Invertebrate, not small vertebrate, herbivory interacts with nutrient availability to impact tallgrass prairie community composition and forb biomass

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The effects of herbivores and their interactions with nutrient availability on primary production and plant community composition in grassland systems is expected to vary with herbivore type. We examined the effects of invertebrate and small vertebrate herbivores and their interactions with nutrient availability on grassland plant community composition and aboveground biomass in a tallgrass prairie ecosystem. The abundance of forbs relative to grasses increased with invertebrate herbivore removals. This increase in forb abundance led to a shift in community composition, where invertebrate removals resulted in greater plant species evenness as well as a divergence in composition among plots. In contrast, vertebrate herbivore removals did not affect plant community composition or aboveground biomass. Nutrient additions alone resulted in a decrease in plant species richness and an increase in the abundance of the dominant grass, but the dominant grass species did not greatly increase in abundance when nutrient additions were combined with invertebrate removals. Rather, several subdominant forbs came to dominate the plant community. Additionally, the combined nutrient addition and invertebrate herbivore removal treatment increased forb biomass, suggesting that invertebrate herbivores suppress the responses of forb species to chronic nutrient additions. Overall, the release of forbs from invertebrate herbivore pressure may result in large shifts in species composition, with consequences for aboveground biomass and forage quality due to altered grass:forb ratios in grassland systems.

Bottom–up and top–down interactions are known to be important in determining primary production and plant community composition (Power 1992, Pace et al. 1999, Chase et al. 2000, Schmitz et al. 2000, Cebrian et al. 2009). Particularly in grassland systems, nutrient additions generally result in increased primary production and decreased plant species richness (Clark et al. 2007, Gruner et al. 2008, Cleland and Harpole 2010, Harpole et al. 2011, Hillebrand 2011). However, the effects of herbivores and their interactions with nutrient availability on primary production and plant community composition are less consistent across grasslands (Olff and Ritchie 1998, Maron and Crone 2006, Hillebrand et al. 2007, Gruner et al. 2008). These inconsistent effects of herbivores in grassland systems may be due to the wide range of herbivore types within and across these systems (Borer et al. 2005, Bakker et al. 2006). Thus, it is important to examine the independent effects of different suites of herbivores and their interactions with nutrient availability on grassland production and plant community composition.

Functional differences among herbivore species can be important in determining their effects on primary producers (Schmitz et al. 2000, Bakker et al. 2006, Maron and Crone 2006, Schmitz 2008a). Large ungulate herbivores, such as bison and cattle, are known to affect grassland community composition by selectively grazing grasses; the removal of large ungulates typically results in increased grass abundance and reduced forb abundance, as the grasses are released from top–down control and begin to outcompete the forbs (Knapp et al. 1998, Frank 2005, Augustine et al. 2010). While the effects of large vertebrate herbivores are generally well-studied in grasslands and have been shown to be consistent across grassland systems (Bakker et al. 2006), the effects of other guilds of herbivores are less well characterized.

Debate exists over the role that small vertebrate and invertebrate herbivores play in structuring grassland plant communities. Although some empirical work in grasslands has shown support for effects of both invertebrate herbivores (Marquis and Whelan 1994, Fraser and Grime 1998, Coupe and Cahill 2003, Amsberry and Maron 2006, Whiles and Charlton 2006, Schadler et al. 2008, Schmitz 2008a, Allan and Crawley 2011, Blue et al. 2011) and small vertebrate herbivores (Collins and Barber 1985, Brown and Heske 1990, Hulme 1994, Collins et al. 1998, Ritchie et al. 1998,

Edwards and Crawley 1999, Howe et al. 2002, Bakker et al. 2006), these effects vary across space and time. Additionally, the relative strengths of the effects of invertebrate versus small vertebrate herbivores remain unclear, as their effects are rarely investigated within a single experiment. Differences in metabolic efficiency, feeding mode and behavior both within and among vertebrate and invertebrate herbivores can strongly influence the strength of their effects on productivity (Shurin and Seabloom 2005, Bakker et al. 2006, Schmitz 2008b, Hopcraft et al. 2010, Allan and Crawley 2011). Additionally, the spatial distribution of vertebrate and invertebrate herbivores differs, with small vertebrate herbivores tending to be more patchily distributed in the landscape than invertebrate herbivores (Huntly 1991, Throop and Lerdau 2004). In order to understand the role of top–down control in grasslands, we must examine the relative impacts of these less well-studied groups of herbivores.

