

Plants regulate grassland arthropod communities through biomass, quality, and habitat heterogeneity

REBECCA M. PRATHER  † AND MICHAEL KASPARI 

Department of Biology, Graduate Program in Ecology and Evolutionary Biology, University of Oklahoma, Norman, Oklahoma 73019 USA

Citation: Prather, R. M., and M. Kaspari. 2019. Plants regulate grassland arthropod communities through biomass, quality, and habitat heterogeneity. *Ecosphere* 10(10):e02909. 10.1002/ecs2.2909

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.

Received 23 July 2019; **accepted** 4 September 2019. Corresponding Editor: Uffe N. Nielsen.

Copyright: © 2019 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** rebeccaprather@ou.edu

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 (sparse or dense), and vegetation spacing (clumped or uniform) combine to generate habitat heterogeneity (Landis et al. 2000, Langelotto 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 (Tilman 1986, LeBauer and Treseder 2008, Stevens et al. 2015) and/or plant nutrient quality (Haddad et al. 2000, Borer et al. 2015, 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, Langelotto and Denno 2004). Fertilization can also increase food quality (Borer et al. 2015), decreasing plant carbon to nitrogen ratios (Tilman 1986, 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 (Langelotto 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 (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 [CV] in plant height per m², 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 *Andropogon ternarius* as the dominant grasses and *Croton glandulosus* and *Juncus marginatus* 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: Figs. S1, S2). Each treatment combination was replicated 15 times, resulting in 90 plots, each 4 m² (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-m² quadrats. We clipped vegetation from two diagonal 1-m² 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·m⁻²·yr⁻¹ by elemental mass and a micronutrient mixture applied at a rate of 100 g·m⁻²·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/m²), magnesium (3 g/m²), sulfur (12 g/m²), boron (0.1 g/m²), water-soluble copper (1 g/m²), water-soluble iron (17 g/m²), water-soluble manganese (2.5 g/m²), molybdenum (0.05 g/m²), and water-soluble zinc (1 g/m²).

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, and 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 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: Tables 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 Development 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 \times plant biomass) and total plot N (average %N \times 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 ($^{\circ}$ C), and average humidity (%) using the lmer function in the lme4 R package (Bates et al. 2014). We checked response variables 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. Relative importance values 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 ($^{\circ}C$), average surface temperature ($^{\circ}C$), minimum surface temperature ($^{\circ}C$), and

average humidity (%). We identified environmental variables explaining significant amounts of variation in arthropod compositional differences between clipping and fertilization treatments using the *ordstep* 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 on the significance of the m^2 statistic on 999 permutations.

RESULTS

Clipping and fertilization treatments 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

