting in the southeast corner and traveling counterclockwise around each plot. Pasture disk meters indirectly measure plant biomass by measuring the height that a thin aluminum disk (0.5 m diameter) is supported by vegetation when dropped from a constant height. The recorded height is correlated with plant biomass using linear regression (Bransby and Tainton 1977). We calibrated the disk once per month by recording the settling height, clipping the vegetation under the disk, drying it at 60°C, then weighing it before

creating a regression linking plant biomass to measurement height. Disk calibration took place in the same prairie, at least 10 m from any plot, and we used 20 disk drops per calibration.

We measured habitat heterogeneity every month on plots by calculating the coefficient of variation (CV) of plant height from the four height measurements taken per plot using the pasture disk meter. We used CV of plant height as a measurement of habitat heterogeneity because it varied in a predictable way with our clipping treatments (i.e., was highest on half-clipped plots; Appendix S1: Fig. S3).

We measured plant quality (i.e., %C and %N) in August 2017. We clipped and dried vegetation at 60°C for 48 h from twelve unclipped plots—six fertilized and six unfertilized. Once dry, we separated vegetation into grasses and forbs. We then ground and weighed samples to the nearest 0.001 mg and sent them to the Cornell Nutrient Analysis Laboratory where they were analyzed for %C and %N using combustion analysis.

Monitoring changes in the arthropod community

We sampled the arthropod community monthly from May to August 2017 using two complementary sampling methods: vacuum sampling and pitfall traps (Standen 2000). Vacuum sampling is good at catching smaller flying or vegetation-dwelling arthropods (Mommertz et al. 1996), while pitfall traps are better at catching organisms walking along the ground or residing in the litter layer (Spence and Niemelä 1994; Roeder et al. 2018). Additionally, vacuum sampling measures instantaneous arthropod activity while pitfall traps, because they run for 48 h, measure arthropod activity density.

Because precipitation can alter arthropod activity, we sampled arthropods on clear days preceded by three dry days. We used an inverted leaf-blower (Husqvarna 125BVX, The

Husqvarna Group, Stockholm, Sweden) to vacuum sample each plot for 50 s. Vacuum samples were put on ice and kept frozen until sorting. To control for disturbance, we started pitfall traps two days after vacuum sampling. We placed one pitfall trap in the center of each plot and left it open for 48 h. Pitfall traps consisted of plastic deli cups 11.2 cm in diameter, 13.9 cm deep, and filled with a 100 mL solution containing 50% ethanol, 50% water, and a drop of scentless detergent. Pitfall samples were rinsed and stored in 95% ethanol until identified.

For each sample, we counted and identified all arthropods to major taxonomic group (Appendix S1: Table S1, S2) and then assigned species or morphospecies within each of those groups. Morphospecies are a reliable estimate of species richness for invertebrate community analyses (Oliver and Beattie 1996). Each plot and month thus generated a measure of arthropod abundance (via vacuum sampling) and activity (via pitfall traps), arthropod diversity (taxon-level; Shannon's *H*), and morphospecies richness. Six samples were excluded from analyses because they were unusable—one May vacuum sample was lost in the field and five pitfall traps were destroyed by boars (three in May and two in August).

Analysis: effects of clipping and fertilization on microclimate

All statistical analyses were conducted in R version 3.5.1 (R Core Team 2016). To test whether fertilization increased nitrogen content of either grasses or forbs in our plots, we used a Welch's *t* test to separately compare the C:N ratios of fertilized and unfertilized grasses and forbs. To account for the increased plant biomass, and thus increased C and N on fertilized plots, we also used a *t* test to compare the total plot C (average %C × plant biomass) and total plot N (average %N × plant biomass) for fertilized and unfertilized plots.

Linear mixed effect models were used to test our hypothesis that fertilization and clipping would change plant biomass and microclimate, including average light incidence (LUX), average surface temperature (°C), and average humidity (%) using the lmer function in the lme4 R package (Bates et al. 2014). Response variables were checked for normality, then $\log_{10}$ transformed average plant biomass, average light incidence, and CV plant height before running analyses. Driver variables consisted of fertilization, CV plant height, and their interaction. To account for repeated sampling, we included plot and month as random factors in our models. We performed model selection using Akaike's Information Criterion (AIC_c; Burnham and Anderson 2003) to determine which driver variables most influenced plant biomass and microclimate and used the MuMIn package (Barton 2016) to perform model comparisons. If models had a $\Delta AIC_c < 2$, they were considered equally parsimonious (Burnham and Anderson 2003). Residuals of the top model were plotted using quantile-quantile plots to check for homoscedasticity.

Analysis: effects of clipping and fertilization on community composition

Because ants composed 3.7% and 88.9 % of vacuum and pitfall samples, respectively, we tested Pearson correlation coefficients to check for a relationship between ant abundance/activity and the abundance/activity, diversity, and richness of non-ant arthropods. We found significant positive correlations for all relationships, so we decided to leave ants in all arthropod analyses, grouped with bees and wasps (i.e., Hymenoptera; Appendix S1: Fig. S4).

To better partition the separate and coupled effects of plant biomass, habitat heterogeneity, and plant quality on our community indices (arthropod abundance, activity, diversity, and richness), we ran additional linear mixed effects models. We had six models—three each for vacuum- and pitfall-sampled arthropods. We checked for normality then $\log_{10}$

transformed arthropod abundance, activity, and richness before running analyses. Driver variables were fertilization, CV plant height, and plant biomass (g), and we included plot and month as random factors in our models. We performed model selection using AIC_c as above and checked residuals of the top model for homoscedasticity.

All models were compared using Relative Importance Values (RIVs), a summed and standardized indicator of predictor variable rank across all possible models. RIVs are the sum of Akaike weights (w_i) of fertilization, CV plant height, and plant biomass predictor variables for each of the six arthropod community responses we examined (Burnham and Anderson 2003). When predictor variables had RIV > 0.45 in models, we performed simple linear regressions and Welch's *t* test.

Effect sizes (Cohen's *d*) were used to help visualize the magnitude of the responses of microclimate variables and arthropods to our factorial clipping and fertilization treatments. Cohen's *d* is an effect size measure that standardizes the direction and magnitude of response variables (Cohen 1988). We define a medium effect as $d = |0.5|$ and a large effect as $d \geq |1.0|$ (Cohen 1988).

Analysis: effects of microclimate on community composition

We separately analyzed the response of arthropods caught using vacuum sampling and pitfall traps to our experimental treatments using a Canonical correspondence analysis (CCA; Tabachnick and Fidell 2007). Canonical correspondence analysis uses raw richness and abundance data to plot both sample points and community composition in multivariate space (the ordination of arthropod taxa was our primary interest). Unlike other ordination techniques, CCA constrains the ordination by a multiple regression of environmental variables provided *a priori*

(Tabachnick and Fidell 2007). We discarded any taxa with fewer than three individuals recorded from our dataset, reducing the number of taxa by two for vacuum samples (Diplopoda and Phasmatodea) and one for pitfall samples (Neuroptera).

In the CCA, we examined environmental variables including average plant biomass per plot (g), average light incidence (LUX), maximum surface temperature (°C), average surface temperature (°C), minimum surface temperature (°C), and average humidity (%). We identified environmental variables explaining significant amounts of variation in arthropod compositional differences between clipping and fertilization treatments using the ordistep stepwise forward selection function in the vegan package (Oksanen et al. 2015). Stepwise forward selection chooses the most parsimonious environmental variable combination explaining the assemblage structure (Oksanen et al. 2015). Variance inflation factors (VIF) were calculated for environmental drivers in our final models using a cutoff of VIF < 3.5 and no evidence of multicollinearity was found (Zuur et al. 2010). We tested the significance of the stepwise-chosen environmental variables on community composition using an *F* distribution based on 999 permutations performed by the anova.cca function in the vegan package (Oksanen et al. 2015).

We compared the arthropod assemblage caught with vacuum sampling and pitfall traps using a Procrustes analysis (Jackson 1995; Peres-Neto and Jackson 2001). This analysis searches for the best fit between two matrices (low sum of squares distances) by rotating one matrix to fit the other. The m^2 statistic ranges from 0 to 1, with 0 indicating the communities are almost identical (Jackson 1995; Peres-Neto and Jackson 2001). We performed this analysis with the matrices from our two CCAs (vacuum and pitfall) using the protest function in the vegan package (Oksanen et al. 2015) and based the significance of the m^2 statistic on 999 permutations.

Results

Clipping and fertilization changed plant biomass, light incidence, temperature, and humidity

We predicted changes in arthropod abundance and diversity through the manipulation of biotic and abiotic variables. Relative importance values demonstrated that fertilization, CV plant height, and their interaction were all important drivers of plant biomass and microclimate (Appendix S1: Table S3). Plant biomass was reduced on both fully clipped and half-clipped plots (Fig. 1a; Appendix S1: Table S3) but this reduction was ameliorated by a one-time fertilization with NPK and micronutrients. Fertilization also increased plant biomass on unclipped plots (Fig. 1a). We measured plant carbon and nitrogen in both forbs and grasses. We found that fertilization did not significantly change the C:N ratio of grasses or forbs (Welch's *t* test, $t = -1.21$, df = 7.77, $P = 0.261$ and $t = 1.02$, df = 5.47, $P = 0.352$, respectively; Appendix S1: Figure S5). Fertilization significantly increased total plot C but did not significantly change total plot N (Welch's *t* test, $t = -3.41$, df = 8.88, $P = 0.008$ and $t = -2.21$, df = 8.34, $P = 0.057$; Appendix S1: Figure S5).

Clipping and fertilization treatments generated changes in some but not all microclimate measures. Clipping significantly increased average light penetrating to ground level (Fig. 1a; Appendix S1: Table S3) but did not increase average temperature. In contrast, fertilization reduced plot temperatures (Fig. 1a; Appendix S1: Table S3). Clipping alone did not change average humidity (Appendix S1: Table S3). However, fertilization increased average humidity on both fully clipped and unclipped plots while not changing humidity on half-clipped plots.

Clipping increased arthropod activity; fertilization increased arthropod abundance

We collected 159,543 arthropods: 20,280 from vacuum sampling and 139,263 from pitfall traps (for taxon list see Appendix 1: Table S1, S2). Plant biomass had the highest RIV (thus was

consistently the strongest predictor compared to fertilization and CV of plant height) for both arthropod abundance (vacuum samples) and arthropod activity (pitfall samples, Appendix S1: Tables S4, S5; Fig. S6).

However, beyond the effects of plant biomass, RIVs reveal that fertilization had significant effects on vacuum-sampled arthropod abundance (Appendix S1: Table S4) and clipping had significant effects on pitfall-sampled arthropod activity (Appendix S1: Table S5). The one-time fertilization with NPK and micronutrients increased arthropod abundance on both unclipped and half-clipped plots (Fig. 1b; Appendix S1: Fig. S7) but did not significantly affect arthropod activity (Fig. 1c; Appendix S1: Fig. S7). Clipping treatments, in contrast, reduced arthropod abundance measured by vacuum samples (Fig. 1b) and increased arthropod activity from pitfall traps (Fig. 1c). The highest pitfall activity was on fully clipped plots (Fig. 1c; Appendix S1: Fig. S8).

Clipping reduced vegetation and ground arthropod diversity and differentially affected richness

We found no evidence for enhanced diversity or richness of vegetation (vacuumed) or ground arthropods (pitfalls) on the most heterogeneous (i.e. half-clipped) plots. In vacuum samples, while CV plant height was present in the top models for both diversity and richness, RIVs show that clipping was not the most important factor in determining arthropod diversity or richness (Appendix S1: Table S4). However, reducing habitat heterogeneity (through fully clipping plots) decreased both diversity and richness, with the largest reductions seen on fully clipped plots (Fig. 1b).

In pitfall samples, clipping had effects beyond that of decreasing plant biomass. Clipping resulted in decreased arthropod diversity but did not change arthropod richness (Appendix S1:

Table S5). Specifically, diversity was lower on fully clipped and half-clipped unfertilized plots (Fig. 1c; Appendix S1: Fig. S8).

Fertilization increased both vegetation and ground arthropod richness but did not significantly change diversity

For vegetation arthropods, consistent with our predictions, fertilization increased richness relative to unfertilized plots (Fig. 1b; Appendix S1: Table S4 and Fig. S7) but did not significantly change arthropod diversity (Fig. 1b; Appendix S1: Table S4).

For ground arthropods, fertilization increased richness (Fig. 1c; Appendix S1: Table S5 and Fig. S7) but did not significantly change arthropod diversity (Fig. 1c; Appendix S1: Table S5). However, fertilization did increase arthropod diversity on half-clipped plots relative to unfertilized half-clipped plots (Fig. 1c).

Clipping and fertilization treatments changed vegetation and ground arthropod community composition

Our treatments significantly altered plant biomass and microclimate, which then had strong effects on arthropod community composition. The CCA of arthropod taxa and associated biplots of microclimate were different for vacuum and pitfall samples (Fig. 2), with significant taxa by microclimate correlations on the first axis for vacuum samples and the first three axes for pitfall samples (vacuum: $F = 9.30$, $P = 0.001$; pitfall: $F = 15.44$, $P = 0.001$; $F = 6.12$, $P = 0.005$; and $F = 4.42$, $P = 0.009$). Procrustes analysis revealed that vacuum and pitfall arthropod communities were very different, with few similarities between the two matrices ($m^2 = 0.995$; $P = 0.249$).

Community composition for vacuum and pitfall samples was driven by several microclimate variables which we altered through our experimental treatments. For vegetation arthropods, community composition was significant affected by average light incidence ($F_{1,348}$ = 4.09, P = 0.004), average temperature ($F_{1,348}$ = 2.94, P = 0.017), maximum temperature ($F_{1,348}$ = 3.75, P = 0.006), and average humidity ($F_{1,348}$ = 2.87, P = 0.01). Specifically, Acari and Mantodea were associated with higher average light on fully clipped plots (Fig. 2; Appendix S1: Table S6). For ground arthropods, community composition patterns were correlated with average plant biomass ($F_{1,348}$ = 6.88, P = 0.001), average light incidence ($F_{1,348}$ = 4.57, P = 0.004), minimum temperature ($F_{1,348}$ = 4.95 P = 0.006), and average humidity ($F_{1,348}$ = 9.36, P = 0.001). Specifically, Hymenoptera were associated with less plant biomass while Blattodea, Coleoptera, Hemiptera, and Orthoptera were associated with more plant biomass found on unclipped plots (Fig. 2; Appendix S1: Table S6).

Discussion

Here, we experimentally confirm that plant biomass, habitat heterogeneity, and plant quality are important drivers of grassland arthropod communities. Clipping had effects beyond reducing plant biomass and resulted in modified microclimate and reduced food availability for arthropods. Plant biomass removal via clipping promoted increased light incidence. In contrast, fertilization increased plant biomass, consequently reducing average surface temperature and increasing average humidity. Fertilization also had effects beyond increasing plant biomass, altering plant quality through increasing plot N and significantly increasing plot C (Appendix S1: Fig. S5). The indirect effects of changing microclimate and plant biomass promoted shifts in

arthropod community composition. Altering vegetation structure led to changes in abundance, activity, diversity, and richness of vegetation and ground arthropods.

Arthropod abundance and activity

Our results are consistent with arthropod abundance being constrained by plant biomass, habitat heterogeneity, and food quality in this mixed-grass prairie. While both of our treatments altered plant biomass, they also had separate effects. Fully clipping a plot and decreasing habitat heterogeneity led to decreased vegetation arthropod abundance, likely through reduced food quantity, a response similar to other studies (Morris 2000; Haysom et al. 2004; Woodcock et al. 2007). Fertilization increased the abundance of vegetation arthropods beyond its effects on plant biomass. A likely reason for the greater arthropod abundance was an increase in the quality of plant tissue. We failed to find a significant increase in plant N in response to our fertilization as has been found by others (Tilman 1986; Siemann, 1998; La Pierre and Smith 2016; Kaspari et al. 2017), although we did see a slight increase in total plot N and a significant increase in total plot C. One explanation is that our NPK + micronutrient fertilization enhanced one or more other limiting nutrients, like P.

Ground arthropod activity increased with reduced habitat heterogeneity (through fully clipping a plot) and increased light incidence, but not temperature as we had predicted (Gillooly et al. 2001; Dell et al. 2011). Because there was an effect of habitat heterogeneity in addition to a plant biomass effect, the simplest explanation for the overall increase in arthropod activity is that clipping, by creating a homogenous surface on either all of the plot (fully clipped) or half of the plot (half-clipped), removed barriers to movement of ground arthropods. Reducing plant biomass did not change average temperature. Instead, we saw a decrease in temperature as fertilization

increased plant biomass levels over those seen on unfertilized plots. The explanation for fertilization reducing temperature may be that our site was nutrient-limited. Bare soil patches were present on all plots not receiving fertilization, resulting in high overall average surface temperature regardless of biomass removal. Surface temperature and solar radiation were reduced only on plots with increased plant biomass (Fig. 1a), perhaps explaining the increased activity of the ant *Crematogaster lineolata*—which made up >50% of pitfall captures and has a relatively high thermal tolerance (Penick et al. 2017)—on plots with less plant biomass.

Arthropod diversity and morphospecies richness

Changes in abundance were accompanied by changes in diversity and richness as predicted (Srivastava and Lawton 1998; Kaspari et al. 2003). Fertilization increased richness of both vegetation and ground arthropods and fertilization was actually the best predictor of ground arthropod richness. While previous work has shown short-term fertilization often increases arthropod diversity (Siemann 1998; Morris 2000; Woodcock et al. 2009), we found no effect of fertilization on the diversity of either vegetation or ground arthropods. However, vegetation removal resulted in decreased arthropod diversity, a finding consistent with other studies (Debano 2006; van Klink et al. 2015).

Arthropod community composition

Arthropod herbivore and predator presence changed with plant biomass, habitat heterogeneity, plant quality, and microclimate. Both vegetation and ground arthropods were affected by habitat modification and the resulting change in microclimate, showing abiotic factors are important and influence which taxa are present. Specifically, herbivores such as Lepidoptera and Hemiptera

prefer high-quality vegetation and were less abundant in plots with higher average temperatures and reduced plant biomass. This finding is consistent with studies in which herbivore abundance decreased with less plant biomass (Morris 2000; Woodcock et al. 2009). Predators such as Neuroptera and Araneae also increased in abundance with increased plant biomass, decreased light availability, and less humidity. This is consistent with studies demonstrating predator abundance decreases with reduced detritus or vegetation structure (Finke and Denno 2002; Langellotto and Denno 2004).

Caveats

Pitfall traps and vacuum sampling produced complementary results in our study, but neither technique can catch all arthropods. Habitat structure can affect the abundance of arthropods caught in pitfall traps. Specifically, pitfall catch increases as vegetation and litter amount decrease (Melbourne 1999). While this limitation may confound our result of higher arthropod activity on clipped plots, altered microclimate is one hypothesis explaining why pitfall catch changes with clipping. Specifically, clipping should increase temperature and solar radiation while decreasing humidity (Honek 1988). We show vegetation removal increased average light incidence but did not change average humidity or average temperature. Thus, to determine possible effects of microclimate and vegetation density on pitfall trap capture rate, future studies should report microclimate and vegetation density data along with pitfall trap results. In contrast, vacuum sample catch often decreases with increases in vegetation density and arthropod size (Mommertz et al. 1996; Standen 2000). While we collected fewer large arthropods in vacuum samples, we caught many large arthropods in pitfall traps and using both methods together

allowed us to capture the response of most of the arthropod community at our site (as confirmed by our Procrustes analysis).

Conclusions and next steps

We demonstrate how plant biomass, spatial heterogeneity, and nutrient availability shape arthropod communities in separate, non-interacting ways. By reducing vegetation, clipping simplified habitat structure, reducing arthropod abundance and diversity. This reduction appeared to be mediated by fertilizer addition, which increased plant biomass and habitat volume, resulting in higher average humidity and lower average surface temperature. By itself, increasing plant biomass through fertilization increased arthropod abundance, activity, and richness. In addition, we show that changing microclimate and plant biomass shifts arthropod community composition. This experiment, while showing a fertilization effect beyond increasing plant biomass, highlights our uncertainty as to mechanism. Future fertilization experiments should focus on measuring not only plant N and C but also measuring other nutrients in both plant tissue and the soil to work toward understanding which nutrients are vital in shaping arthropod communities.

While we did not find that higher habitat heterogeneity (mimicking patchy ungulate grazing) resulted in higher arthropod diversity, we did find that reducing habitat heterogeneity (mimicking ungulate overgrazing) through fully clipping a 2 × 2 m patch of prairie resulted in increased ground arthropod activity, reduced vegetation arthropod abundance, reduced diversity of all arthropods, and reduced vegetation arthropod richness. These results demonstrate that while patchy ungulate grazing (half-clipping) does not increase grassland arthropod abundance

or diversity, low habitat heterogeneity as caused through ungulate overgrazing can reduce the abundance, diversity, and richness of different grassland arthropod guilds.

It is difficult to tease apart how vegetation characteristics specifically affect arthropods, whether by providing food, habitat structure, predator and parasitoid refuges, or by mediating microclimate. However, by factorially combining vegetation removal and fertilization, we demonstrated the importance of both the direct and indirect effects vegetation has on arthropod communities, driving both arthropod activity and determining arthropod community composition.

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Figure Legends

Figure 1. Response of arthropods and microclimate variables to factorial clipping and fertilization treatments, measured as effect size (Cohen's *d*). **(a)** Effect size of changes in microclimate variables including average plant biomass (g), average light (LUX), and average temperature (°C). Effect size of changes in arthropod abundance/activity, diversity (Shannon's *H*), and richness caught via **(b)** Vacuuming sampling and **(c)** Pitfall trap sampling. Gray circles are fertilized plots, and black triangles are unfertilized plots, around each point is the 95% confidence interval. UC, unclipped; HC, half-clipped; C, clipped. Cohen's *d* was calculated by comparing the five treatments to the control (unfertilized, unclipped). A medium effect is $d = |0.5|$ and a large effect is $d \geq |1.0|$,

Figure 2. Canonical correspondence analysis biplot ordination of arthropod taxa from **(a)** vacuum samples ($n = 11$ taxa) and **(b)** pitfall samples ($n = 11$ taxa), with key microclimate variables displayed. Each point represents the monthly arthropod community from plots ($n = 90$) sampled once a month from May to August 2017. Points are shaded by fertilization treatment (shaded = unfertilized, unshaded = fertilized), and 95% confidence ellipses show clipping treatments (black = clipped, dark gray = half-clipped, light gray = unclipped). Arrows are key microclimate variables, with the length of each arrow corresponding to the variable's importance.

Figure 1.

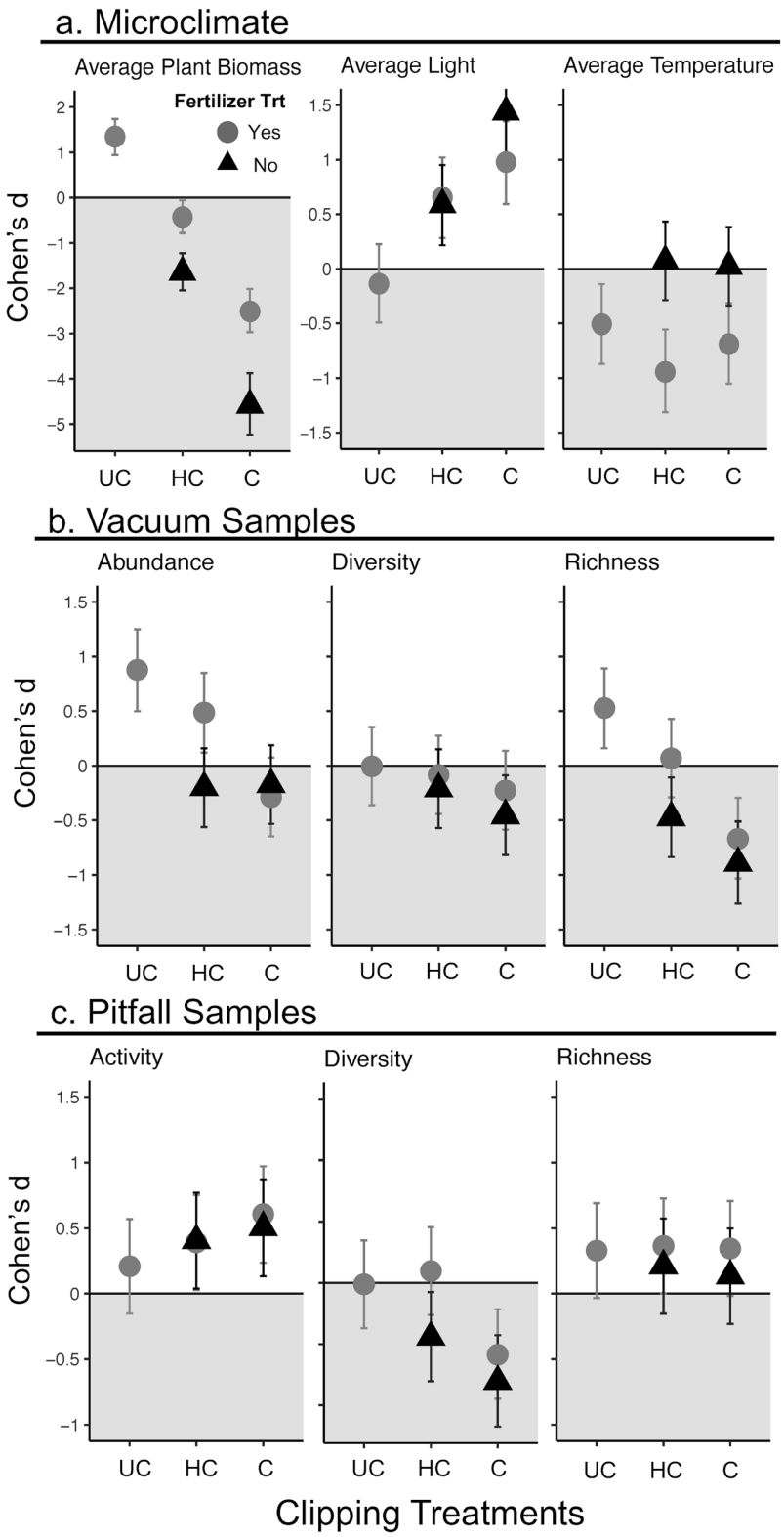
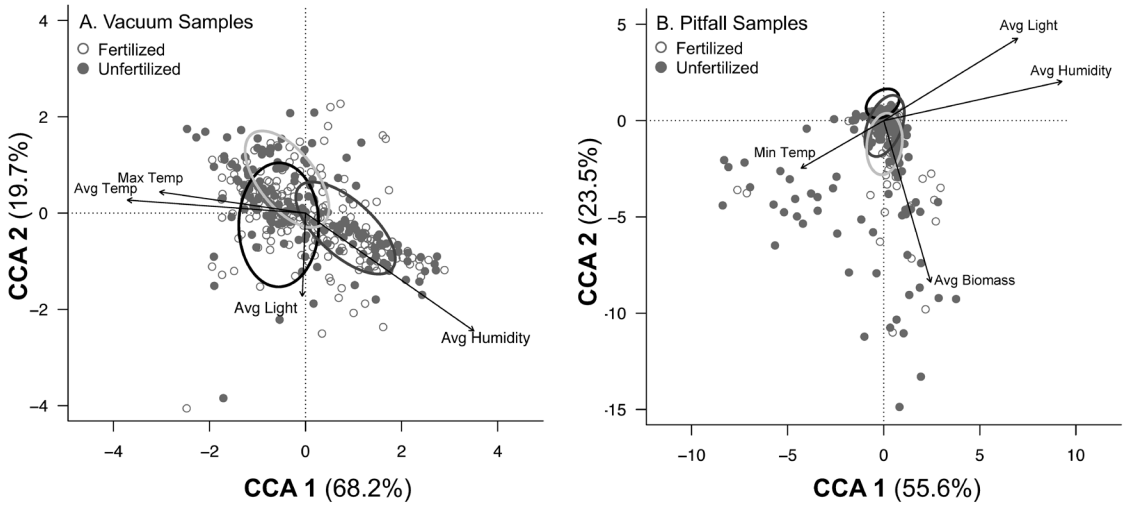

a. Microclimate
Average Plant Biomass
Average Light
Average Temperature
Fertilizer Trt
Yes
No
Cohen's d
UC
HC
C
b. Vacuum Samples
Abundance
Diversity
Richness
c. Pitfall Samples
Activity
Clipping Treatments

Figure 2.