Although nutrient additions are known to affect plant species diversity and primary productivity (Chapin et al. 1986, Gruner et al. 2008, Cleland and Harpole 2010), the role of herbivores in mediating the strength of these effects also remains unclear (Worm et al. 2002, Hillebrand et al. 2007, Gruner et al. 2008). Herbivores may alter plant responses to nutrient additions in several ways. First, herbivores can alter the plant community response to nutrient additions by either selectively feeding on particular groups of species (e.g. grasses versus forbs) or by generally opening up space, allowing for species turnover and immigration (Olff and Ritchie 1998, Proulx and Mazumder 1998, Persson et al. 2001, Eskelinen 2008). Second, feeding by herbivores may reduce the production response to nutrient additions if the plants cannot compensate for tissue lost to herbivory (Agrawal 2000, Meyer 2000, Denyer et al. 2007, McCluney et al. 2012). As the functional effects of vertebrate and invertebrate herbivores on plant community composition and production may vary (Huntly 1991, Hopcraft et al. 2010, Allan and Crawley 2011), the interactive effects of vertebrate versus invertebrate herbivores with nutrient additions may also vary.

Here we experimentally assessed the independent and interactive effects of removing vertebrate and invertebrate herbivores on aboveground productivity and plant community composition in native tallgrass prairie. We further determined whether the removal of vertebrate and invertebrate herbivores interacted with nutrient additions. By doing this, we addressed three related questions: 1) what is the relative strength of the effects of invertebrate versus small vertebrate herbivory in a grassland system; 2) how does herbivory (invertebrate and/or small vertebrate) affect the relative abundances of grasses and forbs, the two dominant plant functional types within the ecosystem; and 3) what are the consequences of these changes in composition for aboveground net primary productivity, an important ecosystem function?

Material and methods

Study system

The study was conducted at the Konza Prairie Biological Station, a native tallgrass prairie site located in northeastern KS, USA. The site receives an average of 835 mm of annual precipitation and is dominated by C_4 grasses, such as *Andropogon gerardii*, *Sorghastrum nutans* and *Schizachyrium scoparium* (Risser et al. 1981, Knapp et al. 1998). The flora is diverse and is thought to be controlled by grazing, fire, and a variable continental climate (Knapp et al. 1998). This experiment was located in experimental watershed 2C (for a map of Konza see < http://kpbs.konza.k-state.edu/treatments.html >), a long-term ungrazed (by bison or cattle), upland watershed with relatively shallow soils that is burned biennially in the spring (mid-April in 2009, 2011, 2013 during this experiment).

Overall, small mammals are relatively common within the Konza Prairie Biological Station (10–13 individuals per trap line; 20 stations with 15-m intervals between stations; Kaufman and Kaufman 1997). As in most tallgrass prairie sites, the small vertebrate herbivore community at the study site is numerically dominated by deer mice *Peromyscus maniculatus* (approximately 8–9 individuals per trapline), with western harvest mice *Reithrodontomus megalotis* and white-footed mice *P. leucopus* also common (approximately one individual per trapline each, Reichman 1987, Kaufman et al. 1998). Prairie voles *Microtus ochrogaster* are less common on Konza than other tallgrass prairie sites (approximately 0.5 individuals per trapline; Kaufman et al. 1998).