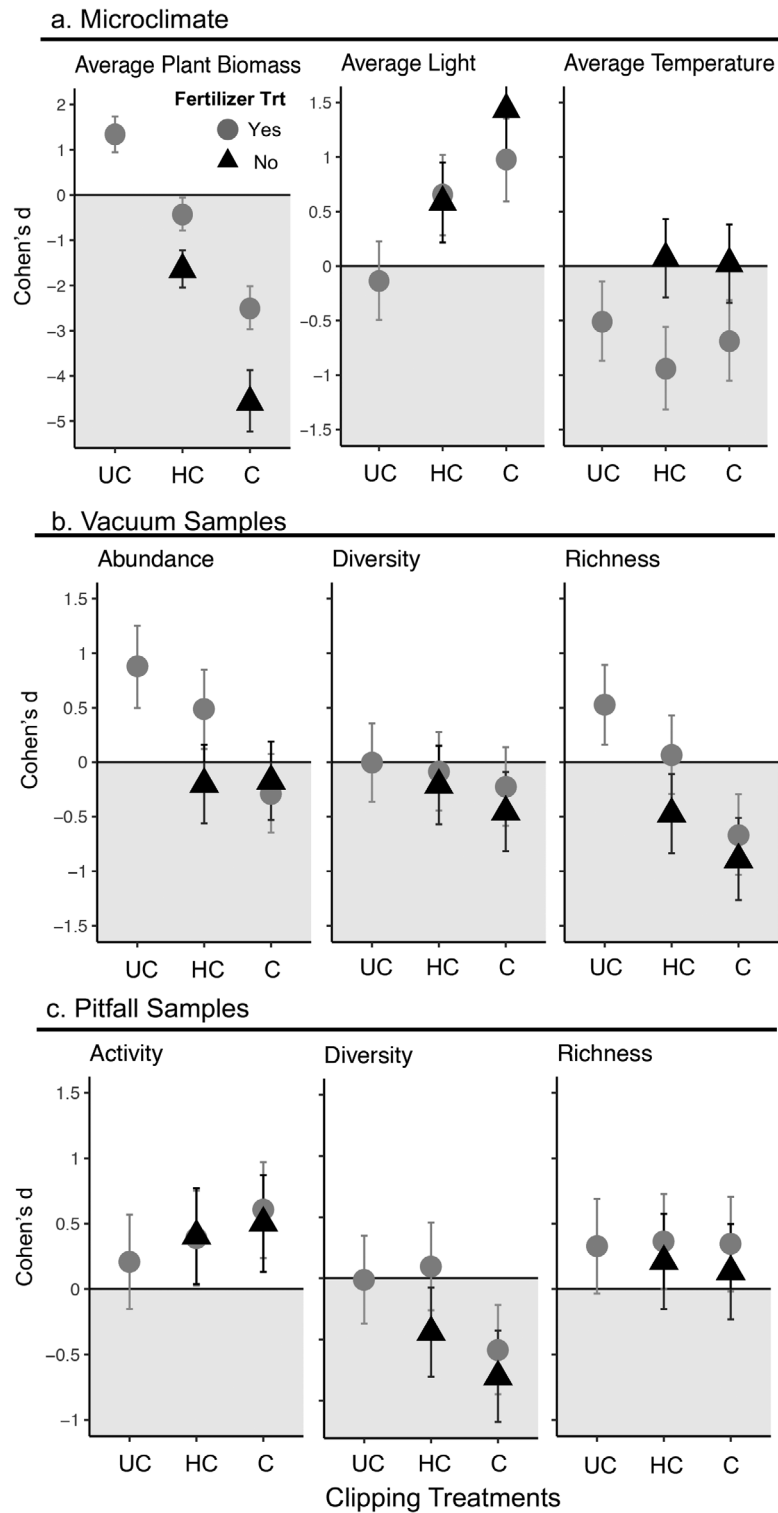


Fig. 1. Response of arthropods and microclimate variables to factorial clipping and fertilization treatments, measured as effect size (Cohen's *d*). (a) Effect size of changes in microclimate variables including average plant

(Fig. 1. *Continued*)

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 $d \geq |1.0|$.

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: Fig. 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: Fig. 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 S1: Tables S1, S2, for summary data, see Data S1). 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 $F = 4.42$, $P = 0.009$). A 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 significantly 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. 2a; 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. 2b; Appendix S1: Table S6).

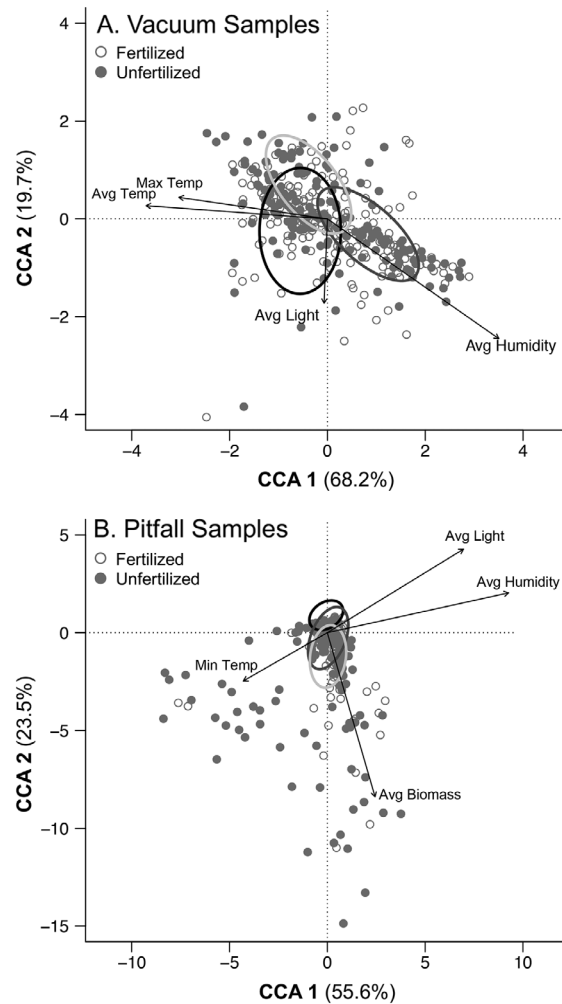


Fig. 2. Canonical correspondence analysis biplot ordination of arthropod taxa from (a) vacuum samples ($n = 11$ taxa) and (b) pitfall samples ($n = 11$ taxa), with key microclimate variables displayed. Each point represents the monthly arthropod community from plots ($n = 90$) sampled once a month from 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.

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 nitrogen and significantly increasing plot carbon (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, constrained by plant biomass, food quality, and habitat heterogeneity 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, 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 homogeneous 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, Langelotto 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

Here, we demonstrate how plant biomass, spatial heterogeneity, and nutrient availability shaped 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.