A. Vacuum Samples
Fertilized
Unfertilized
Max Temp
Avg Temp
Avg Light
Avg Humidity
CCA 2 (19.7%)
CCA 1 (68.2%)
B. Pitfall Samples
Fertilized
Unfertilized
Avg Light
Avg Humidity
Min Temp
Avg Biomass
CCA 2 (23.5%)
CCA 1 (55.6%)

Chapter 2: Appendix S1. Supplemental Data.

Table S1. Summary of arthropods caught using vacuum sampling across all sampling periods, separated by treatment.

Vacuum Samples

	Treatments					
	Fertilized			Unfertilized		
Taxa	**Clipped** Num. Ind.	**Half-clipped** Num. Ind.	**Unclipped** Num. Ind.	**Clipped** Num. Ind.	**Half-clipped** Num. Ind.	**Unclipped** Num. Ind.
Acari	5	4	8	9	8	28
Araneae	100	223	325	99	130	193
Coleoptera	84	117	161	68	91	74
Collembola	13	12	26	2	10	14
Diplopoda	1	0	1	0	0	0
Diptera	234	505	592	159	222	419
Hemiptera	895	1751	2246	684	951	1197
Hymenoptera (ants)	980	1299	1495	1004	956	673
Hymenoptera	70	133	215	44	131	163
Lepidoptera	30	37	54	18	29	53
Mantodea	8	2	4	10	4	11
Neuroptera	9	30	36	4	15	16
Orthoptera	253	217	209	182	111	117
Phasmatodea	0	0	0	0	0	1
Total	**31833**	**24275**	**18948**	**25216**	**23967**	**15024**

Table S2. Summary of arthropods caught using pitfall trap sampling across all sampling periods, separated by treatment.

Pitfall Samples						
	Treatments					
	Fertilized			**Unfertilized**		
Taxa	**Clipped** Num. Ind.	**Half-clipped** Num. Ind.	**Unclipped** Num. Ind.	**Clipped** Num. Ind.	**Half-clipped** Num. Ind.	**Unclipped** Num. Ind.
Acari	204	147	103	92	129	154
Araneae	255	187	155	227	190	144
Blattodea	87	183	122	97	155	149
Coleoptera	305	352	480	225	279	309
Collembola	753	642	600	944	600	446
Diplopoda	4	9	5	2	10	5
Diptera	161	124	116	131	127	67
Hemiptera	153	198	200	110	167	156
Hymenoptera (ants)	28494	21816	16263	22779	21780	12729
Hymenoptera	888	107	309	281	74	352
Lepidoptera	49	0	50	53	54	24
Neuroptera	0	1	0	0	1	1
Orthoptera	480	509	545	275	401	488
Total	**31833**	**24275**	**18948**	**25216**	**23967**	**15024**

Table S3. Top models for relationships between clipping and fertilization and environmental metrics.

Environmental Data Model	Model Variables	AIC_c	LL	df	R^2	ΔAIC_c	w_i
Avg. Plant Biomass	Fertilizer (**0.98**), CV Plant Height (**1**), Interaction (**0.81**)	-25.13	19.72	7	0.81	0.00	0.81
Avg. Light	Fertilizer (**0.91**), CV Plant Height (**1**), Interaction (**0.83**)	-330.75	172.54	7	0.75	0.00	0.83
Avg. Temperature	Fertilizer (**1**), CV Plant Height (**0.82**), Interaction (**0.71**)	-1792.86	903.59	7	0.93	0.00	0.71
Avg. Humidity	Fertilizer (**1**), CV Plant Height (**1**), Interaction (**1**)	-2034.24	1024.28	7	0.93	0.00	1.00

AIC statistics include: *AICc* AIC statistic; *LL* log likelihood; *df* degrees of freedom; R^2 adjusted regression coeffiecient; *ΔAICc* AICc minus top model AICc, and *wi* model weight. RIVs for each variable are presented in (**bold**) next to the model variable the first time it appears (i.e. Fertilizer (0.98), CV Plant Height (**1**)).

Table S4. Top models for relationships between clipping and fertilization and community metrics of arthropods caught in vacuum samples.

Vacuum Samples							
Model	**Model Variables**	**AIC_c**	**LL**	**df**	**R^2**	**ΔAIC_c**	**w_i**
Abundance	Fertilizer (**0.9**), Plant Biomass (**1**)	744.58	-366.17	6	0.42	0.00	0.65
	Fertilizer, Plant Biomass, CV Plant Height (**0.28**)	746.50	-366.09	7	0.43	1.92	0.25
Diversity	Plant Biomass (**1**)	141.20	-65.51	5	0.13	0.00	0.43
	Plant Biomass, CV Plant Height (**0.39**)	142.00	-64.88	6	0.14	0.81	0.28
	Fertilizer (**0.29**), Plant Biomass	142.93	-65.35	6	0.14	1.73	0.18
Richness	Fertilizer (**0.58**), Plant Biomass (**1**)	539.46	-263.61	6	0.47	0.00	0.512
	Plant Biomass	540.22	-265.03	5	0.47	0.76	0.35
	Fertilizer, Plant Biomass, CV Plant Height (**0.29**)	541.40	-263.54	7	0.47	1.94	0.16

AIC statistics include: *AICc* AIC statistic; *LL* log likelihood; *df* degrees of freedom; R^2 adjusted regression coeffiecient; *ΔAICc* AICc minus top model AICc, and *wi* model weight. RIVs for each variable are presented in (**bold**) next to the model variable the first time it appears (i.e. Fertilizer (**0.9**), Plant Biomass (**1**)).

Table S5. Top models for relationships between clipping and fertilization and community metrics of arthropods caught in pitfall traps.

Pitfall Trap Samples							
Model	**Model Variables**	**AIC_c**	**LL**	**df**	**R^2**	**ΔAIC_c**	**w_i**
Activity	Fertilizer (**0.51**), Plant Biomass (**0.76**)	985.70	-486.73	6	0.55	0.00	0.22
	Fertilizer, Plant Biomass, CV Plant Height (**0.62**)	985.84	-485.76	7	0.55	0.14	0.20
	Plant Biomass, CV Plant Height	985.95	-486.85	6	0.55	0.25	0.19
	Plant Biomass	986.43	-488.13	5	0.55	0.73	0.15
	CV Plant Height	986.61	-488.22	5	0.55	0.91	0.14
	Fertilizer, CV Plant Height	987.56	-487.66	6	0.55	1.86	0.09
Diversity	Plant Biomass (**1**)	-288.22	149.19	5	0.21	0.00	0.35
	Plant Biomass, CV Plant Height (**0.5**)	-288.09	150.17	6	0.21	0.12	0.33
	Fertilizer (**0.31**), Plant Biomass, CV Plant Height	-286.65	150.48	7	0.21	1.57	0.16
	Fertilizer, Plant Biomass	-286.46	149.35	6	0.21	1.76	0.15
Richness	Fertilizer (**0.97**)	375.55	-182.69	5	0.53	0.00	0.52

AIC statistics include: *AICc* AIC statistic; *LL* log likelihood; *df* degrees of freedom; R^2 adjusted regression coeffiecient; *ΔAICc* AICc minus top model AICc, and *wi* model weight. RIVs for each variable are presented in (**bold**) next to the model variable the first time it appears (i.e. Fertilizer (**0.51**), Plant Biomass (**0.76**)).

Table S6. Species scores from a canonical correspondence analysis (CCA) performed on vacuum- and pitfall-caught arthropod samples. Species scores over |0.3| have been bolded in the table.

	Vacuum Samples		Pitfall Samples		
Taxa	**Axis 1**	**Axis 2**	**Axis 1**	**Axis 2**	**Axis 3**
Acari	**-0.868**	**0.991**	0.134	-0.079	**0.410**
Araneae	0.006	0.072	-0.066	-0.049	0.093
Blattodea	–	–	-0.102	**-0.709**	0.232
Coleoptera	-0.016	0.049	**0.365**	-0.287	0.156
Collembola	**-0.305**	-0.144	**-0.696**	-0.032	0.058
Diplopoda	–	–	**1.194**	0.232	**0.617**
Diptera	-0.092	-0.041	0.061	-0.008	0.225
Hemiptera	-0.127	-0.030	0.117	**-0.344**	0.100
Hymenoptera	0.148	0.024	0.021	0.024	-0.007
Lepidoptera	0.210	0.098	-0.153	-0.048	**0.365**
Mantodea	**-0.316**	**0.729**	–	–	–
Neuroptera	-0.051	-0.041	–	–	–
Orthoptera	0.157	-0.048	-0.031	**-0.386**	-0.425

Supplemental figure legends

Figure S1. Experimental setup of 90 plots of 2 × 2 m which were established in March 2017 and treated with one of six treatments which included combinations of clipping and NPK + micronutrient fertilization. Treatments were assigned randomly to plots within each of 15 blocks (Rows 1-15). Plots were separated by 10 m on all sides.

Figure S2. Example of plots with both clipping and fertilization treatments.

Figure S3. (a) Average plant height and **(b)** coefficient of variation for plant height for each clipping treatment. Plant height was measured monthly using a disc pasture meter in four locations per plot (n = 90 plots; C = clipped, HC = half-clipped and UC = unclipped). Average plant height is the mean of those four height values per plot. The coefficient of variation was calculated for each plot as the SD of the four height measurements over the average plant height per plot.

Figure S4. Average arthropod abundance and activity **(a** and **d)**, diversity **(b** and **e)**, and richness (ants excluded from all metrics) based on total ant abundance/activity for each plot and month (n = 90). Panels **(a)**, **(b)**, and **(c)** are vacuum samples and while panels **(d)**, **(e)**, and **(f)** are pitfall samples. Points are colored based on clipping treatment (black = clipped, dark gray = half-clipped, and light gray = unclipped). Black line shows linear fit with 95% confidence interval. Pearson's correlation coefficient was used to calculate R^2 and P values.

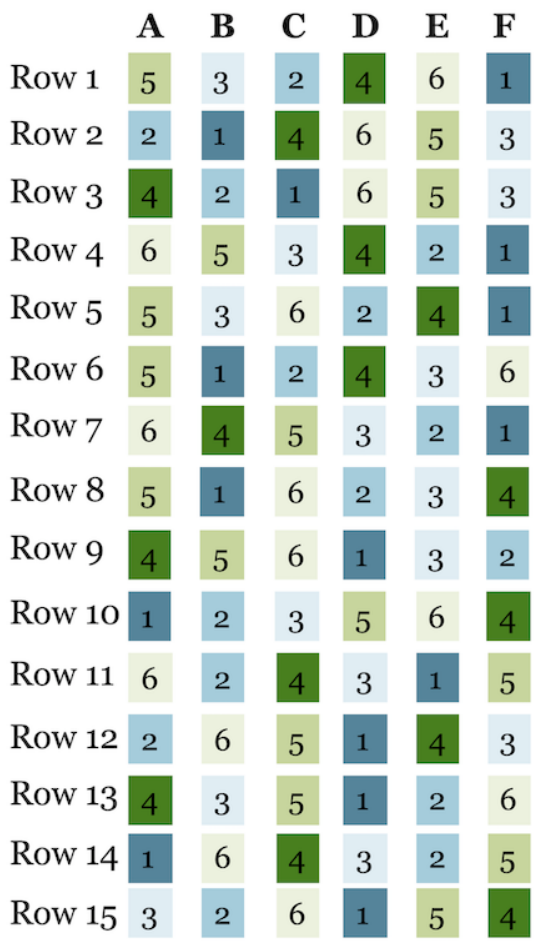
Figure S5. Relationship between fertilization and Total Nitrogen **(a**, **d**, **g)**, Total Carbon **(b, e, h)**, and Total Carbon to Nitrogen Ratio **(c, f, i)**. Panels **(a), (b),** and **(c)** show values for the entire plot, panels **(d)**, **(e)**, and **(f)** show values for forbs, and panels **(g)**, **(h)**, and **(i)** show values for grasses. Asterisk indicate significance differences at the $\alpha < 0.05$ level from a Welch's *t* test.

Figure S6. Relationship between plant biomass and arthropod abundance and activity **(a** and **d)**, diversity **(b** and **e)** and richness **(c)**. Relationships are only shown for variables that had RIV > 0.45 (see Fig. S5 and S6). Panels **(a)**, **(b)**, and **(c)** are vacuum samples and while panels **(d)** and **(e)** are pitfall samples. Black line shows linear fit with 95% confidence interval. A linear model was used to calculate *F*, *P*, and R^2 values.

Figure S7. Relationship between fertilizer application and arthropod abundance and activity **(a** and **c)** and richness **(b** and **d)**. Relationships are only shown for variables that had RIV > 0.45 (see Fig. S6 and S7). Panels **(a)** and **(b)** are vacuum samples and while panels **(c)** and **(d)** are pitfall samples. Results from Welch's *t* test are displayed, and asterisks indicate significance differences at the $\alpha < 0.05$ level.

Figure S8. Relationship between CV plant height and pitfall trap **(a)** arthropod activity and **(b)** richness. Relationships are only shown for variables that had RIV > 0.45 (see Fig. S6 and S7). Black line shows linear fit with 95% confidence interval. A linear model was used to calculate *F*, *P*, and R^2 values.

Figure S1.



Experimental Design

Plot size: 2 x 2 m

15 replicates of each treatment

Each plot separated by 10 m on all sides

Treatment	Clipping	Fertilization
1	Clipped	Unfertilized
2	Half Clipped	Unfertilized
3	Unclipped	Unfertilized
4	Clipped	Fertilized
5	Half Clipped	Fertilized
6	Unclipped	Fertilized

Figure S2.

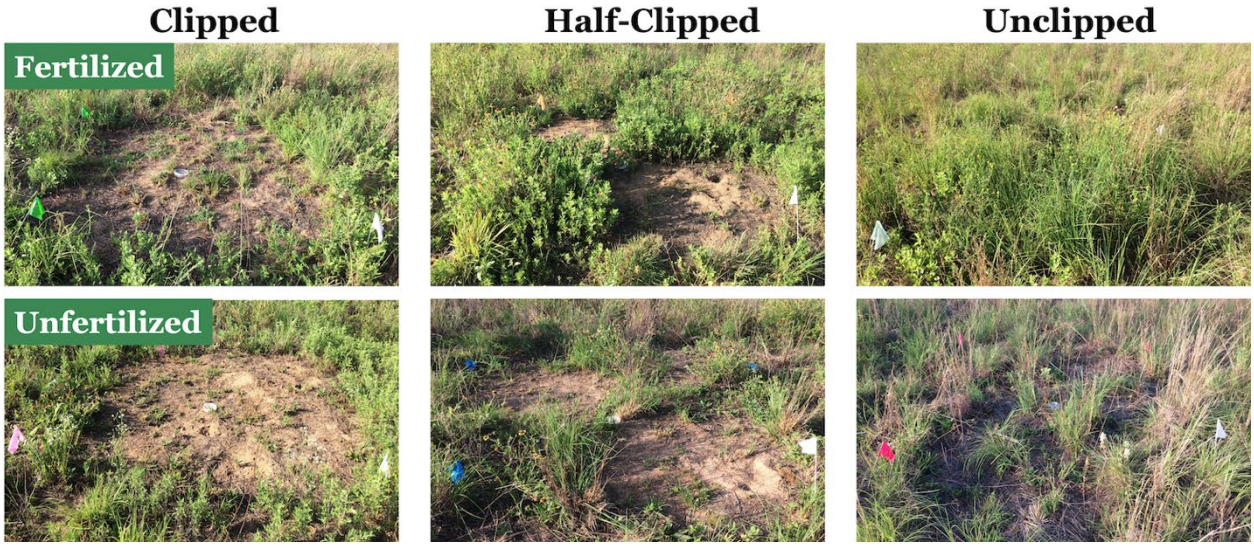


Figure S3.

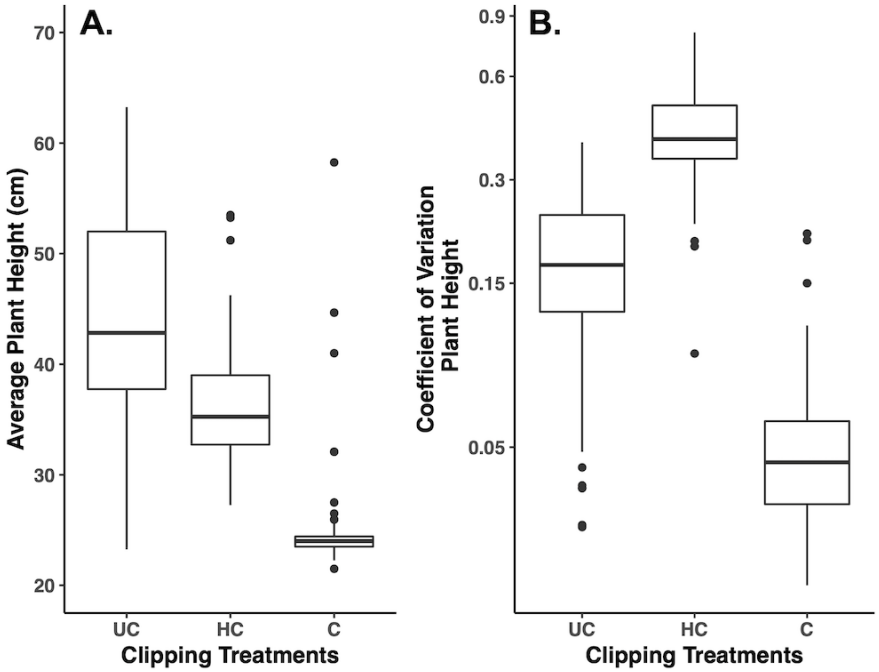

A.
Average Plant Height (cm)
70
60
50
40
30
20
UC
HC
C
Clipping Treatments
B.
Coefficient of Variation
Plant Height
0.9
0.6
0.3
0.15
0.05
UC
HC
C
Clipping Treatments

Figure S4.

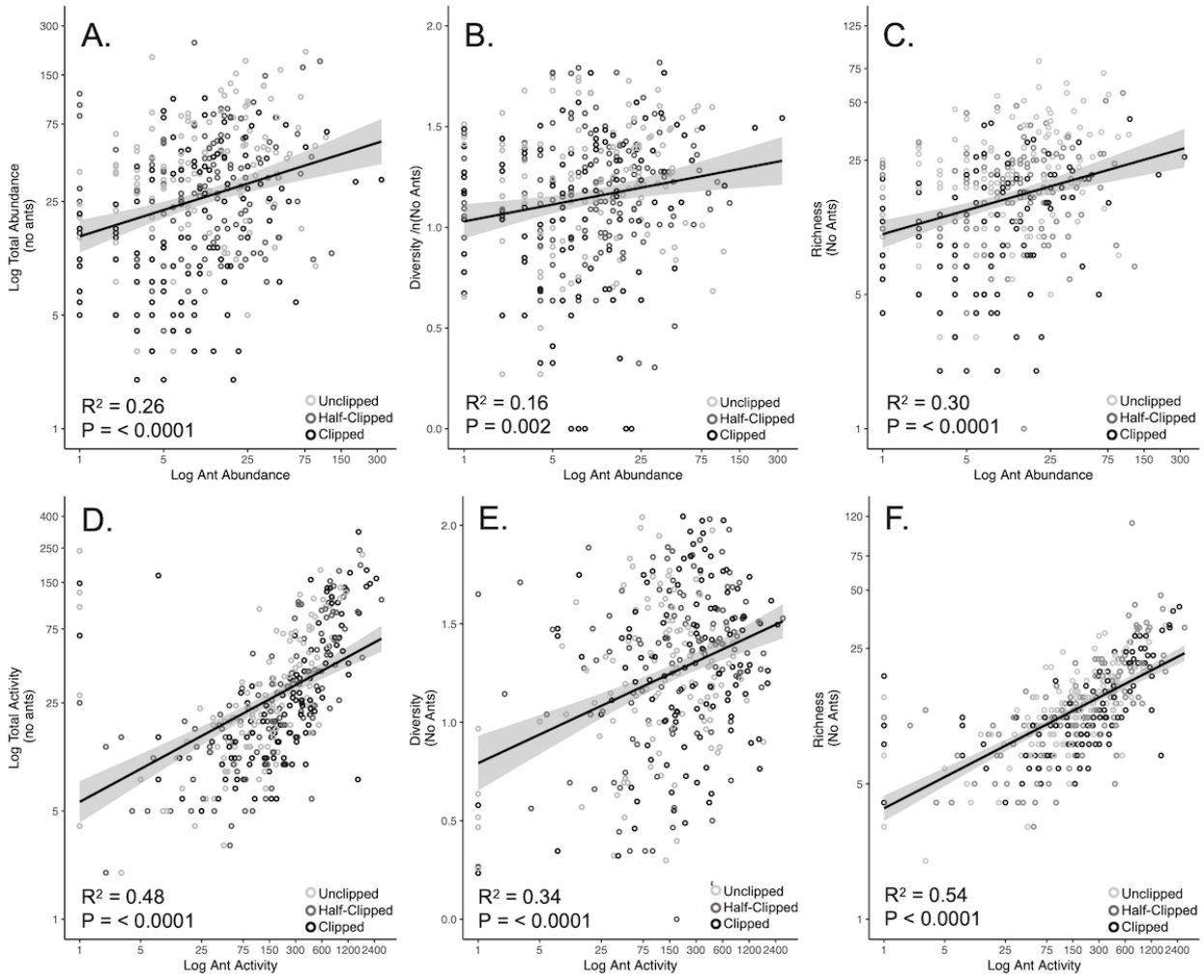

A.
Log Total Abundance (no ants)
Log Ant Abundance
R² = 0.26
P = < 0.0001
B.
Diversity /n(No Ants)
Log Ant Abundance
R² = 0.16
P = 0.002
C.
Richness (No Ants)
Log Ant Abundance
R² = 0.30
P = < 0.0001
D.
Log Total Activity (no ants)
Log Ant Activity
R² = 0.48
P = < 0.0001
E.
Diversity (No Ants)
Log Ant Activity
R² = 0.34
P = < 0.0001
F.
Richness (No Ants)
Log Ant Activity
R² = 0.54
P < 0.0001
Unclipped
Half-Clipped
Clipped

Figure S5.