The invertebrate herbivore community at the study site is numerically dominated by leaf hoppers (Cicadellidae) and moth/butterfly and beetle larvae (Lepidoptera and Coleoptera, respectively; La Pierre unpubl.). While grasshoppers (Acrididae) are less abundant, they make up a large portion of invertebrate herbivore biomass at the site (La Pierre unpubl.). Invertebrate herbivore abundance, primarily that of leaf-chewing herbivores, increases with nutrient additions at the study site, but is unaffected by vertebrate herbivore removals (Supplementary material Appendix 1, La Pierre unpubl.).

Experimental design

Experimental treatments were implemented for five years (2009–2013). Plots were established in a randomized block design (n = 3) with eight treatment combinations per block: control, vertebrate removal (–V), invertebrate removal (–I), all herbivore removal (–V, –I), nutrient addition (+ NPK), vertebrate removal with NPK (–V, + NPK), invertebrate removal with NPK (–I, + NPK), and all herbivore removal with NPK (–V, –I, + NPK). Plots were 4 m² (2 × 2 m) with 1 m wide aisles between each plot and 2 m wide aisles between blocks. The nutrient addition treatment (+ NPK) involved the addition of relatively high levels of nitrogen, phosphorous, and potassium, each applied at a rate of 10 g m^{-2} year^{-1}. Nitrogen was added as time-release urea, phosphorous as calcium phosphate, and potassium as potassium sulfate. These nutrient additions occurred once yearly at the start of the growing season (mid-April after the site was burned).

The vertebrate herbivore removal treatment (–V) involved surrounding the entire plot with a fence consisting of a 1 m high fine mesh with a 0.3 m skirt stapled to the ground to prevent access by burrowing animals. Given that the site only has a single large vertebrate herbivore present at low abun-

dance (white-tailed deer), this treatment primarily excluded small vertebrate herbivores, such as small rodents. Although, rodents that can burrow long distances or climb the 1 m fence may have accessed the plots, no evidence of their presence within the exclosure (e.g. nests, feeding remains, droppings) was observed, while signs of their presence outside of the exclosure were occasionally observed. The fences were erected in May 2009 and remained standing for the duration of the experiment.

The invertebrate herbivore removal treatment (–I) involved the application of insecticide throughout the growing season (April–August). The concentrated insecticide was diluted in water (1 tbs per gallon). The diluted mixture was applied twice monthly to the invertebrate herbivore removal plots using a handheld sprayer at a rate of approximately 300 ml m^{-2}. An equal amount of water was applied using a handheld sprayer to all plots that did not receive the insecticide treatments to account for the effect of water additions. To determine the efficacy of the invertebrate removal treatment, invertebrate samples were collected from a 1 m^2 subsection of each plot using a vacuum sampler twice per growing season (mid-July and mid-August) from 2009–2012. Samples were collected on warm, sunny days between 11 a.m. and 1 p.m. Grasshoppers that hopped out of the plots during sampling were counted by sight and included in the invertebrate abundance data. Invertebrate herbivore abundance data were not collected in 2013. A mixed-model ANCOVA was used to determine the efficacy of the invertebrate removal treatment by examining the proportion of invertebrate herbivores removed, with –I as a fixed factor, –V and + NPK as covariates. The invertebrate removal treatment removed an average of 73.6% of invertebrate herbivores from 2009–2012 compared to the plots where invertebrates were not removed; the efficacy of the –I treatment did not vary with the NPK or vertebrate herbivore removal treatments (Supplementary material Appendix 1–2).

Data collection

To determine the effects of herbivore removals and nutrient availability on grassland diversity and productivity, plant species composition and end-of-season aboveground biomass were sampled. Plant species composition was measured within a permanent 1 m^2 subunit within each experimental plot twice per growing season, once at the beginning (late May) to determine the abundance of early season forbs and C_3 grasses and once at the end (late August) to determine the abundance of late season forbs and C_4 grasses. Percent aerial cover was determined for each species to the nearest 1%. Additionally, plant species richness and evenness were determined for each plot based on maximum cover values for each species (Magurran 2004). Evenness was calculated as follows:

$$J' = \frac{-\sum p_i \ln p_i}{\ln S}$$

where p_i is the proportional abundance of species i in the plot and S is the total number of species.