ACKNOWLEDGMENTS

We thank Andrew Prather, Thad Prather, and Adrian Semones for their help in setting up and fertilizing plots. We thank Tabitha Brown, Amanda Kelly, Ranish Timilsina, and Dalinh Tran for assistance in the field and with sample sorting. We are

grateful to Mariëlle Hoefnagels, Emily Kiehnau, Karl A. Roeder, Michael D. Weiser, Gary Wellborn, and Ellen A. R. Welti for their assistance or helpful discussions. We thank Delmas Northcutt and Richard Page for permission to use their land—Pigtail Alley Prairie. This study was funded by a University of Oklahoma Biological Station Summer Fellowship awarded to R. M. Prather and a NSF grant (DEB-1556280) awarded to M. Kaspari. RMP and MK conceived and designed the experiment. RMP conducted fieldwork, arthropod sorting, analyzed data, and drafted the manuscript. MK provided conceptual advice and edited the manuscript.

LITERATURE CITED

- Adler, P., D. Raff, and W. Lauenroth. 2001. The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* 128:465–479.
- Arnan, X., A. Rodrigo, and J. Retana. 2007. Uncoupling the effects of shade and food resources of vegetation on Mediterranean ants: an experimental approach at the community level. *Ecography* 30:161–172.
- Báldi, A. 2008. Habitat heterogeneity overrides the species–area relationship. *Journal of Biogeography* 35:675–681.
- Barton, K. 2016. MuMIn: model selection and model averaging based on information criteria (AICc and alike). R package version 1.15.6. <http://r-forge.r-project.org/projects/mumin/>
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823 <https://arxiv.org/abs/1406.5823>
- Borer, E. T., E. M. Lind, E. J. Ogdahl, E. W. Seabloom, D. Tilman, R. A. Montgomery, and L. L. Kinkel. 2015. Food-web composition and plant diversity control foliar nutrient content and stoichiometry. *Journal of Ecology* 103:1432–1441.
- Borer, E. T., E. W. Seabloom, D. S. Gruner, and L. R. O'Halloran. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508:517–520.
- Bransby, D., and N. Tainton. 1977. The disc pasture meter: possible applications in grazing management. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa* 12:115–118.
- Burnham, K. P., and D. R. Anderson. 2003. *Model selection and multimodel inference: a practical information-theoretic approach*. Second edition. Springer-Verlag, New York, New York, USA.
- Chase, J. M., and M. A. Leibold. 2003. *Ecological niches: linking classical and contemporary approaches*. First edition. University of Chicago Press, Chicago, Illinois, USA.
- Cohen, J. 1988. *Statistical power analysis for the behavioral sciences*. Second edition. Lawrence Erlbaum Associates, Hillsdale, New Jersey, USA.
- Cole, L., S. Buckland, and R. D. Bardgett. 2008. Influence of disturbance and nitrogen addition on plant and soil animal diversity in grassland. *Soil Biology and Biochemistry* 40:505–514.
- Debano, S. J. 2006. Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodiversity & Conservation* 15:2547.
- Dell, A. I., S. Pawar, and V. M. Savage. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences USA* 108:10591–10596.
- Dennis, P., M. Young, and I. Gordon. 1998. Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. *Ecological Entomology* 23:253–264.
- Finke, D. L., and R. F. Denno. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. *Ecology* 83:643–652.
- Fretwell, S. D. 1987. Food chain dynamics: The central theory of ecology? *Oikos* 50:291–301.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. *Science* 293:2248–2251.
- Greenslade, P. J. 1983. Adversity selection and the habitat templet. *American Naturalist* 122:352–365.
- Haddad, N. M., J. Haarstad, and D. Tilman. 2000. The effects of long-term nitrogen loading on grassland insect communities. *Oecologia* 124:73–84.
- Haddad, N. M., D. Tilman, J. Haarstad, M. Ritchie, and J. M. Knops. 2001. Contrasting effects of plant richness and composition on insect communities: a field experiment. *American Naturalist* 158:17–35.
- Hairton, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. *American Naturalist* 94:421–425.
- Haysom, K. A., D. I. McCracken, G. N. Foster, and N. W. Sotherton. 2004. Developing grassland conservation headlands: response of carabid assemblage to different cutting regimes in a silage field edge. *Agriculture, Ecosystems & Environment* 102:263–277.
- Honek, A. 1988. The effect of crop density and microclimate on pitfall trap catches of Carabidae, Staphylinidae (Coleoptera), and Lycosidae (Araneae) in cereal fields. *Pedobiologia* 32:233–242.