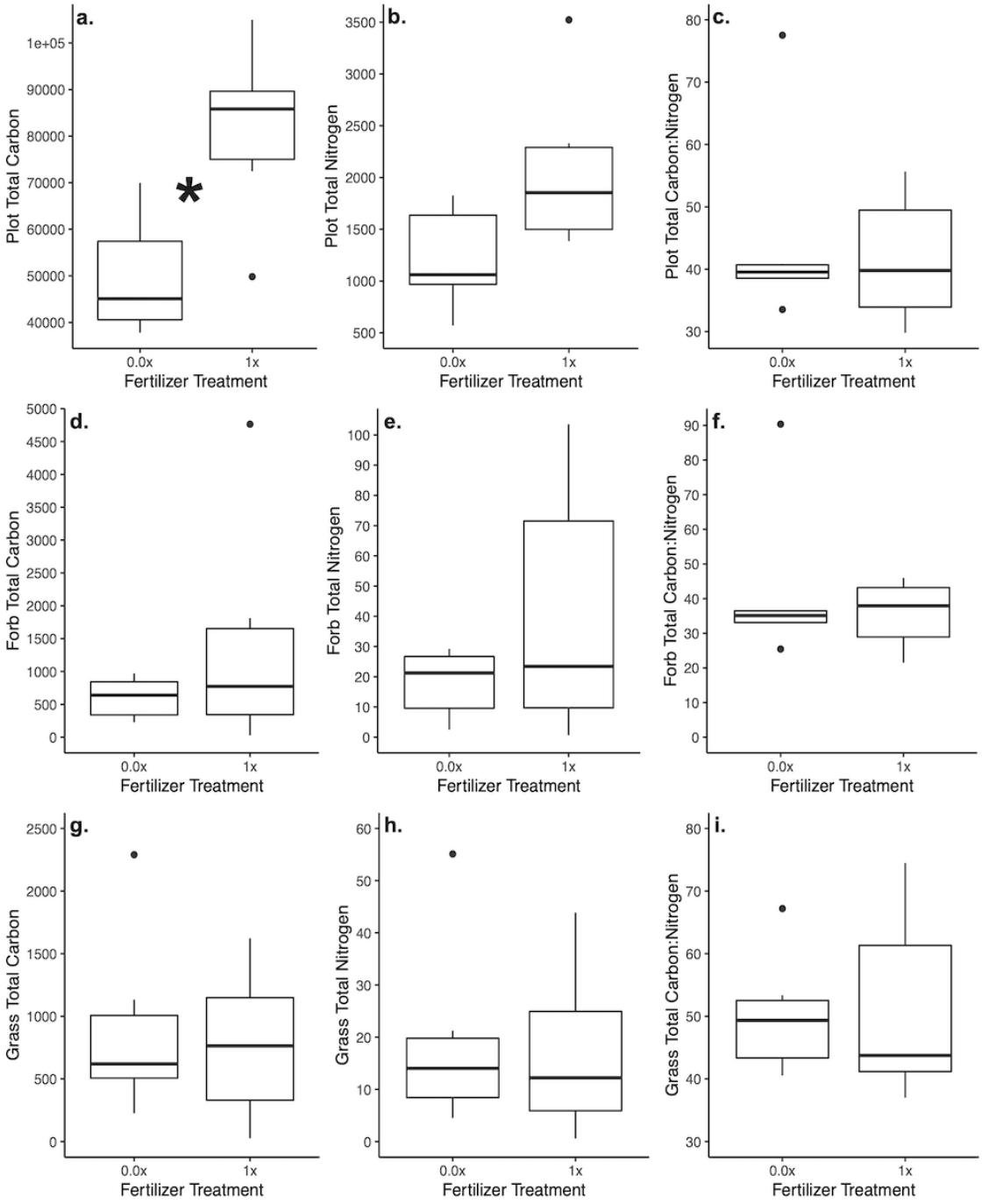

a.
Plot Total Carbon
1e+05
90000
80000
70000
60000
50000
40000
*
0.0x
1x
Fertilizer Treatment
b.
Plot Total Nitrogen
3500
3000
2500
2000
1500
1000
500
0.0x
1x
Fertilizer Treatment
c.
Plot Total Carbon:Nitrogen
80
70
60
50
40
30
0.0x
1x
Fertilizer Treatment
d.
Forb Total Carbon
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.0x
1x
Fertilizer Treatment
e.
Forb Total Nitrogen
100
90
80
70
60
50
40
30
20
10
0
0.0x
1x
Fertilizer Treatment
f.
Forb Total Carbon:Nitrogen
90
80
70
60
50
40
30
20
10
0
0.0x
1x
Fertilizer Treatment
g.
Grass Total Carbon
2500
2000
1500
1000
500
0
0.0x
1x
Fertilizer Treatment
h.
Grass Total Nitrogen
60
50
40
30
20
10
0
0.0x
1x
Fertilizer Treatment
i.
Grass Total Carbon:Nitrogen
80
70
60
50
40
30
0.0x
1x
Fertilizer Treatment

Figure S6.

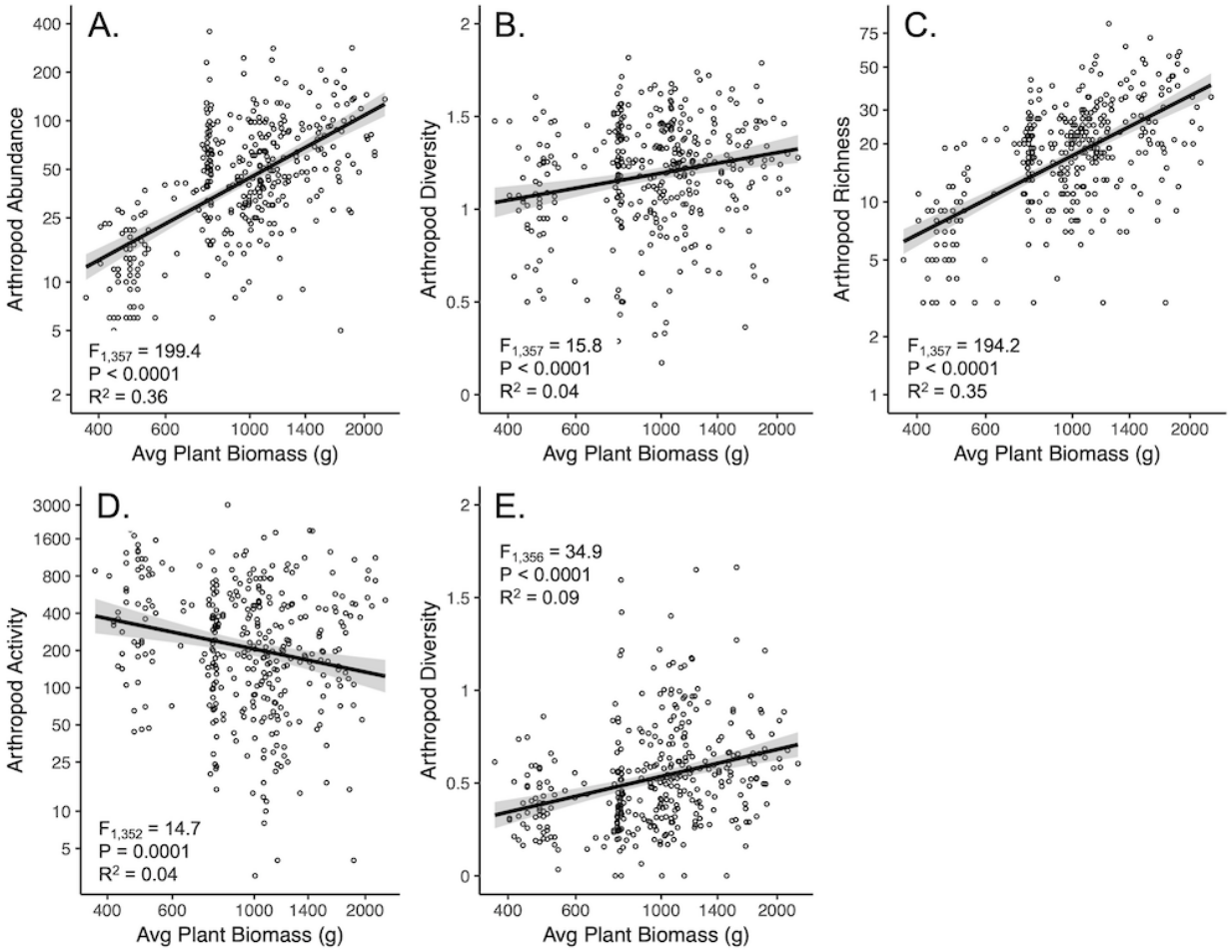

A.
Arthropod Abundance
$F_{1,357}$ = 199.4
P < 0.0001
R^2 = 0.36
Avg Plant Biomass (g)
B.
Arthropod Diversity
$F_{1,357}$ = 15.8
P < 0.0001
R^2 = 0.04
Avg Plant Biomass (g)
C.
Arthropod Richness
$F_{1,357}$ = 194.2
P < 0.0001
R^2 = 0.35
Avg Plant Biomass (g)
D.
Arthropod Activity
$F_{1,352}$ = 14.7
P = 0.0001
R^2 = 0.04
Avg Plant Biomass (g)
E.
Arthropod Diversity
$F_{1,356}$ = 34.9
P < 0.0001
R^2 = 0.09
Avg Plant Biomass (g)

Figure S7.

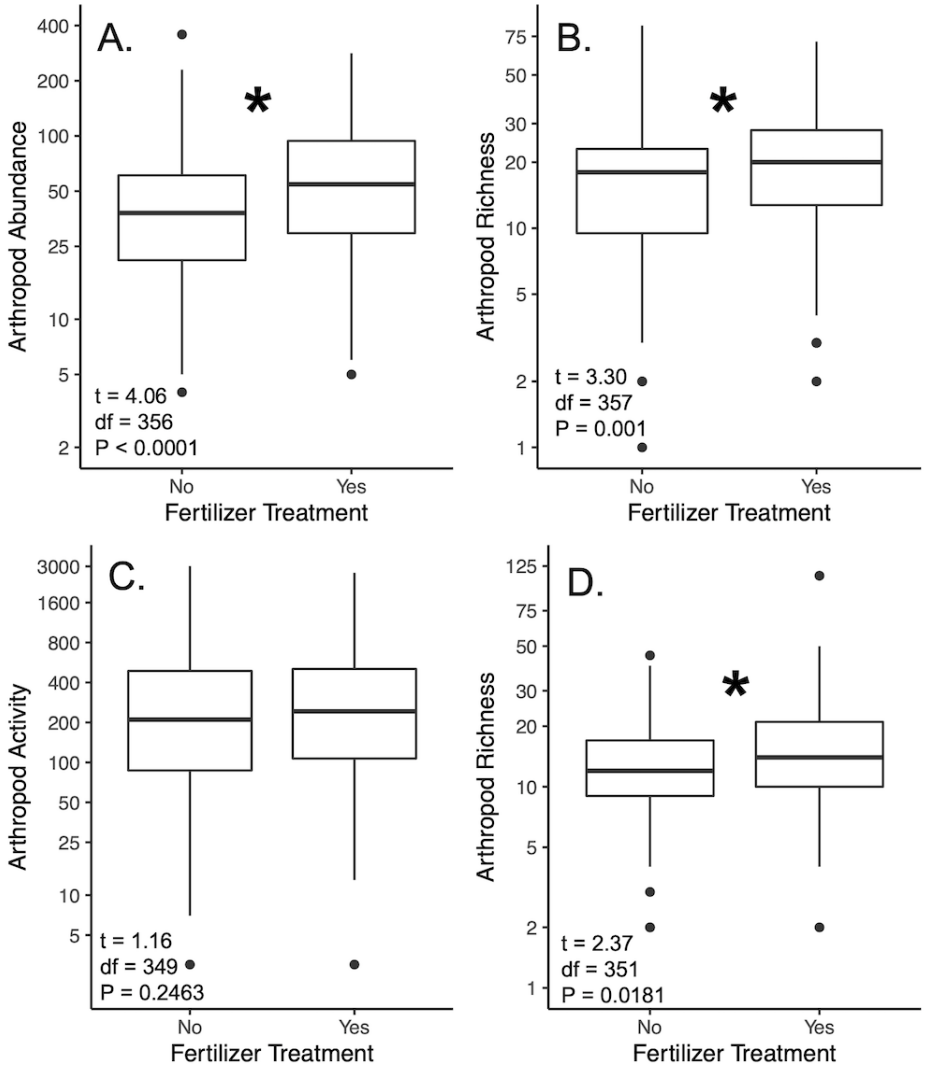
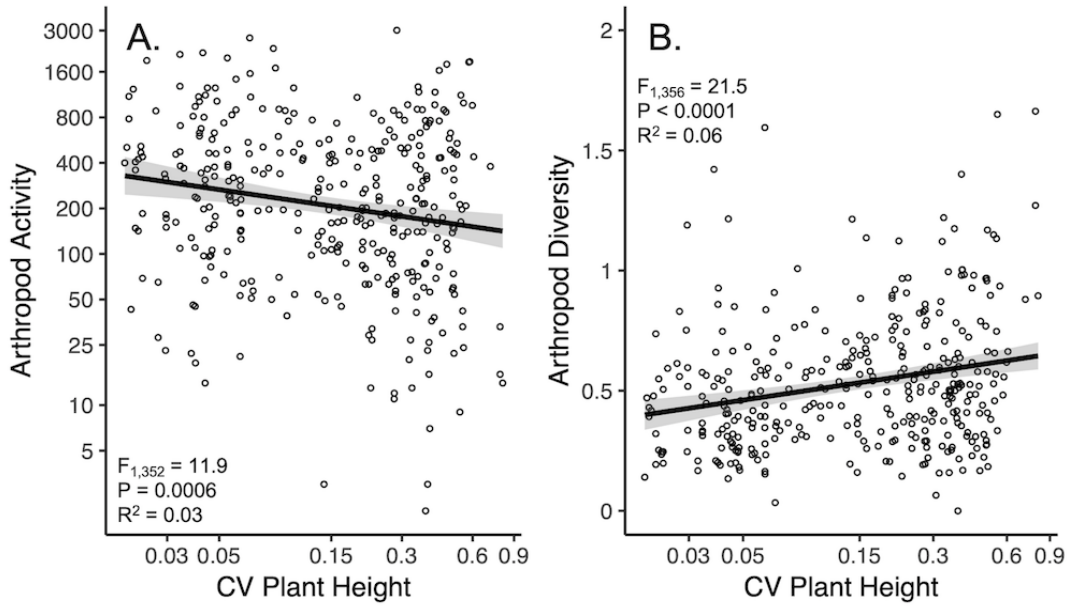

A.
Arthropod Abundance
400
200
100
50
25
10
5
2
t = 4.06
df = 356
P < 0.0001
No
Yes
Fertilizer Treatment
B.
Arthropod Richness
75
50
30
20
10
5
2
1
t = 3.30
df = 357
P = 0.001
No
Yes
Fertilizer Treatment
C.
Arthropod Activity
3000
1600
800
400
200
100
50
25
10
5
t = 1.16
df = 349
P = 0.2463
No
Yes
Fertilizer Treatment
D.
Arthropod Richness
125
75
50
30
20
10
5
2
1
t = 2.37
df = 351
P = 0.0181
No
Yes
Fertilizer Treatment

Figure S8.


A.
Arthropod Activity
3000
1600
800
400
200
100
50
25
10
5
$F_{1,352}$ = 11.9
P = 0.0006
R^2 = 0.03
0.03 0.05 0.15 0.3 0.6 0.9
CV Plant Height
B.
Arthropod Diversity
2
1.5
1
0.5
0
$F_{1,356}$ = 21.5
P < 0.0001
R^2 = 0.06
0.03 0.05 0.15 0.3 0.6 0.9
CV Plant Height

CHAPTER THREE

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ABIOTIC FACTORS AND PLANT BIOMASS, NOT PLANT DIVERSITY, STRONGLY SHAPE GRASSLAND ARTHROPODS UNDER DROUGHT CONDITIONS

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Abstract

Arthropod abundance and diversity often track plant biomass and diversity at the local scale. However, under altered precipitation regimes and anthropogenic disturbances, plant-arthropod relationships are expected to be increasingly controlled by abiotic, rather than biotic, factors. We used an experimental precipitation gradient combined with human management in a temperate mixed-grass prairie to examine (1) how two drivers, altered precipitation and biomass removal, can synergistically affect abiotic factors and plant communities and (2) how these effects can cascade upward, impacting the arthropod food web. Both drought and hay harvest increased soil surface temperature, and drought decreased soil moisture. Arthropod abundance decreased with low soil moisture and, contrary to our predictions, decreased with increased plant biomass. Arthropod diversity increased with soil moisture, decreased with high surface temperatures, and tracked arthropod abundance but was unaffected by plant diversity or quality. Our experiment demonstrates that arthropod abundance is directly constrained by abiotic factors and plant biomass, in turn constraining local arthropod diversity. If robust, this result suggests climate change in the southern Great Plains may directly reduce arthropod diversity.

Keywords: climate change, drought, hay harvest, invertebrate, prairie, precipitation

Introduction

Experimentally increased plant biomass or diversity often increase arthropod abundance and diversity (Siemann 1998, Haddad et al. 2001, Crutsinger et al. 2006, Haddad et al. 2009, Burkle et al. 2013). In contrast, decreasing plant richness can decrease arthropod diversity (Haddad et al. 2000). Global climate change—including increased temperature and atmospheric CO_2, and altered precipitation patterns (de Sassi et al. 2012, Jamieson et al. 2012, Lee et al. 2014)—may also reshape plant–arthropod relationships. This is especially true in grasslands, an ecosystem covering ~37% of Earth's surface that provides many ecosystem services from livestock forage to carbon sequestration (White et al. 2000). Although many grassland organisms are accustomed to limited (and variable) precipitation (Knapp et al. 2002), climate models predict increased precipitation variability in grasslands (Maurer et al. 2020). Reduced precipitation decreases plant biomass (Heisler-White et al. 2009), but increased precipitation variability and soil water content can promote plant diversity (Knapp et al. 2002). Global climate change is co-occurring with anthropogenic disturbances such as hay harvesting, with expected repercussions for both primary producers and arthropods (Xu et al. 2013, Shi et al. 2016). Arthropods comprise the majority of animal biodiversity and provide critical ecosystem services, thus understanding their responses to these multiple stressors is an important step towards maintaining healthy ecosystems (Tscharntke and Greiler 1995, Whiles and Charlton 2006). Here we explore the synergy of changing precipitation and hay removal on the abundance and diversity of grassland arthropods, using a novel experiment to test three, non-exclusive hypotheses.

Drought can affect arthropod communities via its effects on two master regulators: water and temperature. Drought can indirectly affect arthropods by reducing plant turgor pressure and hence foliar water availability (Huberty and Denno 2004). Lower soil moisture can directly

reduce both arthropod abundance and diversity by increasing desiccation risk (Harrison et al. 2012). Likewise, hay removal can expose soil to more insolation, thus increasing surface temperatures and filtering for heat-tolerant species (de Sassi et al. 2012). Combined, drought and hay harvest may result in higher surface temperatures and less moisture than found with drought or hay harvest alone, filtering arthropods and reducing both arthropod abundance and diversity. The abiotic constraint hypothesis (H1) predicts that decreased moisture availability and higher temperatures select for more stress-tolerant arthropods (Greenslade 1983, Chase 1996).

Drought can also reduce primary production (Heisler-White et al. 2009) and alter plant nutrient quality, indirectly affecting arthropod communities. In grasslands, reduced precipitation can increase the productivity of drought-resistant C_4 grasses at the expense of C_3 forbs (Heisler-White et al. 2009) leading to ecosystem-level decreases in plant quality via higher lignin and lower nitrogen content (Caswell et al. 1973, Tscharntke and Greiler 1995). Drought can thus directly reduce the amount of food for herbivores to eat and eat and digestibility at the ecosystem level. However, drought can also increase the concentration of nutrients in individual plants experiencing water stress (Franzke and Reinhold 2011, Grant et al. 2014) while decreasing plant defenses. This may result in increased chewing herbivory on drought-stressed plants (Mattson and Haack 1987, Gutbrodt et al. 2011, Jamieson et al. 2012). Hay removal, by definition, reduces the amount of food for plant consumers. The more individuals hypothesis (H2) predicts that decreases in quantity and quality of forage should also reduce insect abundance, and through doing so, reduce insect diversity (Srivastava and Lawton 1998, Kaspari et al. 2003). Decreases in plant quality may also erode the common positive correlation between plant biomass and arthropod abundance (Siemann 1998, Haddad et al. 2000).

Finally, drought can affect arthropod diversity via changes to their host-plant diversity. The resource heterogeneity hypothesis (H3) predicts increasing plant diversity should directly increase arthropod diversity (Hutchinson 1959, Southwood et al. 1979, Borer et al. 2012). Drought can reduce plant diversity by favoring drought-resistant plant species, filtering out the arthropod species for which host plants become locally extinct (Haddad et al. 2001, Haddad et al. 2009, Borer et al. 2012). In contrast, management such as hay harvest may result in increased plant diversity because it results in more light and space for growth, increasing arthropod diversity despite water stress (Collins et al. 1998).

Here we report results from a novel multi-year factorial field experiment where we manipulated precipitation with rainfall shelters and mimicked management through yearly clipping (hereafter hay harvest). We test the preceding three hypotheses (see Table 1 and Fig. 1A) detailing how direct and indirect effects of drought and hay harvest work synergistically to affect the plant and arthropod assemblages in a mixed-grass prairie with implications for future climatic scenarios.

Methods

Study site

We studied the arthropod community in 2017 and 2018 from June through August at Kessler Atmospheric and Ecological Field Station (KAEFS), a mixed-grass prairie in central Oklahoma, USA (34.59° N, 97.31° W), last farmed >45 yr ago. KAEFS has Nash-Lucien complex soil (Xu et al. 2013) and is dominated by *Schizachyrium scoparium, Sorghastrum nutans*, *Dichanthelium oligosanthes*, *Ambrosia psilostachya,* and *Solidago nemoralis*. Mean annual rainfall is 914 mm and average temperature in July is 27.7°C (Appendix S1: Fig. S1).

Experimental design

To determine the response of arthropods to a precipitation gradient and human management, we used rain shelters to establish a gradient of precipitation and vegetation clipping to mimic hay harvesting. This experimental study is part of Drought-Net, a coordinated global network examining terrestrial ecosystem sensitivity to drought. We used a randomized block split-plot design with seven precipitation treatments five water exclusion levels [–20%, –40%, –60%, –80%, and –100%], water addition [+50%], and control [0% change in precipitation]) replicated three times for a total of 21, 2 × 2 m plots. Rain-out shelters were established in Spring 2016 and reduced rain but not sunlight. We combined precipitation treatments with two clipping treatments (clipped or unclipped subplot) to mimic hay harvest, initiated in September 2016. Clipping occurred in the same subplot each fall, with biomass clipped down to 10 cm and removed (see Appendix S1: Fig. S2 for experimental layout).

Microclimate sampling

To determine how our manipulations affected abiotic factors, we measured soil moisture and surface temperature. Soil probes (Decagon 5TM, ICT International) were installed at a depth of 10 cm in each clipped and unclipped subplot and continuously measured percent volumetric water content (VWC) from May 2017 to September 2018. For each arthropod sampling event (n = 6), we averaged the VWC from the two weeks prior to sampling to determine how the precipitation and clipping treatments affected soil moisture. We used temperature loggers (iButton®, Maxim Integrated) to measure soil surface temperature continuously from May 2017 to September 2018 and averaged the data to obtain monthly mean surface temperature for each subplot. We excluded August 2017 data because rodents disrupted the temperature loggers.

Plant sampling

To determine the effects of a precipitation gradient and land management on plant communities, we measured plant foliar cover and Shannon's diversity each year in May and August using a modified Braun-Blanquet scale (Braun-Blanquet 1932; Castillioni et al. 2020). We estimated aboveground net primary productivity (ANPP) at the end of each growing season (September) by clipping plants, sorting to functional groups, drying, and weighing them. Plant %N was measured in 2018 using combustion analysis at the OSU Soil, Water, and Forage Lab (http://soiltesting.okstate.edu/). We calculated average plant quality (%N) per plot for each functional group weighted by group proportion present using the formula: Plant Quality = ((%N C_3 plants * % C_3 plants/plot) + (%N C_4 plants * % C_4 plants/plot))/2). To see if water stress affected arthropod herbivory, we measured plant herbivory on four plant species per plot (*S. nutans*, *S. scoparium*, *Chamaecrista fasciculata*, and *A. psilotachya*) in August 2018 following the Nutrient Network herbivory protocol. See Appendix S2 for detailed plant sampling protocols.

Arthropod sampling

To measure the arthropod response to our manipulations, we sampled arthropods once per month from June through August 2017 and 2018 on clear days proceeded by at least two dry days (n = 6; Appendix S1: Table S1). We waited at least 1 d between sampling clipped and unclipped subplots to minimize disturbance effects. To sample arthropods, we used an inverted leaf-blower (Husqvarna 125BVX) for 50 s per plot. Samples were put on ice in the field and kept frozen until sorted. We counted and identified all arthropods to family or major taxonomic group and recorded the number of unique species or morphospecies per taxonomic group (Appendix S1: Table S4). We calculated arthropod abundance and diversity for each plot and month (taxon-

level; Shannon's *H*) except August 2017, when did not have corresponding surface temperature data.

Statistical Analysis

All statistics were performed using R version 3.6.1 (R Core Development Team 2016). We used a piecewise Structural Equation Model (SEM) (1) to examine which hypotheses regulate arthropod abundance and diversity under drought and hay harvest conditions and (2) to examine the direct and indirect effects of a precipitation gradient and hay harvest on arthropod abundance and diversity. In comparison to traditional SEM, piecewise SEMs are less restricted by the number of links per sample size and Fisher's *C* is used as the goodness-of-fit statistic (Shipley 2013, Lefcheck 2016). Analogous to traditional SEM, a non-significant *P* value indicates a well-fit model. In our *a priori* model (Fig. 1A), we predicted drought and hay harvest would indirectly affect arthropod abundance and diversity through their effects on surface temperature, soil moisture, ANPP, plant quality, and plant diversity. We kept precipitation treatment as a numerical variable and $\log_{10}$ transformed arthropod abundance and ANPP to meet normality assumptions. In order to resolve pseudo-replication due to repeated sampling, we included plot as a random variable in all model regressions. We used a single piecewise SEM model based on our *a priori* model and did not remove non-significant links. Piecewise SEMs were conducting using the piecewiseSEM (Lefcheck 2016) and nlme (Pinheiro et al. 2013) packages in R.

Results

Abiotic responses to drought and hay harvest

Both drought and hay harvest treatments changed the abiotic environment. Soil surface temperature increased linearly with drought and was about 2.5°C higher on 100% drought plots relative to control plots. Hay harvest led to an average temperature increase of 1.1°C (Fig. 1B; Appendix S1: Fig. S3). The drought gradient linearly decreased soil moisture. Moisture on 100% drought plots was 14% lower than control plots, and soil moisture on water addition treatments was 7% higher (Fig. 1B; Appendix S1: Fig. S4).

Plant responses to drought and hay harvest

Total ANPP on the 100% drought plots was 65% lower than controls. Biomass of C_4 plants decreased by 59% with drought (Appendix S1: Fig. S5). Surprisingly, biomass of C_3 plants was highest on three disparate treatments: 100% drought, control, and water addition plots (Appendix S1: Fig. S5). Hay harvest had no significant effect on ANPP, declining on average by 10% (Fig. 1B; Appendix S1: Fig. S5). Neither drought intensity nor hay harvest significantly affected plant %N (Fig. 1B; Appendix S1: Fig. S6).

We recorded 28 plant species in 2017 and 29 plant species in 2018. Plant diversity was 8% lower on 100% drought plots relative to control. Water addition increased plant diversity by 7.6% relative to control plots (Fig. 1B; Appendix S1: Fig. S7). Hay harvest increased plant diversity by 5.7% (Fig. 1B; Appendix S1: Fig. S7).

Arthropod abundance

Arthropod abundance varied 100-fold among plots in both years. We collected 3,431 arthropods

in 2017 (excluding 865 in August—see Methods) and 10,153 arthropods in 2018. In 2017, the number of arthropods varied from 1 to 96 per plot (mean ± SE; 28.9 ± 1.7); in 2018 the number varied 3 to 335 (83.1 ± 5.5). Our a priori piecewise SEM had a good fit (Fisher's $C = 31.58$, Akaike's information criterion, corrected [AIC_c] = 141.84, $P = 0.207$) and accounted for between 7% (arthropod abundance) and 43% (arthropod diversity) of the variation in the arthropod community response (Fig. 1B; Appendix S1: Tables S2 and S3). The abiotic environment and plant biomass drove arthropod abundance. Consistent with the abiotic constraint hypothesis (H1), higher soil moisture increased arthropod abundance (Fig. 1B; Appendix S1: Fig. S8). Contrary to the more individuals hypothesis (H2), increased plant biomass reduced arthropod abundance (Fig. 1B; Appendix S1: Fig. S8).

Arthropod diversity

In both years, arthropod diversity (Shannon's *H*) varied threefold across plots. In 2017, arthropod diversity per plot varied from 0.4 to 1.7 (1.2 ± 0.02); in 2018 the diversity varied from 0.58 to 1.9 (1.4 ± 0.03). Arthropod diversity changed with abiotic drivers and arthropod abundance. First, consistent with the abiotic constraint hypothesis (H1), increasing soil moisture increased arthropod diversity while increasing surface temperatures reduced diversity (Fig. 1B; Appendix S1: Fig. S9). Second, consistent with the more individuals hypothesis (H2), arthropod diversity increased with arthropod abundance. Plots added one species on average for every 16 more individuals (Fig. 1B; Appendix S1: Fig. S10). Third, contrary to the resource heterogeneity hypothesis (H3) plant and arthropod diversity were uncorrelated and plant quality did not increase either arthropod abundance or diversity (Fig. 1B; Appendix S1: Figs. S6 and S10).

Discussion

Our experiment demonstrated that arthropod abundance responded strongly to changes in plant productivity and soil moisture caused by drought. Arthropod diversity at the 2 × 2 m grain tracked changes in arthropod abundance and increased with higher soil moisture but decreased with temperature. Surprisingly, arthropod diversity did not track plant diversity. As current climate change predictions for the Great Plains include increased frequency and duration of severe droughts, our experimental results suggest future declines in arthropod diversity.

Our results suggest precipitation amount regulates arthropod abundance and diversity while temperature regulates arthropod diversity. Both precipitation reduction and human management (hay harvest) increased ground-level light penetration and surface temperature, a result similar to other studies (Collins et al. 1998, Xu et al. 2013). Drought increased surface temperatures more than simulated haying. Higher surface temperatures reduced arthropod diversity, suggesting high temperatures may have filtered for species that could tolerate hot patches, while not reducing overall arthropod abundance (Barton and Schmitz 2009, de Sassi et al. 2012). Increasing soil moisture promoted both higher arthropod abundance and diversity. Due to the high desiccation risk with arthropods' high surface to volume ratio (Harrison et al. 2012), we would expect soil moisture to filter both the species present and their abundance. Arthropods can deal with reduced moisture by relocating, burrowing in the soil, or building shelters (Berridge 2012). At the spatial scale of our experiment, arthropods likely emigrated from or avoided low moisture plots, options not available at the larger spatial scale of continental droughts.

As predicted, we found that drought conditions decreased overall plant biomass. Contrary to other studies, we found lower abundance of all arthropod trophic guilds at higher levels of

plant biomass (i.e., on plots with less water reduction; Lee et al. 2014, Torode et al. 2016). This unexpected relationship could be due to several reasons. First, on plots with less water reduction (–40% to +50%) we saw a higher proportion of C_4 warm-season grasses. C_4 grasses are less palatable to arthropods than C_3 plants (Caswell et al. 1973, Heisler-White et al. 2009), likely reducing both herbivore abundance and the abundance of predators tracking prey abundance on plots with slight water reduction (Appendix S1: Fig. S11). Second, water reduction can lower plant turgor pressure, increasing the difficulty of sucking arthropods to feed (Huberty and Denno 2004). However, we did not see a change in sucking damage with plant biomass (Appendix S1: Fig. S12). Third, drought can increase the concentration of nutrients in plants experiencing water stress while decreasing plant defenses resulting in increased chewing herbivory on drought-stressed plants (Mattson and Haack 1987, Franzke and Reinhold 2011, Gutbrodt et al. 2011). In fact, we saw a decrease in chewing damage as ANPP increased (correlated with less soil moisture; Appendix S1: Fig. S12). However, we saw no significant change in plant quality with reduced precipitation nor did plant quality significantly affect arthropod abundance or diversity. The specific mechanism driving the increase in arthropod abundance with reduced plant biomass remains unclear, but as drought and ANPP are negatively correlated it deserves further exploration.

Plant diversity, which varied twofold (1.3 to 2.7) across our 30 plots, was uncorrelated with arthropod diversity (Fig. 1B; Appendix S1: Fig. S10). This could be due to low overall plant diversity as both reduced precipitation and plant biomass had strong negative effects on plant diversity (Fig. 1B). Alternatively, if the abundance of C_4 plants continues to increase at the expense of C_3 plants on plots with medium water reduction, we may see a larger decrease in plant diversity and a corresponding reduction in arthropod diversity. Additionally, other studies

reporting a positive relationship between plant and arthropod diversity either experimentally increased plant diversity (Crutsinger et al. 2006, Haddad et al. 2009, Burkle et al. 2013, Welti et al. 2017) or ran their experiment over a longer period, that is, 10+ years (Siemann 1998, Haddad et al. 2009). Although we had interannual variation in our response variables, we sampled our plots after 1–2 yr of treatment, a period perhaps too short to detect a substantial change in plant diversity, which experiences a slower turnover rate than arthropod diversity. Our hay harvesting treatment increased plant diversity due to higher ground surface light, but the comparative effect was not large. Our results demonstrate that under drought conditions, plant diversity may not be as important at constraining arthropod diversity as abiotic factors and arthropod abundance.

Conclusions and Future Directions

Experiments examining the response of arthropods to precipitation manipulation typically use only one or two levels of rainfall reduction or addition (e.g. Suttle et al. 2007, Lee et al. 2014, Griffith and Grinath 2018, Tamburini et al. 2018), or look at combinations of rainfall frequency (Suttle et al. 2007, Grant et al. 2014, Mariotte et al. 2016, Torode et al. 2016). Because climate projections are inexact, our experiment utilized a seven-level precipitation manipulation gradient in combination with hay harvest. This experimental design led to insights relevant to multiple possible future precipitation regimes. As our pulse experiment transitions into a press experiment over the next years, we will see if some of the strongest effects in our results (e.g. decreases in insect abundance with increases in plant biomass) are transitory. Although we documented no effect of drought or hay harvest on %N (our measure of plant quality), other nutrients such as P, K, or micronutrients could be changing with our treatments. A better understanding of the plant above- and belowground stoichiometry across treatments will further address shifts in the

dynamics of plant–arthropod interactions under future climates. Here we show evidence for the importance of moisture and temperature in regulating community abundance and diversity among arthropods, an abundant taxon (Bar-On et al. 2018) in one of Earth's dominant ecosystems (White et al. 2000).

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Table 1. Proposed hypotheses regulating arthropod abundance and diversity in grasslands.

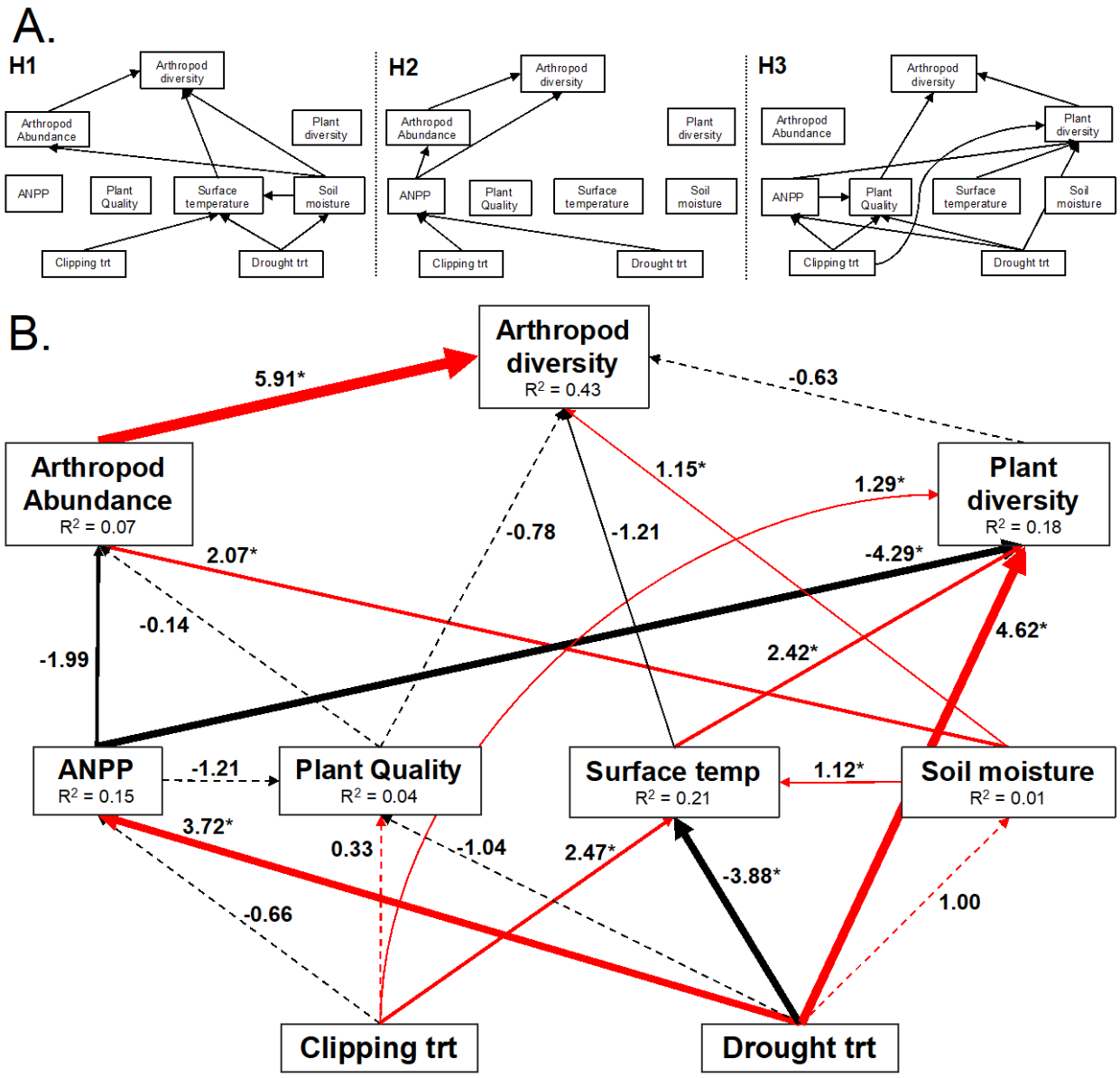
No.	Hypothesis Name	Definition
H1	Abiotic constraint hypothesis	Abiotic factors (moisture availability and temperature) select for a subset of stress-tolerant/intolerant arthropods[1,2]
H2	More individuals hypothesis	Increasing primary producer biomass increases consumer abundance which in turn increases consumer diversity[3,4]
H3	Resource heterogeneity hypothesis	Increasing plant diversity should increase arthropod diversity because of increased niches and diet variety[5,6,7]

Notes: References are given by the numbers above: [1]Greenslade (1983), [2]Chase (1996), [3]Srivastava and Lawton (1998), [4]Kaspari et al. (2003), [5]Hutchinson (1959), [6]Southwood et al. (1979), [7]Borer et al. (2012).

Figure Legends:

Figure 1. Piecewise structural equation model (SEM) depicting the direct and indirect effects of drought and clipping on arthropod abundance and diversity. (A) Conceptual a priori models for each of the three hypotheses examined within our piecewise SEM (see Table 1 for hypotheses descriptions). (B) Piecewise SEM based on our a priori model. The piecewise SEM fit our data well: Fisher's $C = 31.58$, $AIC_c = 141.84$, $P = 0.207$. Partial R^2 values are under each predicted variable and standardized path estimates are provided next to each path with line thickness scaled based on the magnitude of the estimate (see Methods for variable descriptions). Red and black arrows indicate positive and negative relationships, respectively. Dashed arrows represent nonsignificant paths ($P > 0.05$). Asterisks (*) indicate significance ($P < 0.05$). Model estimates, standard errors, and P values are provided in Appendix S1: Table S2.

Figure 1.


A.
H1
Arthropod diversity
Arthropod Abundance
Plant diversity
ANPP
Plant Quality
Surface temperature
Soil moisture
Clipping trt
Drought trt
H2
Arthropod diversity
Arthropod Abundance
Plant diversity
ANPP
Plant Quality
Surface temperature
Soil moisture
Clipping trt
Drought trt
H3
Arthropod diversity
Arthropod Abundance
Plant diversity
ANPP
Plant Quality
Surface temperature
Soil moisture
Clipping trt
Drought trt
B.
Arthropod diversity
R2 = 0.43
Arthropod Abundance
R2 = 0.07
Plant diversity
R2 = 0.18
ANPP
R2 = 0.15
Plant Quality
R2 = 0.04
Surface temp
R2 = 0.21
Soil moisture
R2 = 0.01
Clipping trt
Drought trt
5.91*
-0.63
1.15*
1.29*
-0.78
-1.21
2.07*
-4.29*
-0.14
-1.99
2.42*
4.62*
-1.21
1.12*
3.72*
0.33
-1.04
2.47*
-3.88*
-0.66
1.00

Chapter 3: Appendix S1. Supplemental Data.

Table S1. Table of when experimental manipulations occurred (e.g., "haying") and when variables were collected on plots (e.g., soil temperature, plant diversity). An (X) indicates either a manipulation or a sampling event. The asterisk (*) indicates missing data due to disruptions of the soil temperature loggers and thus the removal of all August 2017 data (e.g. soil moisture and the arthropod response). Variables are described in detail in the Methods.

	May-17	Jun-17	Jul-17	Aug-17	Sep-17	May-18	Jun-18	Jul-18	Aug-18	Sep-18
Clip "Hay" Plot					X					X
Soil Temperature	X	X	X	*	X	X	X	X	X	X
Soil Moisture	X	X	X	X*	X	X	X	X	X	X
Plant Diversity	X			X		X			X	
Plant ANPP					X					X
BugVac Plots		X	X	X*			X	X	X	
Forb Herbivory									X	
Grass Herbivory									X	
Plant %C and %N										X

Table S2. Model estimates, standard errors (SE), and p-values (*P*) from the single piecewise Structural Equation Model (Fig. 1) depicting the direct and indirect effects of precipitation reduction and clipping on arthropod abundance and diversity. Significant *P* values are bolded.

Response	**Predictor**	**Estimate**	**SE**	***P***
log(ANPP)	Drought Trt (prop Precipitation)	0.372	0.129	**0.010**
log(ANPP)	Clipping Trt (clip code)	-0.065	0.055	0.236
log(Num Arthropods)	Avg Moisture	0.207	0.068	**0.003**
log(Num Arthropods)	log(ANPP)	-0.199	0.078	**0.011**
log(Num Arthropods)	Plant Quality (Weighted %N)	-0.014	0.074	0.852
Surface Temp	Clipping Trt (clip code)	0.247	0.053	**0.000**
Surface Temp	Drought Trt (prop Precipitation)	-0.388	0.126	**0.006**
Surface Temp	Avg Moisture	0.112	0.055	**0.043**
Plant Quality (Weighted %N)	log(ANPP)	-0.121	0.079	0.124
Plant Quality (Weighted %N)	Drought Trt (prop Precipitation)	-0.104	0.130	0.431
Plant Quality (Weighted %N)	Clipping Trt (clip code)	0.033	0.062	0.595
Avg Moisture	Drought Trt (prop Precipitation)	0.100	0.071	0.173
Plant Diversity_H	log(ANPP)	-0.429	0.064	**0.000**
Plant Diversity_H	Surface Temp	0.242	0.066	**0.000**
Plant Diversity_H	Drought Trt (prop Precipitation)	0.462	0.156	**0.008**
Plant Diversity_H	Clipping Trt (clip code)	0.129	0.051	**0.013**
Arthropod Diversity_H	log(Num Arthropods)	0.591	0.058	**0.000**
Arthropod Diversity_H	Avg Moisture	0.115	0.056	**0.044**
Arthropod Diversity_H	Surface Temp	-0.121	0.056	**0.032**
Arthropod Diversity_H	Plant Quality (Weighted %N)	-0.078	0.056	0.164
Arthropod Diversity_H	Plant Diversity_H	-0.063	0.057	0.269

Table S3. Direction of the effects of clipping and drought treatments, ANPP, soil moisture, surface temperature, and plant quality on arthropod diversity, arthropod abundance, and plant diversity based on results of piecewise SEM. Indirect effects are in parenthesis, e.g. (+). Cells are blank when no relationship between the two factors was put into the piecewise SEM.

	Arthropod Diversity	Arthropod Abundance	Plant Diversity
Clipping	(–)	(+)	+
Drought	(–)	(–)	+
ANPP	(–)	–	–
Soil Moisture	+	+	(+)
Surface Temp	–		+
Plant Quality	–	–	
Arthropod Abundance	+		
Plant Diversity	–		

Table S4. Functional group assignments for invertebrate taxa. Trophic categorization was assigned from descriptions in Triplehorn & Johnson (2005).

Functional Group	Class	Order	Family	Detail
Chewing Herbivore	Insecta	Coleoptera	Bruchidae	
	Insecta	Coleoptera	Cleridae	
	Insecta	Coleoptera	Chrysomelidae	
	Insecta	Coleoptera	Curculionidae	
	Insecta	Coleoptera	Elateridae	
	Insecta	Coleoptera	Mordellidae	
	Insecta	Coleoptera	Phalacridae	
	Insecta	Hymenoptera	Mutillidae	
	Insecta	Lepidoptera	Psychidae	
	Insecta	Lepidoptera		larva
	Insecta	Orthoptera	Acridiae	
	Insecta	Phasmatodea		
Detritivore	Insecta	Collembola		
Omnivore	Insecta	Hymenoptera	Formicidae	
	Insecta	Hymenoptera	Dryinidae	
	Insecta	Hymenoptera	Ichneumonidae	
	Insecta	Orthoptera	Gryllidae	
Pollinator	Insecta	Hymenoptera	Apidae	
	Insecta	Hymenoptera	Apoidea	
	Insecta	Hymenoptera	Halictidae	
	Insecta	Lepidoptera		
Predator	Arachnida	Acari		
	Arachnida	Araneae		
	Arachnida	Opiliones		
	Insecta	Coleoptera	Coccinellidae	
	Insecta	Coleoptera	Staphylinidae	
	Insecta	Hemiptera	Anthocoridae	
	Insecta	Hemiptera	Geocoridae	
	Insecta	Hemiptera	Nabidae	
	Insecta	Hemiptera	Phymatidae	
	Insecta	Hemiptera	Reduviidae	
	Insecta	Hemiptera	Tingidae	
	Insecta	Hymenoptera	Vespidae	
	Insecta	Mantodea	Mantidae	
	Insecta	Neuroptera	Chrysopidae	

	Insecta	Neuroptera	Hemerobiidae	
	Insecta	Neuroptera	Myrmeleontidae	
Sucking Herbivore	Insecta	Hemiptera	Aphidae	
	Insecta	Hemiptera	Berytidae	
	Insecta	Hemiptera	Blissidae	
	Insecta	Hemiptera	Caliscelidae	
	Insecta	Hemiptera	Cercopidae	
	Insecta	Hemiptera	Cicadellidae	
	Insecta	Hemiptera	Dictyopharidae	
	Insecta	Hemiptera	Lygaeidae	
	Insecta	Hemiptera	Membracidae	
	Insecta	Hemiptera	Psyllidae	
	Insecta	Hemiptera	Rhopalidae	
	Insecta	Hemiptera	Rhyparochromidae	
	Insecta	Hemiptera	Scutelleridae	
	Insecta	Hemiptera	Thyreocoridae	
Unknown	Insecta	Coleoptera		larva
	Insecta	Coleoptera		
	Insecta	Diptera		
	Insecta	Hemiptera		tiny nymph
	Insecta	Hemiptera	Pentatomidae	
	Insecta	Hemiptera	Miridae	
	Insecta	Thysanoptera	Thysanoptera	

Literature Cited

Triplehorn, C. A., & Johnson, N. F. 2005. Borror and Delong's Introduction to the Study of Insects. Belmont: Thomson Brooks/Cole.

Supplemental figure legends

Figure S1. Average rainfall (cm) each month for the duration of the experiment (2016-2018). Rainfall data downloaded from https://www.mesonet.org/ and is from Station 99 which is located at Kessler Atmospheric and Ecological Field Station (McClain County, OK). Light gray is 2016 data, dark gray is 2017 data, and black is data from 2018.

Figure S2. (a) Experimental setup of 21 plots, each 2 × 2 m, established in Spring 2016 and treated with one of seven water manipulation treatments which included control (–0%), –20 %, –40 %, –60 %, –80%, and –100% water exclusion, and +50% water addition. This was combined with two clipping treatments (clipped or unclipped) and replicated three times. Treatments were assigned randomly to plots within each of 3 blocks. **(b)** Detailed plot set-up showing the subplots for clipping (to mimic haying), measuring plant ANPP, sampling arthropods, and measuring plant diversity. Area covered by rain shelter was 3.6 × 3.6 m and within that a 0.8 m buffer surrounded the measurement plot on all sides.

Figure S3. Soil surface temperature (°C) across **(a)** drought and **(b)** clipping treatments. Boxes show median, 25-75% quartiles, and range.

Figure S4. Soil moisture (VWC) across drought treatments. Boxes show median, 25-75% quartiles, and range.

Figure S5. ANPP across drought and clipping treatments. Panels **(a)** and **(b)** show total ANPP based on drought treatment **(a)** and clipping **(b)**. Panels **(c)** and **(d)** show ANPP for C_3 **(c)** and C_4 **(d)** plants based on drought treatment. Boxes show median, 25-75% quartiles, and range.

Figure S6. Relationship between plant quality (Weighted %N – see Methods) and **(a)** drought, **(b)** clipping treatments, **(c)** arthropod abundance, and **(d)** arthropod diversity. Boxes show median, 25-75% quartiles, and range.

Figure S7. Relationship between plant diversity (Shannon's *H*) and **(a)** drought and **(b)** clipping treatments. Boxes show median, 25-75% quartiles, and range.

Figure S8. Relationship between arthropod abundance and **(a)** soil moisture (VWC) and **(b)** plant biomass (g). Black line shows a linear fit (solid = significant fit; dashed = non-significant fit). A linear model was used to calculate *P* and R^2 values.

Figure S9. Relationship between arthropod diversity (Shannon's *H*) and **(a)** surface temperature (°C), **(b)** soil moisture (VWC), and **(c)** arthropod abundance. Solid black line shows a significant linear fit. A linear model was used to calculate *P* and R^2 values.

Figure S10. Relationship between arthropod diversity (Shannon's *H*) and plant diversity (Shannon's *H*). Panel **(a)** and **(b)** show the same data, but panel **(b)** shows data colored by arthropod abundance (red = high arthropod abundance while yellow = low arthropod abundance). The dashed red lines in panel **(b)** correspond to the 0.25, 0.5, 0.75, and 0.99 quantiles.

Figure S11. Relationship between arthropod abundance and **(a)** C_3 biomass and **(b)** C_4 biomass. Black line shows a linear fit (solid = significant fit; dashed = non-significant fit). A linear model was used to calculate *P* and R^2 values.

Figure S12. Relationship between ANPP and **(a)** C_3 sucking damage, **(b)** C_4 sucking damage, **(c)** C_3 chewing damage, and **(d)** C_4 chewing damage. Black line in panel **(c)** shows a significant linear fit.

Figure S1.

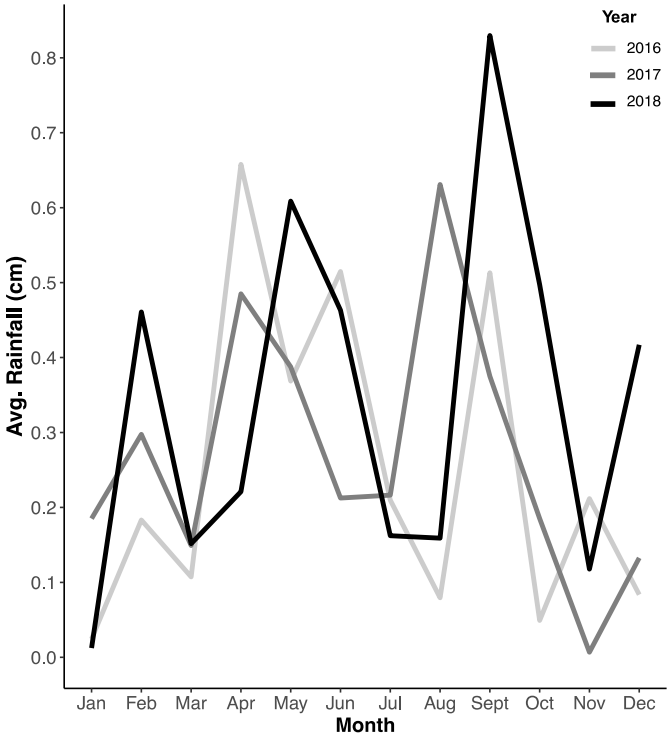

Year
2016
2017
2018
Avg. Rainfall (cm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Month

Figure S2.