- Hutchinson, G. E. 1961. The paradox of the plankton. *American Naturalist* 95:137–145.
- Jackson, D. A. 1995. PROTEST: a PROcrustean randomization test of community environment concordance. *Ecoscience* 2:297–303.
- Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. Van der Hammen. 2007. Habitat structure affects intraguild predation. *Ecology* 88:2713–2719.
- Kaspari, M., K. A. Roeder, B. Benson, M. D. Weiser, and N. J. Sanders. 2017. Sodium co-limits and catalyzes macronutrients in a prairie food web. *Ecology* 98:315–320.
- Kaspari, M., M. Yuan, and L. Alonso. 2003. Spatial grain and the causes of regional diversity gradients in ants. *American Naturalist* 161:459–477.
- La Pierre, K. J., and M. D. Smith. 2016. Soil nutrient additions increase invertebrate herbivore abundances, but not herbivory, across three grassland systems. *Oecologia* 180:485–497.
- Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:175–201.
- Langellootto, G. A., and R. F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia* 139:1–10.
- Lassau, S. A., and D. F. Hochuli. 2004. Effects of habitat complexity on ant assemblages. *Ecography* 27:157–164.
- LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89:371–379.
- McNaughton, S. J. 1984. Grazing lawns: animals in herds, plant form, and coevolution. *American Naturalist* 124:863–886.
- Melbourne, B. A. 1999. Bias in the effect of habitat structure on pitfall traps: an experimental evaluation. *Australian Journal of Ecology* 24:228–239.
- Mommertz, S., C. Schauer, N. Kösters, A. Lang, and J. Filser. 1996. A comparison of D-Vac suction, fenced and unfenced pitfall trap sampling of epigeal arthropods in agroecosystems. *Annales Zoologici Fennici* 33:117–124.
- Moran, M. D., and A. R. Scheidler. 2002. Effects of nutrients and predators on an old-field food chain: interactions of top-down and bottom-up processes. *Oikos* 98:116–124.
- Morris, M. G. 2000. The effects of structure and its dynamics on the ecology and conservation of arthropods in British grasslands. *Biological Conservation* 95:129–142.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. O'hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2015. Vegan: community ecology package. <http://CRAN.R-project.org/package=vegan>
- Oliver, I., and A. J. Beattie. 1996. Invertebrate morphospecies as surrogates for species: a case study. *Conservation Biology* 10:99–109.
- Penick, C. A., S. E. Diamond, N. J. Sanders, and R. R. Dunn. 2017. Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants. *Functional Ecology* 31:1091–1100.
- Peres-Neto, P. R., and D. A. Jackson. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* 129:169–178.
- Post, D. M., M. L. Pace, and N. G. Hairston Jr. 2000. Ecosystem size determines food-chain length in lakes. *Nature* 405:1047.
- R Development Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Roeder, K. A., D. V. Roeder, and M. Kaspari. 2018. Disturbance mediates homogenization of above and belowground invertebrate communities. *Environmental Entomology* 47:545–550.
- Rosenzweig, M. L. 1995. Species diversity in space and time. First edition. Cambridge University Press, Cambridge, UK.
- Savolainen, R., and K. Vepsäläinen. 1988. A competition hierarchy among boreal ants: impact on resource partitioning and community structure. *Oikos* 51:135–155.
- Siemann, E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology* 79:2057–2070.
- Spence, J. R., and J. K. Niemelä. 1994. Sampling carabid assemblages with pitfall traps: the madness and the method. *Canadian Entomologist* 126:881–894.
- Srivastava, D. S., and J. H. Lawton. 1998. Why more productive sites have more species: an experimental test of theory using tree-hole communities. *American Naturalist* 152:510–529.
- Standen, V. 2000. The adequacy of collecting techniques for estimating species richness of grassland invertebrates. *Journal of Applied Ecology* 37:884–893.
- Stevens, C. J., et al. 2015. Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology* 96:1459–1465.
- Tabachnick, B. G., and L. S. Fidell. 2007. Using multivariate statistics. Volume 5. Pearson Education, Boston, Massachusetts, USA.
- Tilman, D. 1986. Nitrogen-limited growth in plants from different successional stages. *Ecology* 67:555–563.
- van Klink, R., F. van der Plas, C. G. E. T. van Noordwijk, M. F. WallisDeVries, and H. Olf. 2015. Effects

- of large herbivores on grassland arthropod diversity. *Biological Reviews* 90:347–366.
- Wan, S., Y. Luo, and L. Wallace. 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biology* 8:754–768.
- Woodcock, B., S. Potts, E. Pilgrim, A. Ramsay, T. Tscheulin, A. Parkinson, R. Smith, A. Gundry, V. Brown, and J. Tallowin. 2007. The potential of grass field margin management for enhancing beetle diversity in intensive livestock farms. *Journal of Applied Ecology* 44:60–69.
- Woodcock, B. A., S. G. Potts, T. Tscheulin, E. Pilgrim, A. J. Ramsey, J. Harrison-Cripps, V. K. Brown, and J. R. Tallowin. 2009. Responses of invertebrate trophic level, feeding guild and body size to the management of improved grassland field margins. *Journal of Applied Ecology* 46:920–929.
- Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1:3–14.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2909/full>