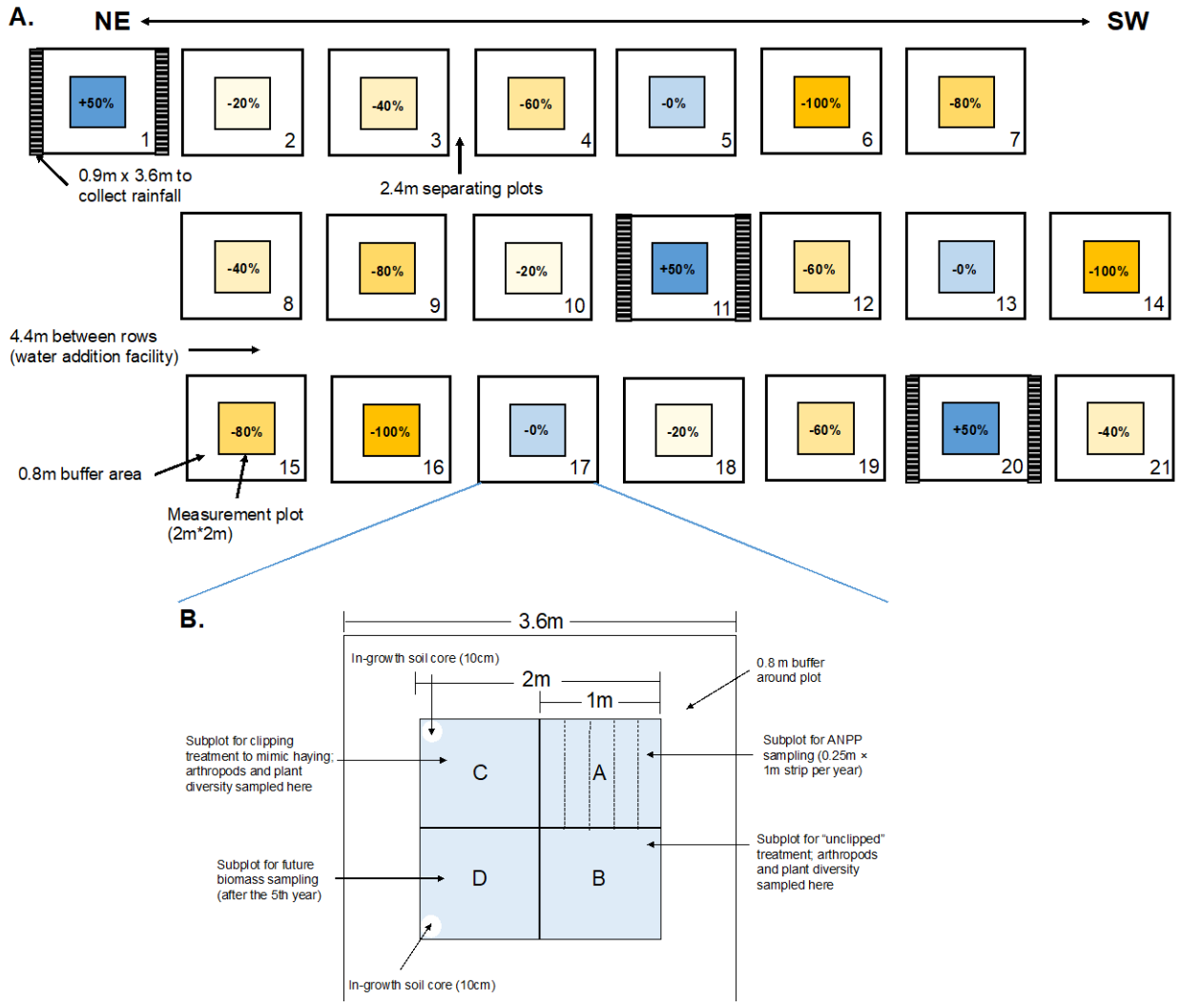

A.
NE
SW
+50%
1
-20%
2
-40%
3
-60%
4
-0%
5
-100%
6
-80%
7
0.9m x 3.6m to collect rainfall
2.4m separating plots
-40%
8
-80%
9
-20%
10
+50%
11
-60%
12
-0%
13
-100%
14
4.4m between rows (water addition facility)
-80%
15
-100%
16
-0%
17
-20%
18
-60%
19
+50%
20
-40%
21
0.8m buffer area
Measurement plot (2m*2m)
B.
3.6m
In-growth soil core (10cm)
2m
1m
0.8 m buffer around plot
Subplot for clipping treatment to mimic haying: arthropods and plant diversity sampled here
C
A
Subplot for ANPP sampling (0.25m × 1m strip per year)
Subplot for "unclipped" treatment; arthropods and plant diversity sampled here
Subplot for future biomass sampling (after the 5th year)
D
B
In-growth soil core (10cm)

Figure S3.

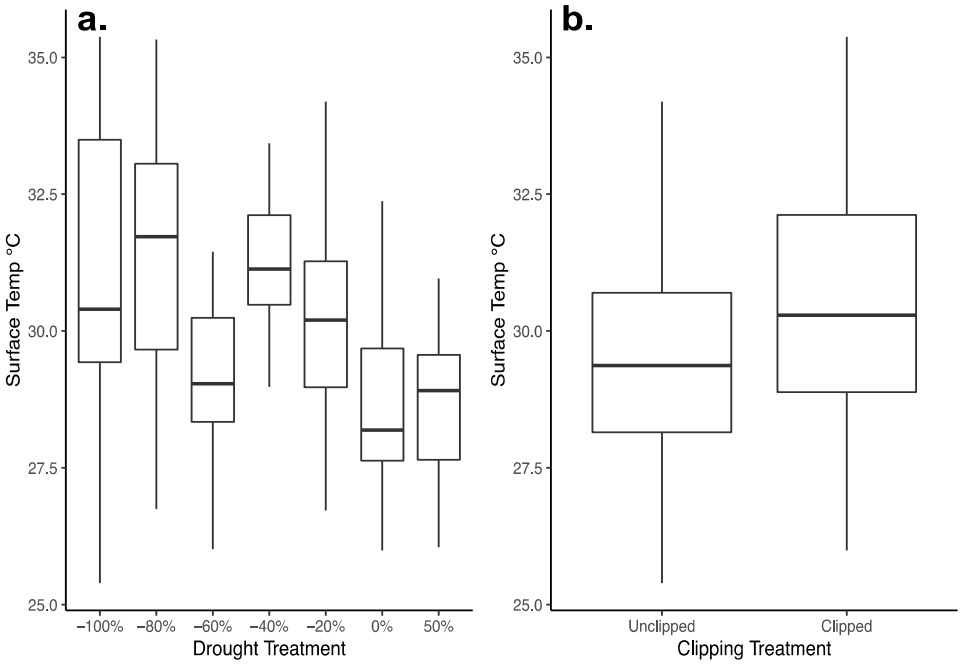

a.
35.0
32.5
30.0
27.5
25.0
Surface Temp °C
−100%
−80%
−60%
−40%
−20%
0%
50%
Drought Treatment
b.
35.0
32.5
30.0
27.5
25.0
Surface Temp °C
Unclipped
Clipped
Clipping Treatment

Figure S4.

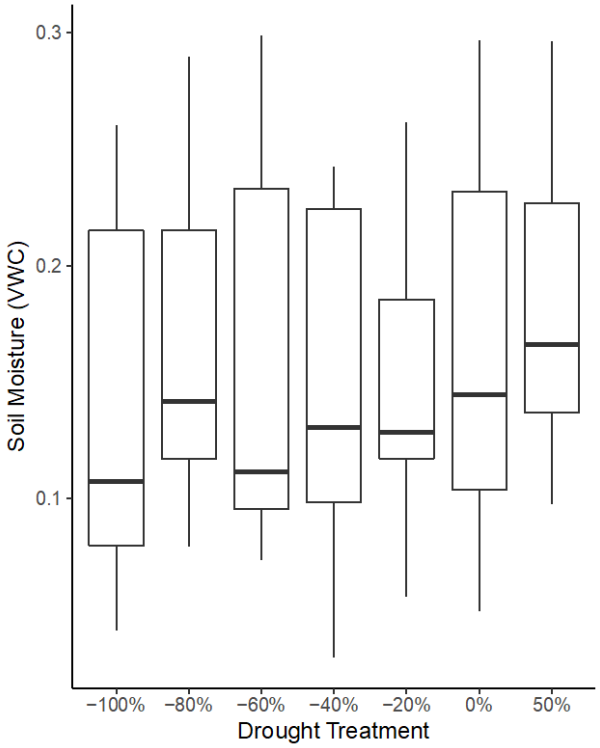

0.3
0.2
0.1
Soil Moisture (VWC)
−100%
−80%
−60%
−40%
−20%
0%
50%
Drought Treatment

Figure S5.

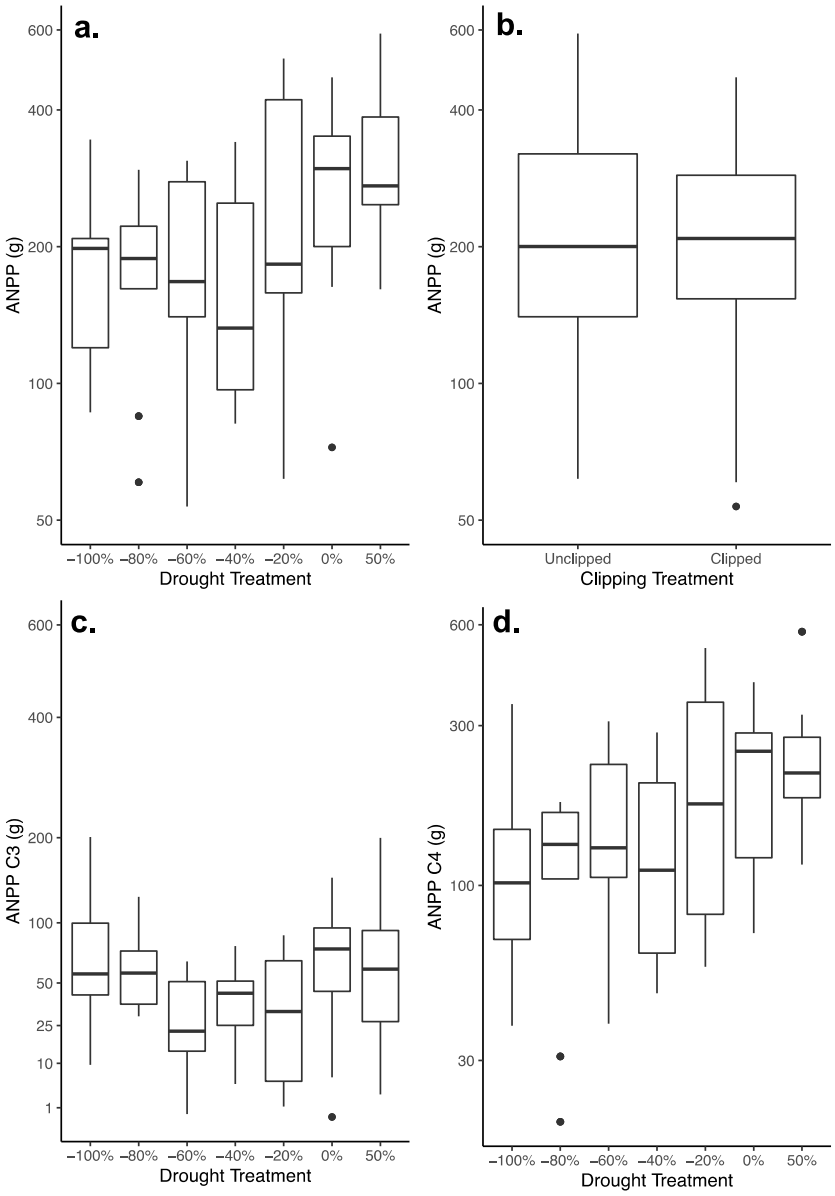

a.
b.
c.
d.
ANPP (g)
ANPP C3 (g)
ANPP C4 (g)
Drought Treatment
Clipping Treatment
−100%
−80%
−60%
−40%
−20%
0%
50%
Unclipped
Clipped

Figure S6.

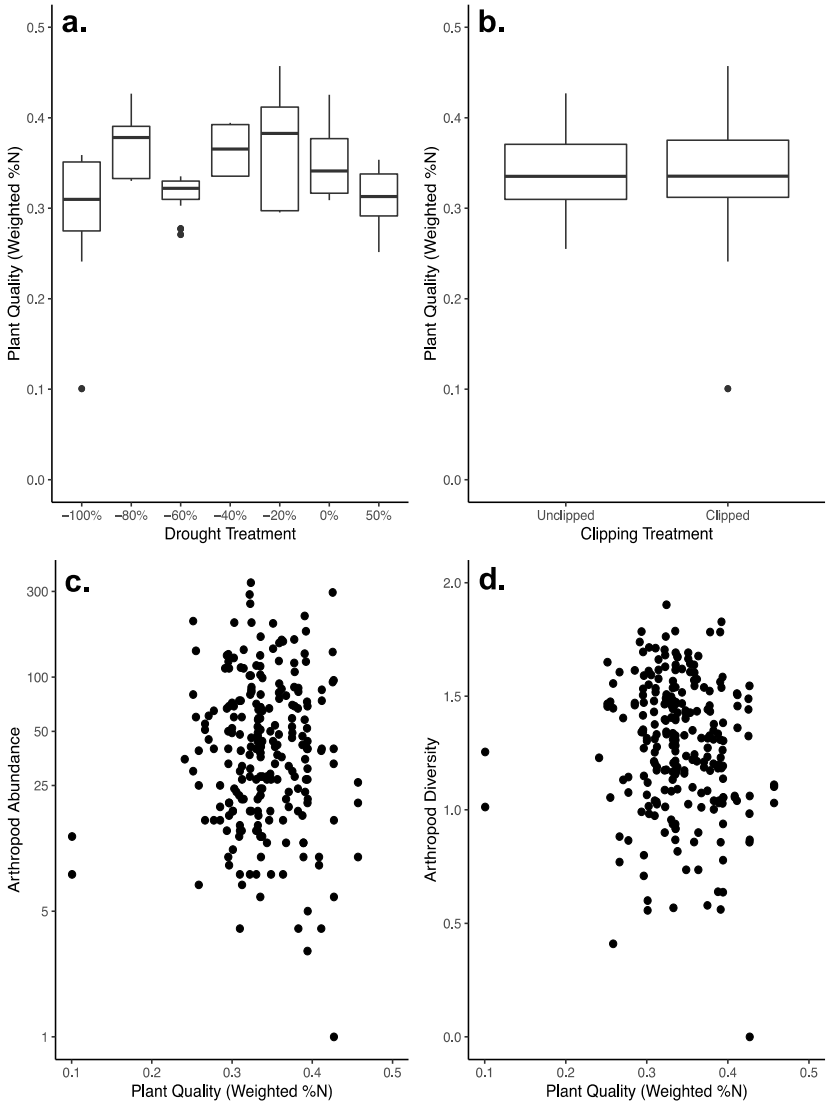

a.
b.
c.
d.
Plant Quality (Weighted %N)
Drought Treatment
−100%
−80%
−60%
−40%
−20%
0%
50%
Clipping Treatment
Unclipped
Clipped
Arthropod Abundance
Arthropod Diversity

Figure S7.

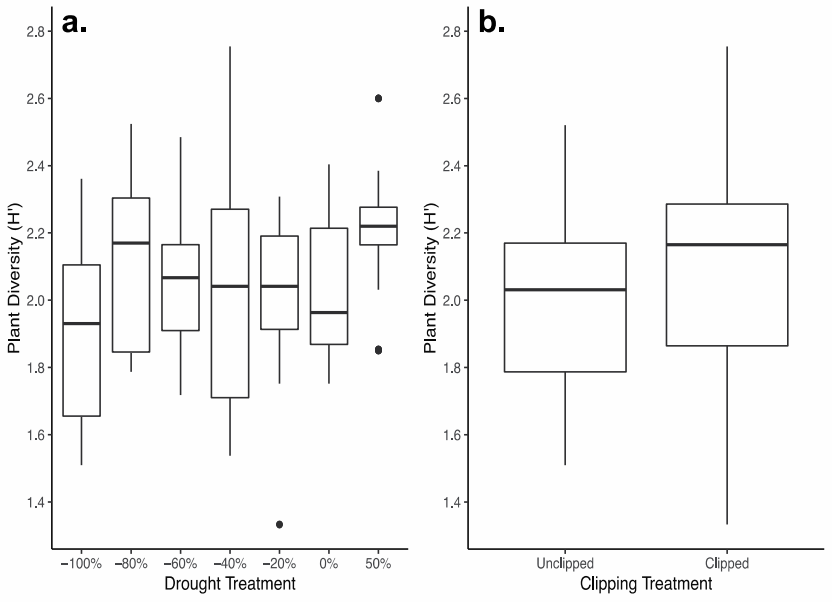

a.
b.
Plant Diversity (H')
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
−100%
−80%
−60%
−40%
−20%
0%
50%
Drought Treatment
Unclipped
Clipped
Clipping Treatment

Figure S8.

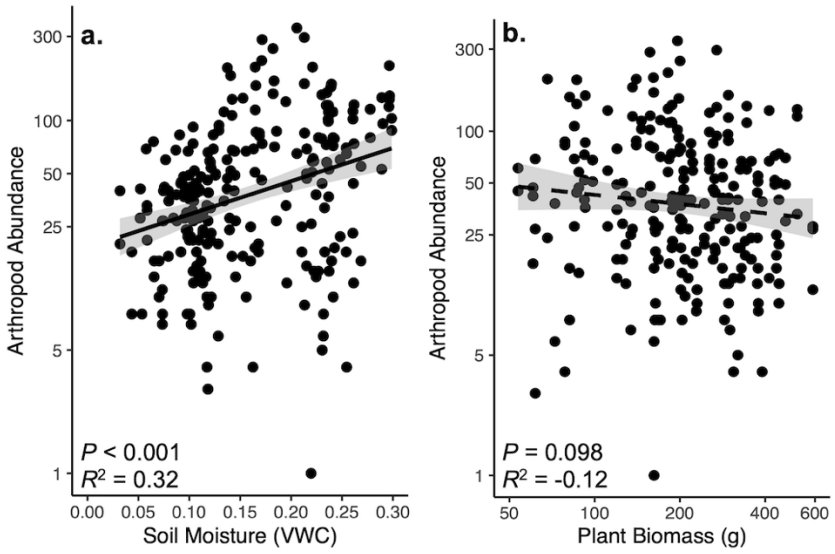

a.
b.
Arthropod Abundance
300
100
50
25
5
1
P < 0.001
R2 = 0.32
P = 0.098
R2 = -0.12
0.00 0.05 0.10 0.15 0.20 0.25 0.30
Soil Moisture (VWC)
50 100 200 400 600
Plant Biomass (g)

Figure S9.

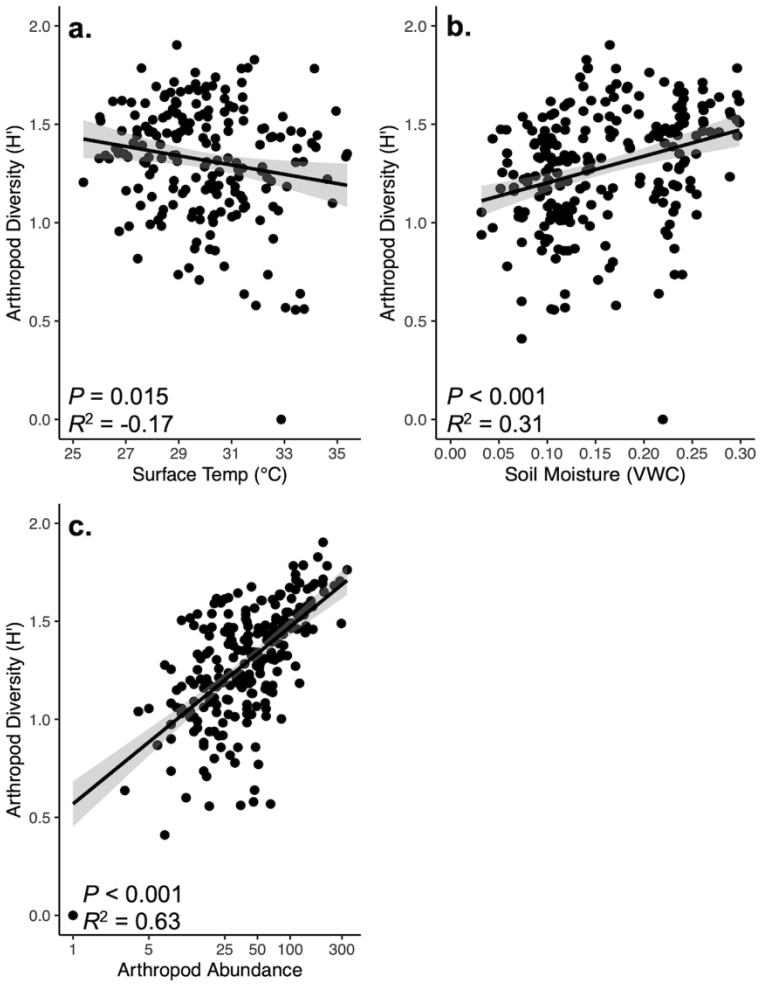

a.
Arthropod Diversity (H')
2.0
1.5
1.0
0.5
0.0
P = 0.015
R2 = -0.17
25
27
29
31
33
35
Surface Temp (°C)
b.
Arthropod Diversity (H')
P < 0.001
R2 = 0.31
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Soil Moisture (VWC)
c.
Arthropod Diversity (H')
P < 0.001
R2 = 0.63
1
5
25
50
100
300
Arthropod Abundance

Figure S10.

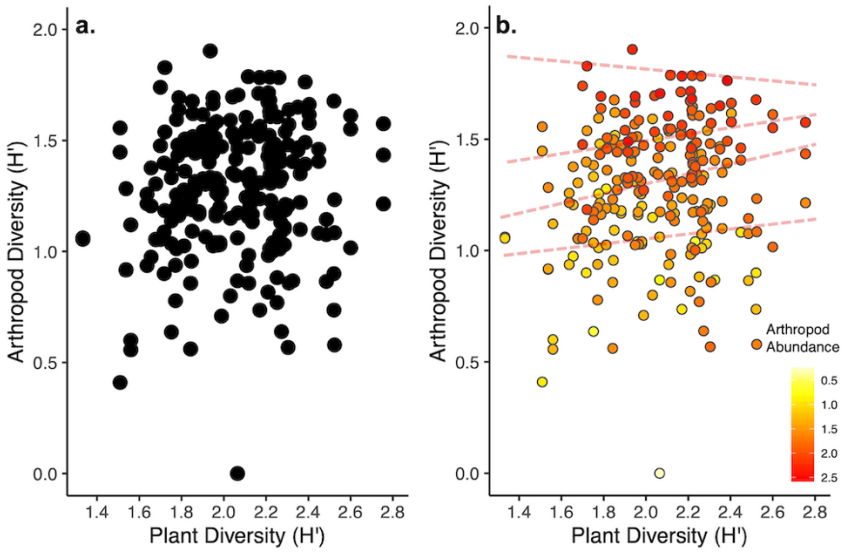

a.
b.
Arthropod Diversity (H')
Plant Diversity (H')
Arthropod Abundance
0.5
1.0
1.5
2.0
2.5
0.0
0.5
1.0
1.5
2.0
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8

Figure S11.

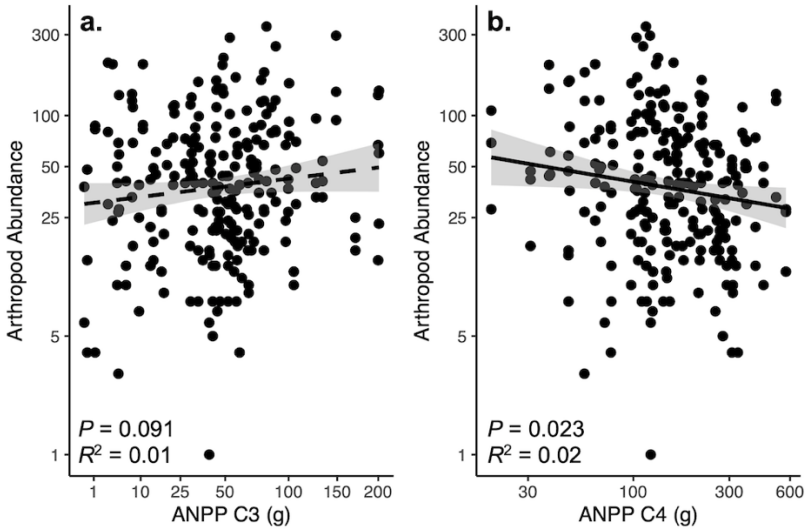
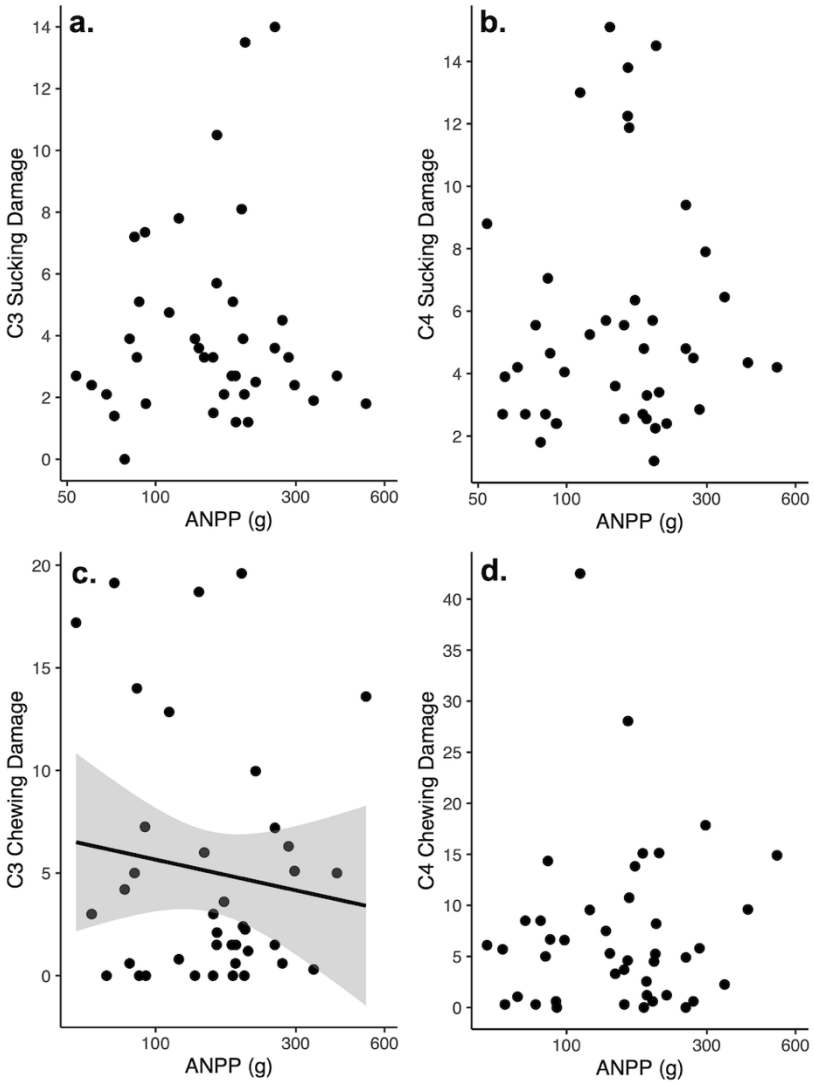

a.
b.
Arthropod Abundance
300
100
50
25
5
1
P = 0.091
R2 = 0.01
P = 0.023
R2 = 0.02
1
10
25
50
100
150
200
30
100
300
600
ANPP C3 (g)
ANPP C4 (g)

Figure S12.


a.
b.
c.
d.
C3 Sucking Damage
C4 Sucking Damage
C3 Chewing Damage
C4 Chewing Damage
ANPP (g)
50
100
300
600

Chapter 3: Appendix S2. Supplemental Methods.

Plant Diversity Protocol

The Braun-Blanquet method is a classic survey method for vegetation abundance. It is a method of cover-abundance rating and we used a modified Braun-Blanquet scale to estimate foliar cover (Braun-Blanquet 1932, Wikum and Shanholtzer 1978, Podani 2006). Specifically, we estimated percent foliar cover (e.g., vegetative cover including stems and leaves) using a modified Braun-Blanquet cover-abundance scale that included seven categories of percent foliar cover:1 = 0–1%, 2 = 1–5%, 3 = 5–25%, 4 = 25–50%, 5 = 50–75%, 6 = 75–95%, 7 = 95–100% (Braun-Blanquet 1932, Castillioni et al. 2020). In replacement of cover scales, we used the median of each assigned cover class as the abundance for each species in a subplot. We then used these values to calculate Shannon Wiener Diversity (*H*) for each subplot (clipped and unclipped; $n = 42$).

Plant Aboveground Net Primary Productivity Protocol

We determined the total aboveground plant primary productivity (ANPP) at the end of each growing season (September) by clipping plants to ground level within the designated ANPP subplot using a 0.25 m × 1 m quadrat (see Appendix S1: Fig. S2). We sorted individuals into functional groups (C_3 or C_4), oven dried them at 60°C for 48 h, and weighed them to the nearest 0.1g.

Herbivory Protocol

We used a herbivory protocol modified from the Nutrient Network herbivory assay https://nutnet.umn.edu/methods/leaf-damage). We measured chewing and sucking damage on four plant species per plot (*Sorghastrum nutans*, *Schizachyrium scoparium*, *Chamaecrista fasciculata*, and *Ambrosia psilotachya*) in August 2018. To do this, we used five leaves per

species on each plot (or as many available if there were fewer than five). Each leaf was from a different individual of the species (thus, when possible we used 20 individuals per plot – five individuals each of four species). We tried to minimize bias towards larger plants by choosing the first five individuals of each species that we encountered. We standardized leaf age by choosing leaves half-way up the stem. Within the middle of the plant, we randomly chose leaves so as to not bias towards measuring leaves with particularly heavy or light damage.

We defined chewing damage as holes that go all the way through the leaf and sucking/mining damage as damage that does not go all the way through the leaf. We used ten categories to assess damage: 0 = 0%, 1 = 1–5%, 2 = 5–10%, 3 = 10–20%, 4 = 20–30%, 5 = 30–40%, 6 = 40–50%, 7 = 50–60%, 8 = 60–70%, 9 = 80–90%, and 10 = 90–100% damage. To assess total herbivory damage on each plot by photosynthetic pathway (C_3 or C_4), we separately averaged chewing and sucking damage for C_3 and C_4 plants.

Literature Cited

Braun-Blanquet, J. 1932. Plant sociology: the study of plant communities. First ed. McGraw-Hill, New York.

Castillioni, K., K. Wilcox, L. Jiang, Y. Luo, C. G. Jung, and L. Souza. 2020. Drought midly reduces plant dominance in a temperatre prairie ecosystem across years. Ecology and Evolution **10**:6702-6713.

Podani, J. 2006. Braun-Blanquet's legacy and data analysis in vegetation science. Journal of Vegetation Science **17**:113-117.

Wikum, D. A., and G. F. Shanholtzer. 1978. Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. Environmental Management **2**:323-329.

CHAPTER FOUR

Formatted for publication in the *Journal of Ecology*

EXPERIMENTAL GRADIENTS OF SOIL FERTILITY REVEAL SHIFTING DRIVERS OF INVERTEBRATE HERBIVORY IN A GRASSLAND FOOD WEB

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Abstract

1. Herbivory is an important population interaction regulated by many factors. Anthropogenic nutrient deposition is rapidly increasing and produces numerous interacting effects on grassland food webs. It simultaneously changes soil and plant fertility, plant and arthropod communities, and ultimately, herbivory. Understanding how increased nutrient deposition will change these interactions is important for maintaining grassland communities in the future.
2. We used a gradient of fertilization to track nutrient addition through a grassland food web, measuring changes to soil and plant nutrients, plant biomass and richness, and arthropod abundance. Our end goal was to quantify the amount and type of herbivory plants experience. We compared the response of the aforementioned variables to a nutrient pulse vs. press in a single year on two sets of plots. Using a multi-year experiment, we worked to uncover the mechanisms driving herbivory within and across fertilizer quantities and durations.
3. Nutrient addition increased soil fertility 100-fold and generated 1.3-fold increases in herbivory. However, this herbivory increase weakened over time—from a 1-year pulse experiment to a 2-year press experiment—and shifted herbivory damage from sucking herbivores to chewing herbivores. Additionally, we saw decreases over time in the role that herbivore abundance plays in predicting herbivory levels. Overall, we found the rather paradoxical result that fertilization increased herbivore abundance but decreased herbivory.
4. We demonstrate the rippling effects of changing soil fertility on the abundance and function of a prairie food web, predicting herbivore abundance and herbivory. This research fills knowledge gaps about the shifting response of food webs in the years immediately following

nutrient addition. The mechanisms we uncover could help predict responses to long-term fertilization.

Keywords: arthropod, fertilization, herbivore, insect, nitrogen, NPK, nutrient limitation, prairie

Introduction

Nutrients shape not only plant and arthropod abundance, but also their interactions. Through eutrophication, humans have increased the availability of limiting macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Galloway et al. 2003, Steffen et al. 2015). Fertilization has both short- and long-term effects on soil fertility which ripple through the food web, affecting both plants and their consumers (Tilman 1987, Haddad et al. 2000, Ritchie 2000, Suding et al. 2005, Elser et al. 2007, Harpole et al. 2011, Murphy et al. 2012, Fay et al. 2015, La Pierre and Smith 2016, Lind et al. 2017, Prather et al. 2020a). Studying the effects of fertilization on arthropod food webs, and ultimately herbivory, can be challenging. Plant chemistry can rapidly change with fertilization, increasing arthropod abundance (Kaspari et al. 2017, Prather et al. 2018, Welti et al. 2019, Prather et al. 2020a). Plant richness has a slower response to nutrient addition – a pulse of nutrients will quickly increase plant biomass, but species may not be lost until two or more years of nutrient addition – a nutrient press (Tilman 1987, Haddad et al. 2000, Suding et al. 2005, Clark and Tilman 2008, Midolo et al. 2019, Prather and Kaspari 2019, Seabloom et al. 2020). Invertebrate herbivores can track changes in plant nutrients, biomass, and diversity, taking all factors into account when choosing a patch of food. By designing a concurrent nutrient pulse and press experiment (one versus two years of fertilization), we work

to tease apart the direct and indirect effects of increased soil fertility on herbivory and begin to understand the mechanisms underlying herbivore food choice.

Herbivores respond to vegetation quantity and quality. Fertilization rapidly increases plant biomass, increasing food quantity available to herbivores (Wedin and Tilman 1996, Gough et al. 2000, Harpole et al. 2011, Fay et al. 2015, Prather and Kaspari 2019). Fertilization also increases food quality in two ways, either through improving plant palatability for herbivores (Sedlacek et al. 1988, Loranger et al. 2013) or via increasing plant nutrient concentrations (Cleland and Harpole 2010, La Pierre and Smith 2016, Lind et al. 2017, Firn et al. 2019, Prather et al. 2020a). First, eutrophication can decrease the abundance of unpalatable and silicon-rich grasses while enhancing N-rich forb abundance, raising plant palatability. However, this mechanism based on species turnover is slow. Decreasing the abundance of low nutrient-use groups such as legumes and unpalatable C_4 grasses can take several years after nutrients are applied (Tilman 1987, Foster and Gross 1998, Siemann 1998, Suding et al. 2005, Anderson et al. 2018, Seabloom et al. 2020). Second, fertilization can increase the nutrient density and/or decrease the defenses of all plant functional groups. Herbivores can track increases in plant quantity and quality, altering their feeding patterns to selectively eat nutrient-rich food from larger patches (Bernays et al. 1994, Chambers et al. 1996, Schmitz 2008b, Behmer 2009, Loaiza et al. 2011). More herbivores all selectively eating on fertilized plots could increase overall herbivory but decrease per-capita herbivory as each herbivore meets their nutrient requirements with less total food (Mattson Jr 1980, Chambers et al. 1996, Throop and Lerdau 2004, Berner et al. 2005, Schmitz 2008b, Chen et al. 2010, Loaiza et al. 2011, La Pierre and Smith 2016).

Yet, when fertilization increases food quantity, it can counterintuitively reduce herbivore abundance and herbivory via several mechanisms. Fertilization-increased plant biomass results

in higher habitat complexity and volume which can support more predators and parasitoids (Power 1992, Post et al. 2000, Schmitz 2008a, Welti et al. 2020a). More predators can reduce herbivore abundance indirectly via fear (Schmitz 2008a) or directly via predation, ultimately suppressing herbivory. Nutrient addition can reduce food quality for herbivores two ways, via nutrient dilution or increased plant defenses. First, increased plant biomass can cause plant nutrient dilution—thinly spread nutrients through more biomass—disincentivizing herbivores from feeding on fertilized plots (Fan et al. 2008, McLauchlan et al. 2010, Welti et al. 2020b). Second, nutrient addition can increase plant concentrations of secondary metabolites, defenses which deter herbivores (Coley et al. 1985, Rosenthal and Berenbaum 2012, Mur et al. 2017). If fertilization provides enough excess N and carbon (C), plants may use those nutrients to increase secondary metabolite production, decreasing herbivory on plots with high levels of nutrient addition. Less herbivores all selectively eating undefended, high-nutrient plants in an expanse of nutrient dilution could increase per-capita herbivory as each herbivore eats more to meet their nutrient requirements (Mattson Jr 1980, White and Whitham 2000, Throop and Lerdau 2004, Schmitz 2008b, Loaiza et al. 2011).

Altogether, gradients of nutrient availability produce numerous interacting effects on plant and arthropod communities. To tease apart how biogeochemistry shapes food webs in a south Great Plains grassland, we used a gradient of fertilization to manipulate soil and plant nutrients. Our novel experiment compared the response to a nutrient pulse vs. press in a single year on two sets of plots rather than the typical setup which measures the response across years on the same plots. We used a nutrient pulse vs. a nutrient press with the goal of discovering the ecological mechanisms underlying food web responses to eutrophication. We test the direct and

indirect effects of nutrient addition on soil and plant nutrient content, plant biomass, arthropod abundance, and ultimately, the amount and type of herbivory plants experience.

Methods

Site Description

We studied the plant and arthropod assemblage from May 2017 through September 2018 at Pigtail Alley Prairie in Oklahoma, USA (33.89° N, 96.84° W). Pigtail Alley Prairie is a mixed-grass prairie with sandy soil last farmed >20 years ago dominated by the grasses *Andropogon virginicus* and *Vulpia octoflora* and the forbs *Croton glandulosus* and *Agalinis heterophylla*.

Plot Setup and Fertilization

We used a gradient of nutrient addition to affect soil fertility, plant biomass, plant richness, and plant foliar nutrient concentrations. In March 2017 we established and fertilized 35 plots, each 4 m^2 and separated by 10 m. We used seven levels of fertilization (control [0x], 0.1x, 0.5x, 1x, 2x, 3x, and 4x) replicated five times (see Appendix S1: Fig. S1 for plot schematic). Fertilizer consisted of N, P, K, and a micronutrient mixture, and fertilization level was randomly assigned to plots. We added N as time-release humic coated urea, P as super triple phosphate, and K as potassium sulfate. For micronutrients, we used Scott's Micromax fertilizer containing calcium (6 g/m^2), magnesium (3 g/m^2), sulfur (12 g/m^2), boron (0.1 g/m^2), water-soluble copper (1 g/m^2), water-soluble iron (17 g/m^2), water-soluble manganese (2.5 g/m^2), molybdenum (0.05 g/m^2), and water-soluble zinc (1 g/m^2).

Our 1x treatment was based on the Nutrient Network experimental protocols and consisted of N, P, and K applied at a rate of 10 $g \cdot m^{-2} \cdot y^{-1}$ by elemental mass and a micronutrient

mixture applied at a rate of 100 $g \cdot m^{-2} \cdot y^{-1}$ (Borer et al. 2014). Other treatments were based on the 1x treatment so, for example, the 4x treatment receieved N, P, and K at a rate of 40 $g \cdot m^{-2} \cdot y^{-1}$ by elemental mass and micronutrients at a rate of 400 $g \cdot m^{-2} \cdot y^{-1}$ (see Appendix S1: Table S1a for fertilizer amounts). In March 2018, we set up an additional 35 plots (each 4 m^2) fertilized with the same seven levels of nutrient addition (see above). At the same time, we re-fertilized the plots set up in 2017 with N, P, and K, but following Nutrient Network protocols, excluded micronutrients (Appendix S1: Table S1b).

Plant Sampling

To see how increased soil nutrient addition changed plant productivity, we measured above-ground plant biomass each year in mid-August by clipping vegetation within one 1 m × 0.1 m clip strip per plot. Vegetation was sorted into grasses, forbs, and detritus, dried at 60°C for 48 h, and weighed.

We tested plant community changes with nutrient addition by measuring plant richness and plant percent cover within a 1-m^2 subplot on each plot in 2018 using a modified Braun-Blanquet scale with seven categories of percent foliar cover (Braun-Blanquet 1932, Wikum and Shanholtzer 1978, Podani 2006). We used the foliar cover estimates for each species to calculate Shannon Wiener diversity for each subplot (see Prather et al. 2020b for detailed methods).

Soil and Plant Chemistry

To investigate how nutrient addition altered soil fertility, we assessed soil chemistry (Ca, K, Mg, NO_3N, P, S) and pH on a subset of plots (n = 42) using inductively coupled plasma mass spectrometry at the Texas A&M Soil, Water, and Forage Testing Laboratory

(http://soiltesting.tamu.edu/). Soils were collected in August 2018. To characterize soil chemistry, we ran a principal component analysis (PCA) on soil elements and pH. Principle component 1 (PC 1) for soil was strongly negatively correlated with Ca, K, Mg, NO_3N, P, and S (i.e. |correlation| > 0.6) so we used the inverse of PC 1 as an index of soil nutrient content (Appendix 1: Fig. S2).

We tested how increased soil fertility altered plant nutrient concentrations in two ways. We measured grass and forb %N and %C on a subset of plots (n = 39) in August 2018 using combustion analysis. Plant elemental chemistry (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, H, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr, Ti, and Zn) was measured on the same subset of plots using hot plate digestion and inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Cornell Nutrient Analysis Laboratory (https://cnal.cals.cornell.edu/). Grass and forb material were pooled proportionally by weight on each plot to obtain a composite plant sample for elemental chemistry.

To characterize plant chemistry, we ran a PCA based on nutrients added in our fertilization treatments (i.e., B, C, Ca, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, S, and Zn). Principle component 1 (PC 1) for plant nutrient content was strongly negatively correlated with B, Ca, Cu, Mg, Mn, Mo, N, Na, P, S, and Zn, so we used the inverse of PC 1 as an index of plant nutrient content (Appendix 1: Fig. S3). PCAs were conducted in R and visualized using the R package factoextra (Kassambara and Mundt 2017).

To examine whether fertilization had a direct effect on plant nutrient content and whether the shape of that relationship changed similarly across years, we ran a polynomial regression with linear, quadratic, and cubic models. We compared models using Akaike information criterion (AIC) values.

Arthropod Sampling

In order to test whether herbivore abundance was affected by increased soil fertility, we sampled the arthropod community in June 2018 using a vacuum sampler (Husqvarna 125BVX, The Husqvarna Group, Stockholm, Sweden) for 50 s per plot (Stewart and Wright 1995). Samples were put on ice in the field and kept frozen until sorting. We counted and identified all herbivores to major taxonomic group and within that assigned species or morphospecies before assigning herbivores to the functional groups chewing or sucking herbivore.

Herbivory Scoring

To see whether altered plant nutrients and increased plant biomass affected herbivory, we assessed herbivory on nine plant species per plot. Scored grass species consisted of *Andropogon virginicus*, *Dichanthelium oligosanthes*, and *Juncus marginatus*. Scored forb species consisted of *Croton glandulosus*, *Agalinis heterophylla*, *Gaillardia aestivalis*, *Oenothera laciniata*, and *Monarda punctata* while scored legumes consisted of *Chamaecrista fasciculata*. We scored leaves for herbivory using six categories: invertebrate chewing, invertebrate sucking or mining, rust, mildew, fungal unknown, and vertebrate damage. Vertebrate damage was not analyzed as it was seen on <2% of plots. As able, we scored five leaves per plant species per plot. Leaves were assessed for herbivory damage using five categories based on the area of leaf that was damaged: 0–1%, 1–5%, 6–25%, 26–49%, or 50–100%. For each herbivory category, the damage amount for each leaf was the median of the assigned damage category.

We analyzed herbivory by plant functional group, averaging damage across species for the grasses, forbs, and legumes in each plot. Similarly, we averaged herbivory on each plant

species by agent, pooling rust, mildew, and fungal unknown into "fungal damage" which resulted in three herbivory types: chewing, sucking, and fungal damage. We visualized herbivory effect sizes by herbivory type and by plant functional group using Cohen's d (Cohen 1988).

Structural Equation Model

To examine how fertilization affected herbivory both directly and indirectly after one and two years of fertilization, we used a piecewise Structural Equation Model (SEM; Lefcheck, 2016). We built an SEM for each fertilization duration (1 year or 2 years) based on an *a priori* model. We predicted fertilization would indirectly affect herbivory through effects on soil nutrient concentration, grass and forb biomass, plant nutrient concentration, and herbivore abundance. Fertilizer amount, forb biomass, and grass biomass were $\log_{10}$-transformed to meet normality assumptions. We did not remove non-significant links from either SEM but did add a direct link from fertilizer to herbivore abundance in the Year 1 model to account for plant secondary metabolites. Soil nutrient content was the inverse of PC 1 from a PCA done on soil elemental chemistry, and plant nutrient content was the inverse of PC 1 from a PCA done on plant nutrients added in fertilization treatments (see above). We used Fisher's *C* to assess the completeness of the models. Piecewise SEMs were conducting using the R package piecewiseSEM (Lefcheck 2016).

Inferential statistics

The SEMs we constructed did not directly compare total grass and forb biomass, herbivore abundance, and herbivory amounts between one and two years of fertilization but rather explored the mechanisms controlling changes in these variables *within* a year. Thus, we used generalized

linear models to examine changes in grass and forb biomass, herbivore abundance, total herbivory, plant richness, and plant diversity across years. Specifically, we tested the effects of fertilizer quantity, fertilizer duration, and a fertilizer duration × quantity interaction. The herbivore abundance model used a poisson error distribution while all other models used a gaussian error distribution. All statistics were performed using R version 3.6.1 (R Core Development Team 2019).

Results

Both of our piecewise SEMs were a good fit (Year 1 model: $AIC = 72.85$, $P = 0.106$, Fisher $C = 20.85$; Year 2 model: $AIC = 67.48$, $P = 0.355$, Fisher $C = 17.48$). Thus, we were able to predict herbivory across years based on soil fertility, plant biomass, plant nutrient content, and herbivore abundance.

Fertilization increased soil nutrients and plant foliar nutrient content

Our SEMs suggest that fertilization influenced soil and plant nutrients both directly and indirectly. Soil nutrients increased linearly with fertilization after both one and two years of fertilization (Fig. 1; Appendix 1: Table S2). For example, soil NO_3N was 15-fold higher on 4x plots relative to control plots after one year of fertilization while it was 12-fold higher relative to controls after two years.

Fertilization indirectly increased plant nutrient content (i.e., via its effect on soil nutrients) after one, but not two years of fertilization (Fig. 1; Appendix 1: Table S2). Across years, GLMs show plant nutrients increased linearly with fertilizer quantity after one year of

fertilization and varied cubically with fertilizer quantity after two years (Appendix 1: Fig. S4, Table S3).

Nutrient dilution—the decrease in nutrient concentrations with increasing biomass—also varied with one versus two years of fertilization. It was absent after one year (Fig. 1a; Appendix 1: Table S2a). However, after two years of fertilization there was a weak signal of plant nutrient dilution as a 1 g increase in grass biomass caused a linear decrease of 249 ppm K and 61 ppm P (Fig. 1b; Appendix 1: Table S2b).

Fertilization decreased grass biomass and increased forb biomass

Grass and forb biomass were affected differently by fertilization across years. Within and across years, SEMs and GLMs show grass biomass decreased linearly with soil nutrient availability and fertilizer quantity (Figs. 1 and 2). Grass biomass was 3-fold lower on 4x plots relative to control plots after one year of fertilization and 2-fold lower after two years (t = -2.43, P = 0.018; Figs. 1 and 2a). Declines in C_4 grasses drove this decrease; after one year, they were half as abundant on 4x plots as controls. In contrast, forb biomass increased with soil nutrients 5-fold on 4x plots after one year of fertilization, with no effect after two years (Fig. 1). GLMs show fertilizer quantity had no direct effect on forb biomass in either year (t = 0.48, P = 0.634; Fig. 2b).

Like grasses, legume cover decreased from one to two years of fertilization. Legume cover decreased 5-fold on 4x plots after one year of fertilization but did not decrease further on those plots after two years. Rather, legume cover decreased more on 2x and 3x plots after two years by 8-fold and 2-fold respectively.

Fertilization also changed plant richness, but not diversity, in the 2 × 2 m plots. Plant richness did not change with one year of fertilization (t = 1.09; P = 0.28) regardless of fertilizer

amount (t = 1.09; P = 0.28). Plant richness decreased after two years of fertilization with fertilizer quantity (significant duration × quantity interaction; t = -2.45, P = 0.017). Plant species declined on 4x plots from 10 species after one year of fertilization to 7 species after two years (Appendix 1: Fig. S5). However, Shannon Weiner diversity—which also accounts for evenness—did not vary with fertilizer duration (t = -1.39, P = 0.17) or quantity (t = -1.57, P = 0.12).

Herbivore abundance increased with fertilization amount and duration

Herbivore abundance varied 12-fold among plots. In 2018, we collected 867 herbivores on plots fertilized for one year (mean ± SE; 24.8 ± 2.1) and 934 herbivores on plots fertilized for two years (26.7 ± 2.3). Our piecewise SEMs accounted for 72% of the variation in the arthropod community response after one year of fertilization and 30% after two years.

Herbivores were more abundant on plots that had been fertilized for two years (GLM, z = 2.88, P = 0.004). Herbivore abundance increased with fertilizer quantity across years (z = 8.98, P < 0.001; Fig. 2c), but unequally (duration × quantity interaction z = -2.42, P = 0.016; Fig. 2). After one year of fertilization, herbivore abundance was 2.2-fold higher on 3x and 4x plots compared to control plots whereas after two years of fertilization abundance on those plots was only 1.6-fold higher than control plots. Surprisingly, the SEM revealed that fertilizer quantity drove increases in herbivore abundance with little indirect effects via plant nutrients, but only after one year of fertilization (Fig. 1).

Grass and forb biomass affected herbivore abundance, but in different ways (Fig. 1; Appendix 1: Table S2). Grass effects were consistent after one and two years of fertilization: a 10 g decrease in grass biomass resulted on average in the loss of ten herbivores (Fig. 1). Forb

effects were more complex. After one year of fertilization a 10 g increase in forb biomass added two herbivores on average to a plot. After two years, the same magnitude of increase added less than one herbivore (Fig. 1; Appendix 1: Table S2).

Total herbivory varied with nutrient addition but less consistently with herbivore abundance…

Our piecewise SEMs accounted for 48% of the variation in herbivory on the 2 × 2 m plots after one year of fertilization and 55% after two years. We measured herbivory on 981 leaves from plots fertilized for one year (mean damage ± SE; 41.3 ± 1.8) and 1,007 leaves from plots fertilized for two years (31.7 ± 1.4). Overall herbivory was higher in plots exposed to one year of fertilization (t = -4.55, P < 0.001; Fig. 2d). In both years, the quantity of fertilizer drove higher levels of herbivory, which averaged 1.3-fold higher on 4x plots relative to control (t = -3.06, P = 0.003; Fig. 2d).

Herbivory drivers changed for plots that had been fertilized for one year versus two. After one year, herbivory decreased with plant nutrient content; after two years this effect had disappeared (Fig. 1). After one year, herbivore abundance drove variation in herbivory between plots: for each herbivore added, herbivory increased by 7%. After two years this effect, too, disappeared. Instead, after two years of fertilization, grass and forb biomass had the largest effects on herbivory levels: a 1 g increase in grass and forb biomass generated a 2.3% and 0.7% increase in herbivory on average, respectively (Fig. 1).

…and changed with type of herbivory between years…

The different agents of herbivory—chewing herbivores, sucking herbivores, and fungi—generated different levels of damage. Chewing herbivory increased with fertilizer quantity with

an average effect size of +2.8 on 3x and 4x plots (Fig. 3a and 3b). Chewing herbivory also increased with fertilizer duration from an average effect size of +1.7 after one year of fertilization to +2.3 after two years. Sucking herbivory was most abundant on low fertilizer quantities (0.1x to 1x) after one year of fertilization with an average effect size of +2.1 (Fig. 3c). After two years of fertilization, sucking herbivory declined regardless of fertilizer quantity to an average effect size of -0.3 (Fig. 3d). Fungal damage, in contrast, failed to vary with fertilizer quantity or duration (confidence intervals overlap zero; Fig. 3e and 3f).

The levels of herbivory among the three agents were correlated across plots. Fungal damage increased with chewing damage after both one and two years of fertilization ($r = 0.28$ and $r = 0.21$). After one year, but not two, fungal damage decreased with sucking damage on plots ($r = -0.31$; Appendix 1: Fig. S6).

…as well as by plant functional group

Herbivory amount differed across plant functional groups with fertilization duration and quantity. Forb herbivory increased with fertilization duration from an effect size of +0.5 after one year of fertilization to an effect size of +1.6 after two years (Fig. 4a, 4b). Responses to fertilizer quantity varied with fertilizer duration. After one year of fertilization, herbivory was highest at 1x and 2x with an average effect size of +1.2 (Fig. 4a). After two years of fertilization, forb herbivory was greatest on 2x through 4x fertilizer quantities with an average effect size of +2 (Fig. 4b).

Grass herbivory, in contrast, decreased with fertilization duration from an effect size of +1.3 after one year of fertilization to an effect size of +0.7 after two years (Fig. 4c, 4d). Responses to fertilizer quantity varied from plots that had been fertilized for one year versus two.

After one year of fertilization, herbivory was highest on 3x and 4x plots with an average effect size of +2.2 whereas those same treatments suppressed grass herbivory down to +0.3 after two years (Fig. 4c, 4d).

Herbivory on legumes was unaffected by duration or dose of fertilizer (Fig. 4e, 4f). This null result may have resulted from the relative rarity of legumes in our prairie, especially on fertilized plots.

Discussion

Herbivory is a common and economically important population interaction (Branson et al. 2006). Here we manipulated soil fertility 100-fold, generating 1.3-fold increases in herbivory in a south Great Plains grassland. Uniquely, we tracked those added nutrients through the grassland food web, documenting changes in plant biomass, plant nutrient density, herbivorous insect abundance, and three agents of herbivory. This herbivory increase weakened over time—from a 1-year pulse experiment to a 2-year press experiment—as herbivory damage shifted from sucking herbivores (e.g., aphids) to chewing herbivores (e.g., grasshoppers). Overall, we found the rather paradoxical result that adding nutrients to the soil increased herbivore abundance but decreased herbivory. Combined, our experiment details the shifting nature of food web dynamics, and major patterns of cause and effect, that link gradients of soil fertility to levels of herbivory.

Increasing soil fertility continued to change the plant community over two years

Fertilization-induced species loss has unknown long-term ramifications for plant-arthropod interactions, highlighting the need for more multi-decadal fertilization experiments (but see

Hughes et al. 2017, Lind et al. 2017, Seabloom et al. 2020). Consistent with other studies, we found eutrophication resulted in low nutrient-use efficiency plants outcompeting legumes and grasses (Tilman 1987, Foster and Gross 1998, Siemann 1998, Haddad et al. 2000, Anderson et al. 2018, Seabloom et al. 2020). One mechanism changing plant composition is fertilization-increased detritus, which blocks sunlight and prevents legume seeds from sprouting (Foster and Gross 1998, Gough et al. 2000). Detritus increased 2-fold on our plots receiving 30 g N $m^{-2} \cdot y^{-1}$ (Tilman's H treatment) after our first year of fertilization, while Tilman (1987) found equivalent increases only after his third year. A second mechanism by which fertilization changes plant composition is increasing C_3 grass and forb biomass. Consistent with Gough et al. (2000), we found a 2-fold increase in plant biomass with 9 to 13 g N $m^{-2} \cdot y^{-1}$ (equivalent to our 0.5x treatment) and a 3-fold increase in plant biomass with our 4x treatment (90 g N $m^{-2} \cdot y^{-1}$). Interestingly, our biomass increase was driven by forbs rather than C_3 grasses. As little as two years of fertilization can change plant dominance, enhancing abundance of some species while suppressing the abundance of others.

Changes in plant composition were accompanied by decreases in plant richness. Two years of fertilization decreased plant richness, but not plant diversity. Reduction in C_4 grass and legume species, which decreased with fertilization across years, drove declines in plant richness. For example, the C_4 grasses *Andropogon ternarius* and *Eragrostis spectabilis* as well as the legume *Chamaecrista fasciculata* were present on 50% less plots after two years of fertilization. Additionally, four rarer species (two C_4 grasses and two legumes) were completely lost from plots fertilized for two years (Appendix S1: Table S4). Our result of rare species loss with nutrient addition is consistent with Suding et al. (2005) which found that rare plant species have more than a 60% chance of disappearance with fertilization.

Changing plant community composition also likely affected plant nutrient concentration. Plant nutrient concentration increased linearly with fertilizer quantity after one year and varied cubically with fertilizer quantity after two years – increasing at low and high quantities of nutrient addition and dipping at medium quantities. The remaining C_4 grass biomass on 2x and 3x plots in year two may be driving the non-linear response as C_4 grasses have lower %N than other plant functional groups.

Herbivores accumulated on more fertilized plots, but less so after two years

Across years, herbivores increased about 2-fold with fertilizer quantity and 1.2-fold with fertilizer duration. Arthropods can track changes in plant nutrients and biomass, selecting patches with higher food quality and quantity (Prestidge 1982, La Pierre and Smith 2016, Lind et al. 2017, Firn et al. 2019). For example, a meta-analysis examining arthropod responses to fertilization found arthropod abundance increased with NPK and micronutrient fertilization (effect size +1.3), the same fertilizer combination used here (Prather et al. 2020a). This increase was due to immediate increases in plant nutrients and biomass with nutrient addition (Firn et al. 2019, Prather et al. 2020a). However, herbivore abundance began declining slightly at the highest treatment levels (3x and 4x) after two years of fertilization (Fig. 2). One possible explanation is increased predator abundance from higher habitat complexity and volume on fertilized plots (Power 1992, Post et al. 2000). Higher predator abundance can reduce herbivore abundance directly via predation or indirectly via fear—herbivores staying away from those plots to avoid predation (Throop and Lerdau 2004, Schmitz 2008a, Welti et al. 2020a).

We found one of our most surprising results when we narrowed our focus to mechanisms driving herbivore abundance within a year. In the year 1 SEM, fertilizer quantity both indirectly

(via forb biomass) and directly increased herbivore abundance. As herbivores do not eat fertilizer, we initially predicted indirect effects of fertilizer quantity on herbivore abundance via increased plant quality and biomass (Prather and Kaspari 2019). One working hypothesis is that plant secondary metabolites (which deter herbivores) decreased with one year of fertilization. because of increased energy allocation toward plant growth (Coley et al. 1985, Herms 2002, Throop and Lerdau 2004, Tylianakis et al. 2008, Bumgarner et al. 2012). Unless fertilization increases photosynthesis rate, rapidly growing plants have the same amount of available carbon to allocate to production of new leaves and secondary metabolites (Herms and Mattson 1992, Jones and Hartley 1999, Herms 2002). Thus, secondary metabolites can be reduced in plants rapidly growing post-nutrient addition as plants allocate their carbon elsewhere (Herms and Mattson 1992, Herms 2002). This may explain the direct increase in herbivore abundance we saw with fertilization.

The accumulated effects of two years of fertilization lowered herbivory

Our hypothesis going into this study was that anything that increased the number of herbivores on a plot would increase average levels of herbivory. However, despite increases in herbivore abundance with fertilization in both years, overall herbivory was lower after two years of fertilization. At least two mechanisms may generate this decrease in herbivory. First, higher plant nutrient content may allow herbivores to meet their nutrient requirements with less plant tissue, and hence less foraging (Mattson Jr 1980, Schmitz 2008b, Behmer 2009, Gossner et al. 2014, La Pierre and Smith 2016). The macronutrients N, P, and K increased in plant tissue with fertilizer quantity and duration. Phosphorus increased the most, doubling foliar concentration in plants after two years. Our result of increased herbivore abundance but decreased herbivory after two

years of fertilization is consistent with La Pierre and Smith (2016), which found per-capita herbivory rate decreased with N and P fertilization (and higher tissue %N).

Second, a longer duration of nutrient addition may have increased secondary metabolites in forbs, reducing herbivory on plots fertilized for two years. Forb biomass rapidly increased with soil nutrients after one year of fertilization, but this effect disappeared after two years. If forbs were no longer allocating excess carbon to new leaf production, they could instead increase secondary metabolite production after two years of fertilization (Herms and Mattson 1992, Herms 2002). Increased metabolites would deter herbivores, reducing herbivory on plots fertilized for two years (Rosenthal and Berenbaum 2012, Mur et al. 2017).

Herbivory damage type changed with fertilizer quantity and duration

The different agents of herbivory—chewing herbivores, sucking herbivores, and fungi—generated different levels of damage across fertilizer quantities and duration. After both one and two years of fertilization, chewing damage was greatest at the highest fertilization levels (3x and 4x). This response is likely due to herbivores selectively foraging for and eating nutrient-rich, palatable plants (Schmitz 2008b, Behmer 2009, Anderson et al. 2018).

Sucking herbivores that tap directly into a plant's xylem and phloem responded differently from grazers to increased nutrient supply. Sucking herbivory was greatest after one year of fertilization and peaked at low to medium fertilizer levels (0.1x to 1x). Consistent with results from a press vs. pulse experiment done in a salt marsh, we found that longer fertilization durations suppressed sucking herbivory (Murphy et al. 2012). High levels of K dilute plant xylem and phloem, decreasing nutrient and amino acid concentrations (Huberty and Denno 2006,

Butler et al. 2012). Excess plant K after two years of fertilization would explain the decrease in sucking herbivory in year two.

Fungal damage did not change with fertilizer quantity or duration. Excess levels of nutrients might have also determined amount of fungal damage on plants. Specifically, N fertilization can decrease plant fungal infection rate, especially at higher levels of N-addition (Veresoglou et al. 2013).

Fungal and chewing damage were positively correlated in both years. This suggests fungal diseases may increase plant quality to chewing herbivores. Powdery mildew infections can reduce plant volatiles, decreasing parasitism rate for caterpillars (Desurmont et al. 2016). Additionally, rust fungal infections can speed up chewing herbivore development due to increased nutrients and amino acids in fungus-infected leaves (Eberl et al. 2020). These results add to a growing body of literature confirming the important role of plant pathogens in mediating plant-insect interactions (Biere and Tack 2013, Shikano et al. 2017, Rosa et al. 2018).

Herbivory amount switched from grasses to forbs after two years of fertilization

Indirect interactions of the plant community influenced herbivory amount. Forbs were both more common and suffered 1.6-fold more herbivory after two years of fertilization. Higher forb herbivory could be due to increased forb biomass with fertilization (Suding et al. 2005) or because herbivores prefer the higher N content in forbs relative to grasses (Mattson Jr 1980, Behmer 2009). Forb damage in year two was likely driven by chewing damage, which showed a similar pattern of increase with fertilizer amount.

In contrast, grasses had higher herbivory after one year of fertilization, especially at 3x and 4x. Those same treatments suppressed grass damage after two years of fertilization. This

could be due to decreased grass biomass with fertilization (Tilman 1987, Foster and Gross 1998, Siemann 1998, Suding et al. 2005, Anderson et al. 2018, Seabloom et al. 2020). Alternatively, fertilization increases grass fiber and silica content making it more difficult for herbivores to eat (Scherber et al. 2006, Loranger et al. 2013, Gossner et al. 2014).

Fertilizer quantity or duration did not affect legume herbivory. As fertilization increased the N content of all plants, it decreased the attractiveness of N-rich legumes, potentially explaining why we found no change in legume herbivory with fertilization. However, we only had one common legume, so these results are provisional.

Conclusions

By manipulating soil fertility over different timespans, we show the importance of assessing what is happening in the early stages of long-term field experiments. Herbivory drivers shifted over time—from herbivore abundance in year one to amount of plant biomass in year two. Importantly, herbivore abundance inaccurately predicted herbivory amount after two years of fertilization, and despite increased herbivore abundance, we saw decreased herbivory after two years. Herbivores removed more tissue after one year of fertilization, resulting in more plant damage and less nutrient-rich plant material going back into the soil. We demonstrate rippling effects of changing fertility on the abundance and function of a prairie food web, ultimately predicting herbivore abundance and herbivory in a south Great Plains grassland.

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Figure Legends

Figure 1. Piecewise structural equation model (SEM) depicting the direct and indirect effects of fertilization on herbivory after **(a)** one year and **(b)** two years of fertilization. Partial R^2 values are under each predicted variable and standardized path estimates are provided next to each path. Line thickness is based on the magnitude of the estimate (see Methods for variable descriptions). Blue and black arrows indicate positive and negative relationships, respectively. Dashed arrows represent nonsignificant paths ($P > 0.05$) while solid lines indicate significance ($P < 0.05$). Model estimates, standard errors, and *P* values are provided in Appendix S1: Table S2.

Figure 2. Relationship between fertilization level and **(a)** grass biomass, **(b)** forb biomass, **(c)** herbivore abundance, and **(d)** total herbivory damage. Lines show a linear fit (solid = significant fit; dashed = non-significant fit) and are colored by fertilization duration (light green = 1 year; dark green = 2 years).

Figure 3. Herbivory based on fertilization level, fertilization duration, and herbivore type. See Appendix 1: Table S1 for nutrient addition rates and Methods for detailed fertilizer composition. Plots were either fertilized for one year **(a, c, e)** or two years **(b, d, f)**. Herbivores include chewing herbivores **(a, b)**, sucking herbivores **(c, d)**, or fungi **(e, f)**.

Figure 4. Herbivory based on fertilization level, fertilization duration, and plant functional group. See Appendix 1: Table S1 for nutrient addition rates and Methods for detailed fertilizer composition. Plots were either fertilized for one year **(a, c, e)** or two years **(b, d, f)**. Plant functional groups include forbs **(a, b)**, grasses **(c, d)**, or legumes **(e, f)**. See Methods for plant functional group species composition.

Figure 1.

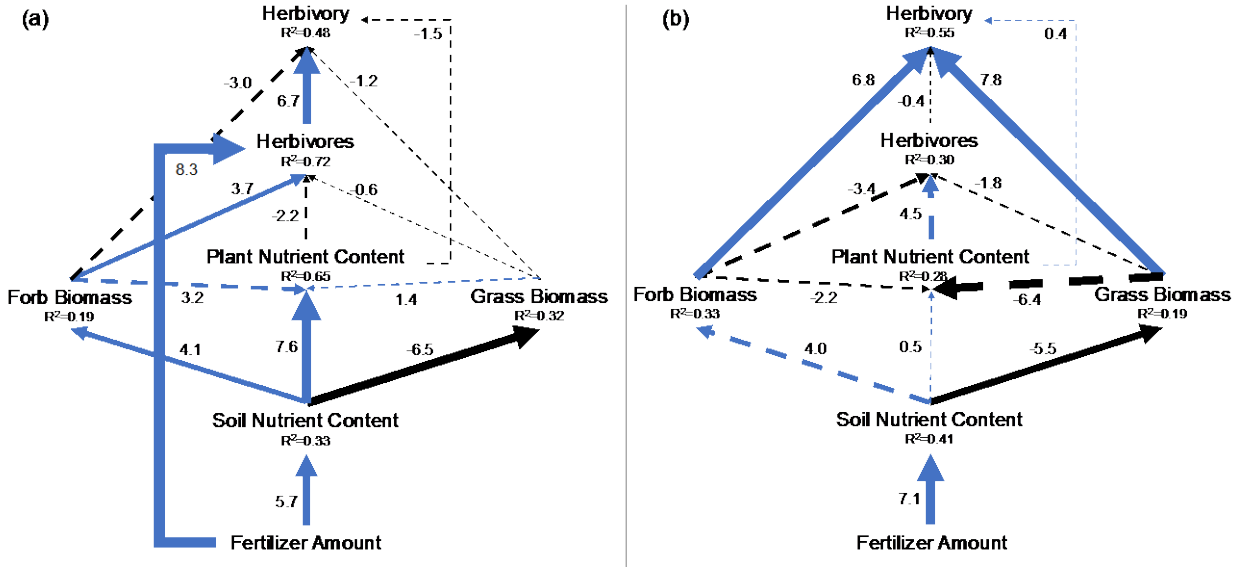

(a)
Herbivory
R²=0.48
-1.5
-3.0
6.7
-1.2
Herbivores
R²=0.72
8.3
3.7
-0.6
-2.2
Plant Nutrient Content
R²=0.65
Forb Biomass
R²=0.19
3.2
1.4
Grass Biomass
R²=0.32
4.1
7.6
-6.5
Soil Nutrient Content
R²=0.33
5.7
Fertilizer Amount
(b)
Herbivory
R²=0.55
0.4
6.8
7.8
-0.4
Herbivores
R²=0.30
-3.4
-1.8
4.5
Plant Nutrient Content
R²=0.28
Forb Biomass
R²=0.33
-2.2
-6.4
Grass Biomass
R²=0.19
4.0
0.5
-5.5
Soil Nutrient Content
R²=0.41
7.1
Fertilizer Amount

Figure 2.

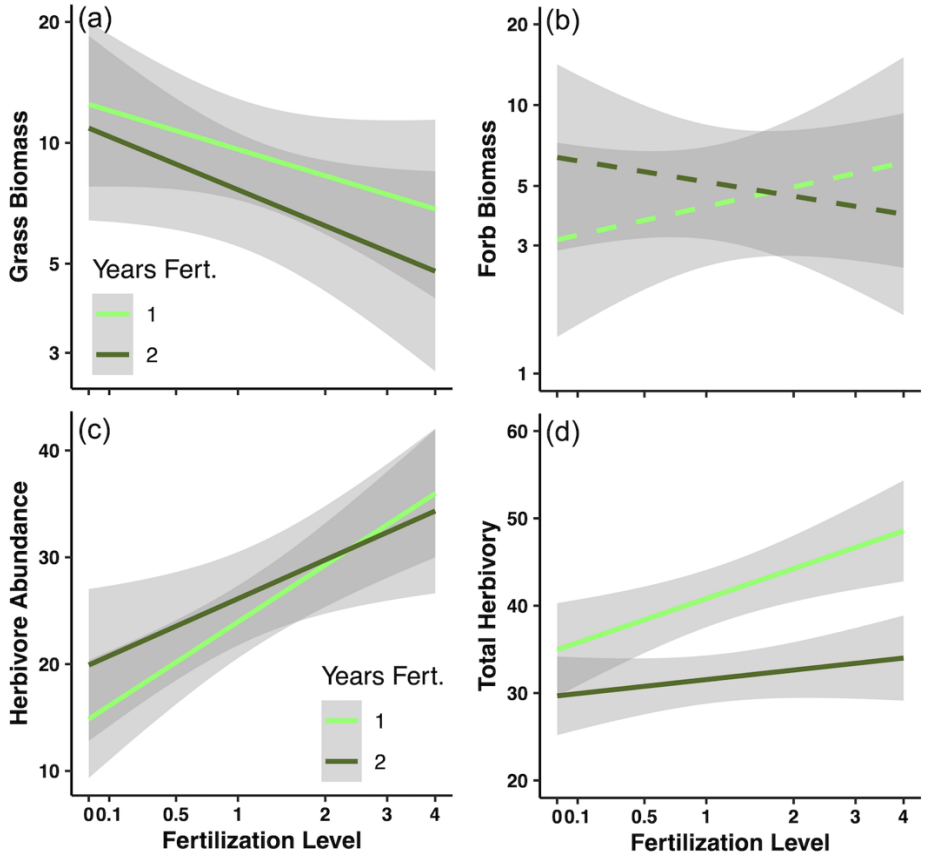

(a)
Grass Biomass
20
10
5
3
Years Fert.
1
2
(b)
Forb Biomass
20
10
5
3
1
(c)
Herbivore Abundance
40
30
20
10
Years Fert.
1
2
0 0.1 0.5 1 2 3 4
Fertilization Level
(d)
Total Herbivory
60
50
40
30
20
0 0.1 0.5 1 2 3 4
Fertilization Level

Figure 3.

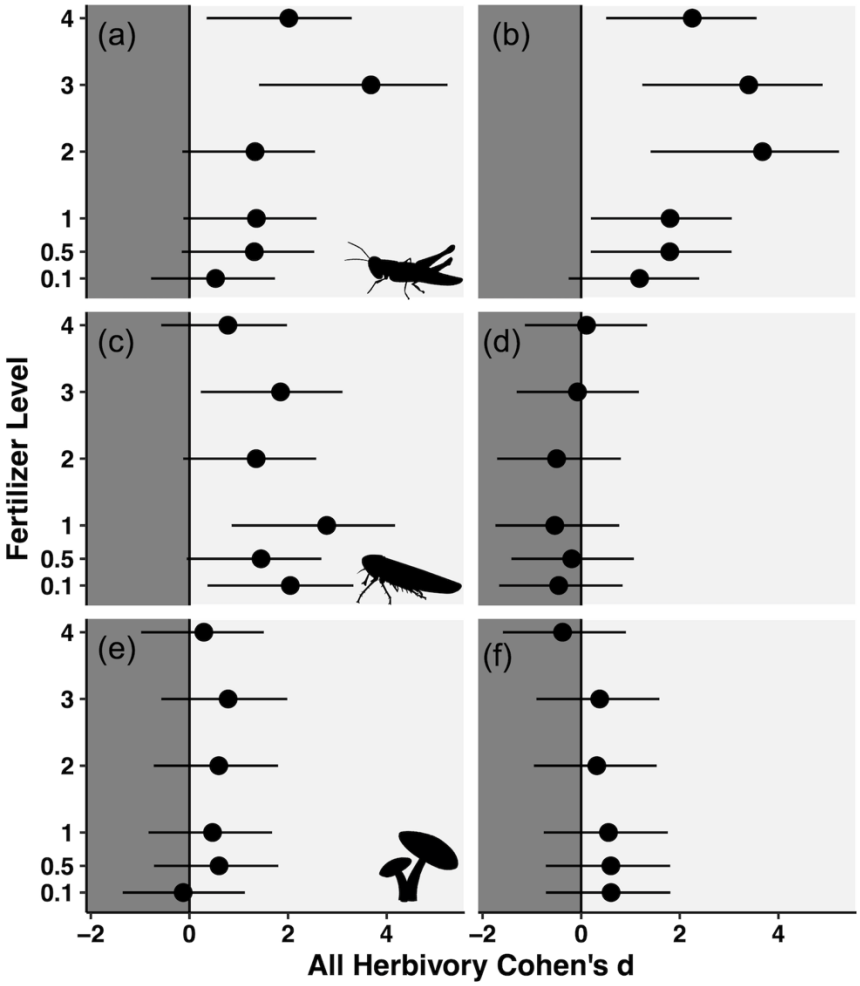
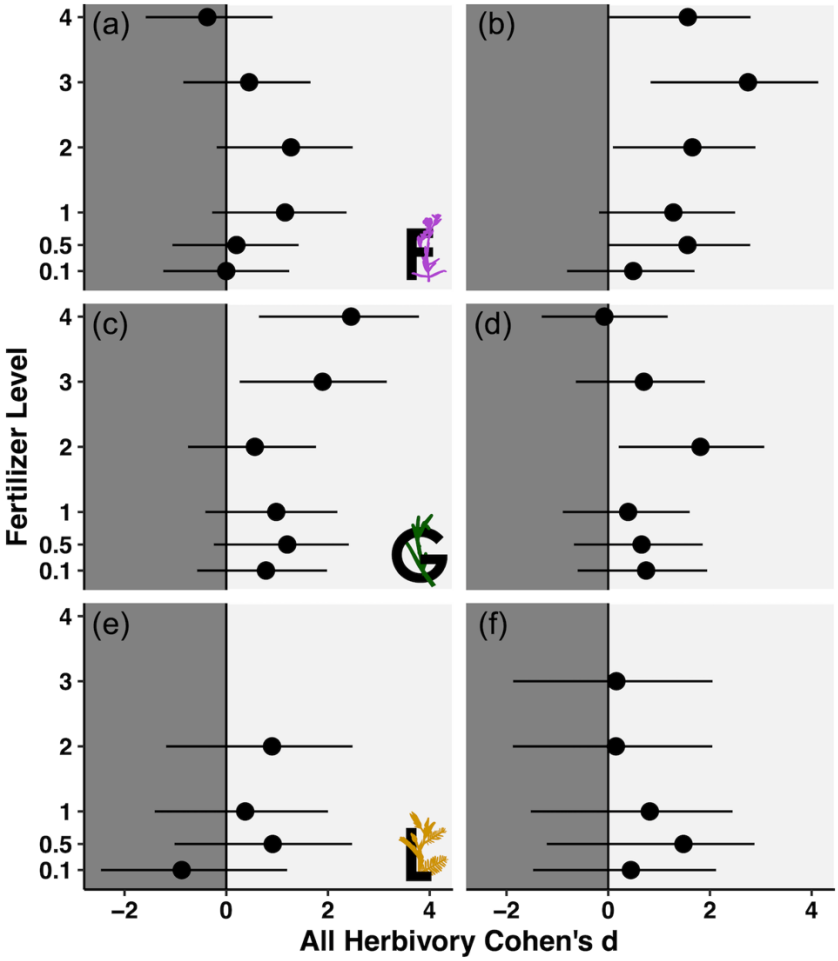

(a)
(b)
(c)
(d)
(e)
(f)
Fertilizer Level
4
3
2
1
0.5
0.1
−2
0
2
4
All Herbivory Cohen's d

Figure 4.


(a)
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Fertilizer Level
4
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2
1
0.5
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0
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4
All Herbivory Cohen's d

Chapter 4: Appendix S1. Supplemental Data.

Table S1. Nutrient addition rates for the seven fertilization treatments for **(a)** year one and **(b)** year two of fertilization. See Methods for detailed fertilizer composition.

a) Nutrient addition year one

Fertilizer Treatment	Nitrogen ($g/m^2/y$)	Phosphorus ($g/m^2/y$)	Potassium ($g/m^2/y$)	Micronutrients ($g/m^2/y$)
0x	0	0	0	0
0.1x	1	1	1	10
0.5x	5	5	5	50
1x	10	10	10	100
2x	20	20	20	200
3x	30	30	30	300
4x	40	40	40	400

b) Nutrient addition year two

Fertilizer Treatment	Nitrogen ($g/m^2/y$)	Phosphorus ($g/m^2/y$)	Potassium ($g/m^2/y$)	Micronutrients ($g/m^2/y$)
0x	0	0	0	0
0.1x	1	1	1	0
0.5x	5	5	5	0
1x	10	10	10	0
2x	20	20	20	0
3x	30	30	30	0
4x	40	40	40	0

Table S2. Model estimates, standard errors (SE), and p-values (*P*) from the piecewise Structural Equation Model (**Fig. 1**) depicting the direct and indirect effects of fertilization on herbivory after **(a)** one year and **(b)** two years of fertilization. Significant *P* values are bolded.

a) One year of fertilization				
Response	**Predictor**	**Estimate**	**SE**	***P***
Soil Nutrient Content	log(Fertilizer Amount)	0.572	0.185	**0.006**
log(Grass Biomass)	Soil Nutrient Content	-0.648	0.219	**0.008**
log(Forb Biomass)	Soil Nutrient Content	0.415	0.197	**0.048**
Plant Nutrient Content	Soil Nutrient Content	0.758	0.188	**0.001**
Plant Nutrient Content	log(Grass Biomass)	0.141	0.177	0.437
Plant Nutrient Content	log(Forb Biomass)	0.320	0.197	0.123
Herbivore Abundance	log(Fertilizer Amount)	0.834	0.168	**0.000**
Herbivore Abundance	Plant Nutrient Content	-0.216	0.189	0.268
Herbivore Abundance	log(Grass Biomass)	-0.059	0.139	0.674
Herbivore Abundance	log(Forb Biomass)	0.365	0.171	**0.047**
Total Herbivory	log(Grass Biomass)	-0.117	0.186	0.536
Total Herbivory	log(Forb Biomass)	-0.301	0.239	0.223
Total Herbivory	Herbivore Abundance	0.672	0.204	**0.004**
Total Herbivory	Plant Nutrient Content	-0.155	0.216	0.482
b) Two years of fertilization				
Response	**Predictor**	**Estimate**	**SE**	***P***
Inverse_Soil_PC1_2018	log(Fertilizer Amount)	0.706	0.159	**0.000**
log(Grass Biomass)	Soil Nutrient Content	-0.554	0.176	**0.005**
log(Forb Biomass)	Soil Nutrient Content	0.401	0.199	0.058
Plant Nutrient Content	Soil Nutrient Content	0.050	0.336	0.884
Plant Nutrient Content	log(Grass Biomass)	-0.638	0.328	0.074
Plant Nutrient Content	log(Forb Biomass)	-0.216	0.356	0.554
Herbivore Abundance	Plant Nutrient Content	0.446	0.272	0.122
Herbivore Abundance	log(Grass Biomass)	-0.183	0.361	0.620
Herbivore Abundance	log(Forb Biomass)	-0.337	0.299	0.278
Total Herbivory	log(Grass Biomass)	0.782	0.290	**0.017**
Total Herbivory	log(Forb Biomass)	0.679	0.248	**0.016**
Total Herbivory	Herbivore Abundance	-0.037	0.206	0.858
Total Herbivory	Plant Nutrient Content	0.040	0.235	0.867

Table S3. Results of linear, quadratic, and cubic regressions quantifying response of plant nutrient content (inverse PC1) to $_{log10}$fertilization level separated by fertilization duration.

Years Fertilized	Test	Terms	df	Estimate	*F*	R^2	*P*	AIC
One	Linear	Fertilization	18	2.96	16	0.5	**0.001**	87.53
One	Quadratic	Fertilization+ Fertilization^2	17	1.14	7.93	0.5	0.539	89.07
One	Cubic	Fertilization+ Fertilization^2+ Fertilization^3	16	4.24	5.54	0.5	0.363	90.00
Two	Linear	Fertilization	17	2.07	4.27	0.2	0.054	94.45
Two	Quadratic	Fertilization+ Fertilization^2	16	1.27	2.16	0.2	0.624	96.15
Two	Cubic	Fertilization+ Fertilization^2+ Fertilization^3	15	13.35	3.69	0.4	**0.033**	92.21

Table S4. Plant species found on experimental plots including their overall frequency (number of plots found on out of 70), Year 1 frequency (number of yr1 plots found on out of 35), Year 2 frequency (number of yr2 plots found on out of 35), maximum percent cover, average percent cover, carbon pathway (C3, C4, or CAM), life span (annual or perennial), functional group (grass, forb, legume), and whether herbivory was assessed on that species.

Species	Overall Frequency	Year 1 Frequency	Year 2 Frequency	Max Cover	Avg. Cover	Carbon Pathway	Life span	Functional Group	Herbivory
Andropogon virginicus	95.71	97.14	94.29	97.5	28.67	C4	Perennial	Grass	Y
Vulpia octoflora	84.29	85.71	82.86	85	20.32	C3	Annual	Grass	
Croton glandulosus	81.43	77.14	85.71	15	3.72	C3	Annual	Forb	Y
Andropogon ternarius	68.57	91.43	45.71	85	22.34	C4	Perennial	Grass	
Juncus marginatus	67.14	57.14	77.14	15	1.145	C3	Perennial	Grass	Y
Agalinis heterophylla	60	62.86	57.14	37.5	4.4	C3	Annual	Forb	Y
Strophostyles leiosperma	52.86	57.14	48.57	15	1.97	C3	Annual	Legume	
Gaillardia aestivalis	40	40	40	37.5	8.07	C3	Perennial	Forb	Y
Oenothera laciniata	35.71	37.14	34.29	15	3.72	C3	Annual	Forb	Y
Erigeron strigosus	35.71	22.86	48.57	15	2.56	C3	Annual	Forb	
Chamaecrista fasciculata	32.86	40	25.71	37.5	7.43	C3	Annual	Legume	Y
Diodella teres	31.43	31.43	31.43	15	4.05	C3	Annual	Forb	
Monarda punctata	30	40	20	15	7.88	C3	Perennial	Forb	Y
Dichanthelium oligosanthes	25.71	20	31.43	62.5	13.92	C3	Perennial	Grass	Y
Heterotheca subaxillaris	24.29	28.57	20	15	3.09	C3	Annual	Forb	
Plantago patagonica	22.86	20	25.71	3	1.13	C3	Annual	Forb	
Eragrostis spectabilis	22.86	31.43	14.29	15	8.84	C4	Perennial	Grass	

Digitaria cognata	20	22.86	17.14	15	7.96	C4	Perennial	Grass
Rumex hastatulus	17.14	11.43	22.86	15	2.33	C3	Perennial	Forb
Croptilon divaricatum	11.44	11.43	11.43	3	1.13	C3	Annual	Forb
Pyrrhopappus carolinianus	10	8.57	11.43	3	0.86	C3	Annual	Forb
Galactia regularis	8.57	8.57	8.57	0.5	0.5	C3	Perennial	Legume
Digitaria ciliaris	7.14	2.86	11.43	97.5	52.6	C4	Annual	Grass
Ambrosia psilostachya	7.14	14.29	20	97.5	48.1	C3	Perennial	Forb
Bromus japonicus	5.71	11.43	20	62.5	17.25	C3	Annual	Grass
Rudbeckia hirta	5.71	8.57	2.86	3	1.125	C3	Annual	Forb
Prunus angustifolia	5.71	2.86	8.57	3	1.125	C3	Perennial	Shrub
Oenothera cinerea	4.29	0	8.57	0.5	0.5	C3	Annual	Forb
Mollugo verticillata	2.86	2.86	2.86	3	3	C3	Annual	Forb
Urochloa texana	1.43	2.86	0	2	2	C4	Annual	Grass
Cyperus retroflexus	1.43	2.86	0	0.5	0.5	C4	Perennial	Grass
Froelichia	1.43	0	2.86	0.5	0.5	C3	Annual	Forb
Desmodium ciliare	1.43	2.86	0	3	3	C3	Perennial	Legume
Desmodium sessilifolium	1.43	2.86	0	3	3	C3	Perennial	Legume
Opuntia humifusa	1.43	2.86	0	3	3	CAM	Perennial	Forb

Supplemental Figure Legends

Figure S1. Experimental setup of 70 plots, each 2 × 2 m and separated by 10 m on all sides. Rows A – G were established in 2017 and rows H – N were established in 2018. Plots received of seven fertilization treatments of N, P, K, and micronutrients when established and those set up in 2017 were fertilized again in 2018 with N, P, and K. Fertilization treatments were 0x (control), 0.1x, 0.5x, 1x, 2x, 3x, or 4x. See Appendix 1: Table S1 for nutrient addition rates and Methods for detailed fertilizer composition.

Figure S2. The Principle Component Analysis of the elemental composition of Ca, K, Mg, NO_3N, P, S, and pH of soil collected from plots in 2018 **(a)** has one significant axis as shown using a broken stick model **(b)**. The significant axis (PC1) is negatively correlated with soil nutrient concentrations so the inverse of PC1 is referred to as soil nutrient content.

Figure S3. The Principle Component Analysis of the elemental composition of nutrients added in the fertilization treatments (B, C, Ca, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, S, Zn) in plant tissue collected from plots in 2018 **(a)** has two significant axes as shown using a broken stick model **(b)**. PC1 is negatively correlated with nutrient concentrations in plant tissue so the inverse of PC1 is referred to as plant nutrient content.

Figure S4. Nutrient content in plants based on fertilizer level after **(a)** one year and **(b)** two years of fertilization. Plant nutrients is the inverse of PC1 from the PCA done on the nutrient concentration in plant tissue of elements added via the fertilization treatments (B, C, Ca, Cu, Fe, K, Mg, Mn, Mo, N, Na, P, S, Zn). Lines show significant fit and results from these models are in Appendix 1: Table S3.

Figure S5. Relationship between fertilization level and **(a)** plant richness and **(b)** plant diversity. Lines show a linear fit (solid = significant fit; dashed = non-significant fit) and are colored by fertilization duration (light green = 1 year; dark green = 2 years).

Figure S6. Correlations among the three measures of herbivory (chewing damage, fungi damage, sucking damage) assessed. Plots were either fertilized for one year **(a, b, c)** or two years **(d, e, f)**. *R* value in each figure is the Pearson correlation coefficient.

Figure S1.

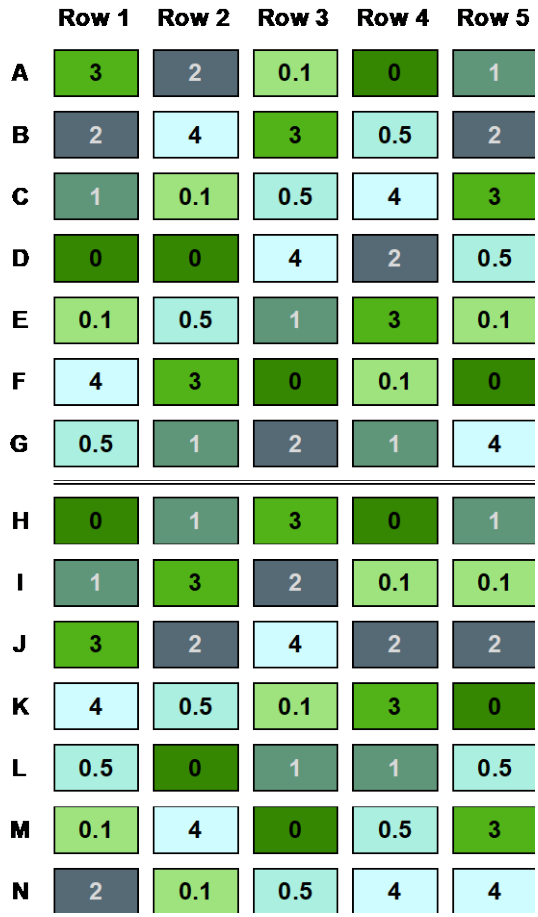

Row 1 Row 2 Row 3 Row 4 Row 5
A 3 2 0.1 0 1
B 2 4 3 0.5 2
C 1 0.1 0.5 4 3
D 0 0 4 2 0.5
E 0.1 0.5 1 3 0.1
F 4 3 0 0.1 0
G 0.5 1 2 1 4
H 0 1 3 0 1
I 1 3 2 0.1 0.1
J 3 2 4 2 2
K 4 0.5 0.1 3 0
L 0.5 0 1 1 0.5
M 0.1 4 0 0.5 3
N 2 0.1 0.5 4 4

Figure S2.

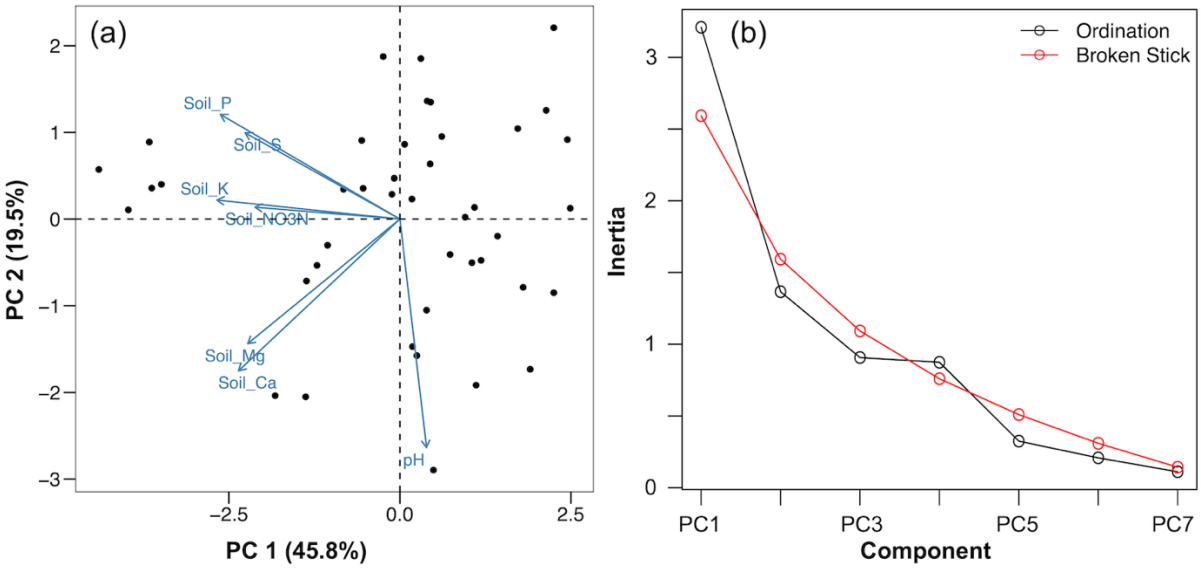

(a)
Soil_P
Soil_S
Soil_K
Soil_NO3N
Soil_Mg
Soil_Ca
pH
PC 2 (19.5%)
PC 1 (45.8%)
(b)
Ordination
Broken Stick
Inertia
PC1
PC3
PC5
PC7
Component

Figure S3.

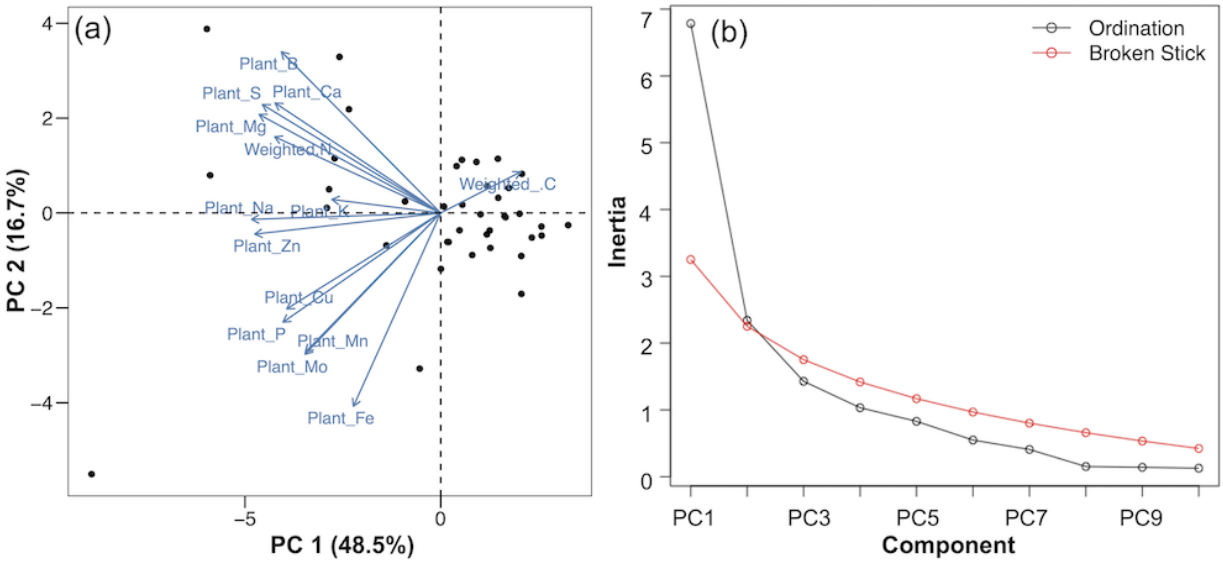

(a)
Plant_B
Plant_S
Plant_Ca
Plant_Mg
Weighted_N
Weighted_.C
Plant_Na
Plant_K
Plant_Zn
Plant_Cu
Plant_P
Plant_Mn
Plant_Mo
Plant_Fe
PC 2 (16.7%)
PC 1 (48.5%)
(b)
Ordination
Broken Stick
Inertia
PC1
PC3
PC5
PC7
PC9
Component

Figure S4.

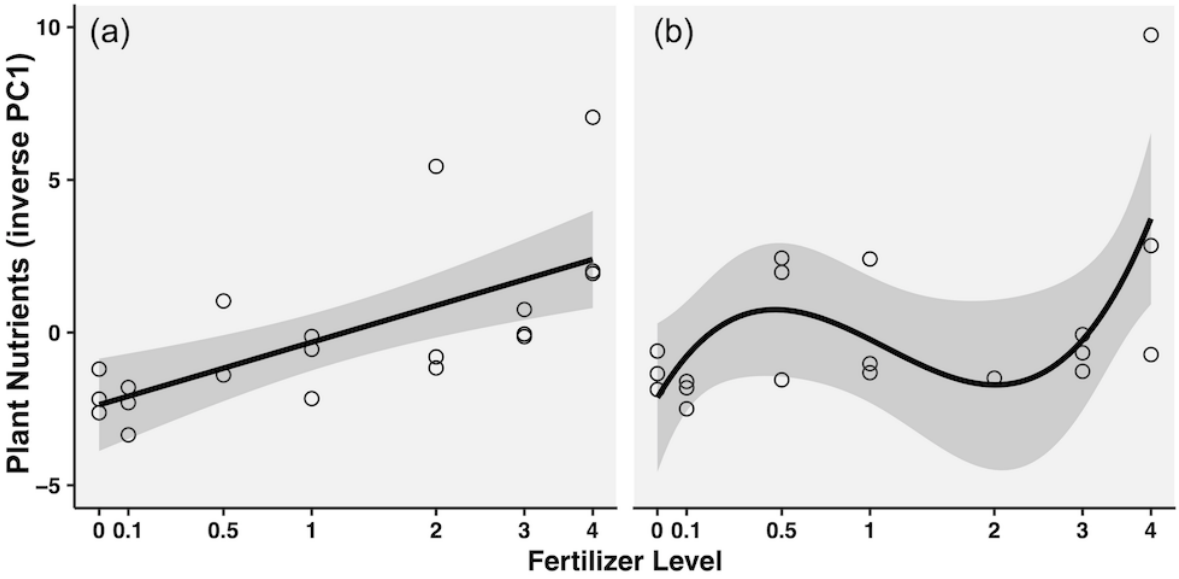

(a)
(b)
Plant Nutrients (inverse PC1)
10
5
0
−5
0 0.1
0.5
1
2
3
4
Fertilizer Level

Figure S5.

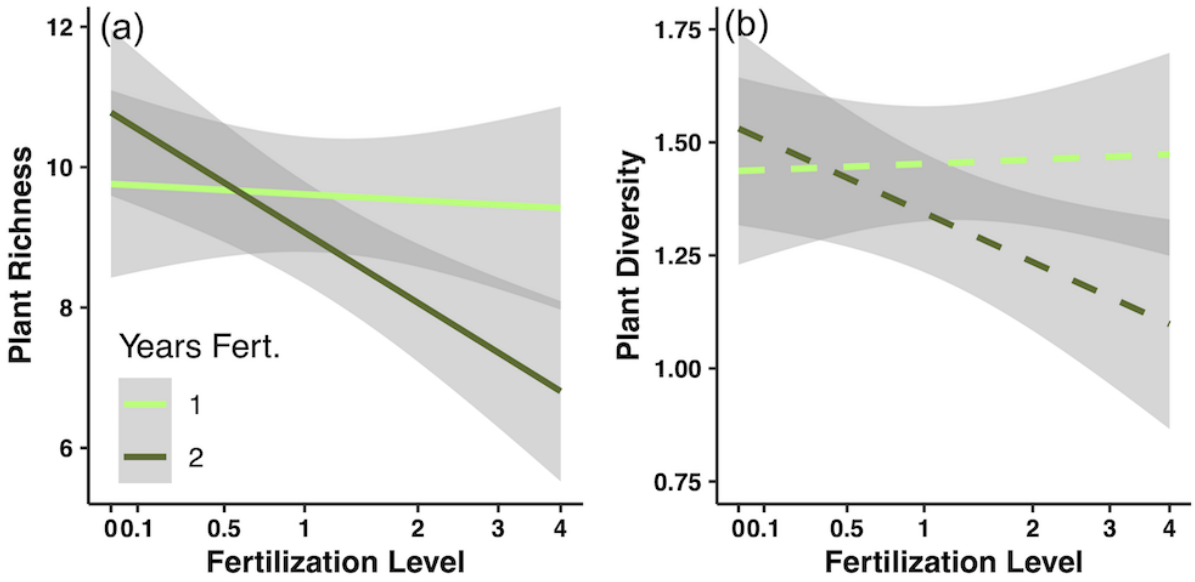
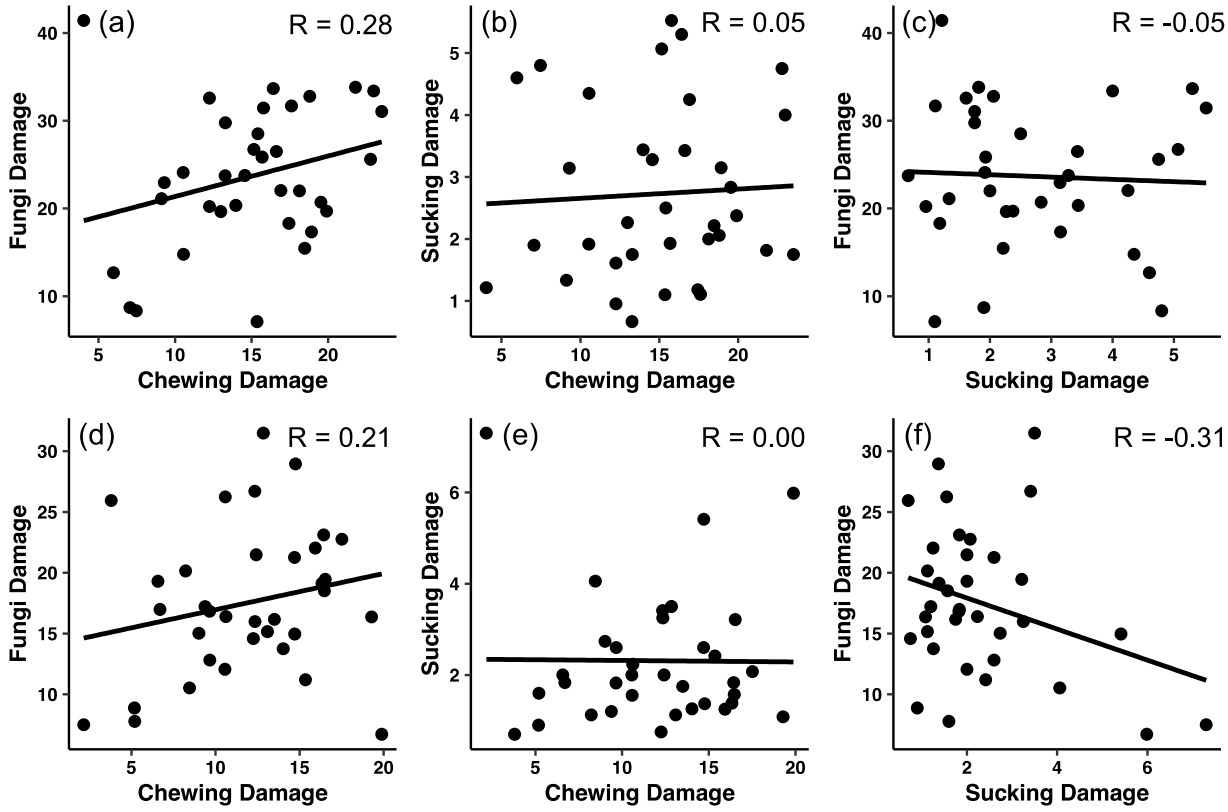

(a)
Plant Richness
12
10
8
6
Years Fert.
1
2
0
0.1
0.5
1
2
3
4
Fertilization Level
(b)
Plant Diversity
1.75
1.50
1.25
1.00
0.75
0
0.1
0.5
1
2
3
4
Fertilization Level

Figure S6.


(a)
R = 0.28
Fungi Damage
Chewing Damage
(b)
R = 0.05
Sucking Damage
Chewing Damage
(c)
R = -0.05
Fungi Damage
Sucking Damage
(d)
R = 0.21
Fungi Damage
Chewing Damage
(e)
R = 0.00
Sucking Damage
Chewing Damage
(f)
R = -0.31
Fungi Damage
Sucking Damage