Aboveground standing crop was sampled once per growing season, at peak biomass (late August). Two 0.1 m^2 (20 × 50 cm) quadrats were sampled in each plot by clip-

ping all aboveground biomass at ground level. The location of the quadrats was moved each year to prevent resampling. Current year's biomass (live and recently senesced material) was separated from previous year's dead material (litter). Current year's biomass was separated to functional group (grass, forb, woody). All biomass was dried at 60°C and weighed.

Statistical analysis

Repeated measures analyses of variance (rmANOVAs) were used to evaluate the abundance responses of the two dominant functional types (grasses and forbs) and the responses of two measures of plant diversity (richness and evenness), with the vertebrate removal, invertebrate removal, and nutrient addition treatments as fixed effects and year as the repeated effect. Satterthwaite degrees of freedom were used to correct for unequal variances among treatments (Satterthwaite 1946).

A distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was used to test for variation in community composition between treatments. The PERMANOVA was performed on a Bray–Curtis resemblance matrix of plant species abundances, with the vertebrate removal, invertebrate removal and nutrient addition treatments and year as fixed factors. Differences in community composition between treatments were visualized using multidimensional scaling (MDS), and a similarity percentage (SIMPER) analysis was used to determine the species that best explained the differences in community composition among treatments. Additionally, a permutational analysis of multivariate dispersion (PERMDISP) was used to test for variation in plant community structure within a treatment. The PERMDISP was performed on a Bray–Curtis resemblance matrix of plant species abundances, with the vertebrate removal, invertebrate removal, and nutrient addition treatments analyzed in separate PERMDISP tests (i.e. no interactions were able to be identified).

Finally, the biomass responses (total aboveground biomass, graminoid biomass, and forb biomass) were determined using rmANOVAs, with the vertebrate removal, invertebrate removal, and nutrient addition treatments as fixed effects and year as the repeated effect.

The PERMANOVA, PERMDISP and SIMPER analyses were performed in PRIMER ver. 6.1.13. All other analyses were performed in SAS ver. 9.3.

Results

Plant diversity and community composition

Nutrient additions alone decreased plant species richness by 2.4 and increased plant species evenness, but had no effect on the grass:forb ratio (Supplementary material Appendix 3, Table 1). The relative abundance of forbs increased by 63.1% with invertebrate herbivore removals, resulting in a decrease in the grass:forb ratio (Fig. 1, Table 1). This shift in species abundances resulted in an increase in evenness with invertebrate herbivore removals (Fig. 1, Table 1). However, plant species richness was not significantly affected by vertebrate or invertebrate herbivore removals or the interaction of these

Table 1. F-statistics from a repeated measures ANOVA (rmANOVA) examining the effects of nutrient additions (+ NPK) and invertebrate and small vertebrate herbivore removals (–I and –V, respectively) on the relative abundances of two dominant functional plant types (grass, forb), the grass:forb ratio (G:F), and two measures of diversity (richness, evenness). Degrees of freedom are shown in parentheses. Bold values indicate significant effects ($p < 0.05$).

	Grass	Forb	G:F	Richness	Evenness
year	**6.47 (4,59)**	**5.01 (4,62)**	**3.76 (4,60)**	**5.12 (4,54)**	**4.50 (4,52)**
+ NPK	**8.47 (1,23)**	**9.30 (1,24)**	1.66 (1,20)	**12.34 (1,22)**	**4.70 (1,22)**
year× + NPK	1.44 (4,59)	2.49 (4,62)	2.40 (4,60)	0.73 (4,54)	1.96 (4,52)
–I	**8.02 (1,23)**	**9.25 (1,24)**	**6.59 (1,20)**	2.04 (1,22)	**7.00 (1,22)**
year×–I	0.72 (4,59)	0.77 (4,62)	0.85 (4,60)	0.64 (4,54)	2.37 (4,52)
+ NPK×–I	0.79 (1,23)	1.63 (1,24)	0.08 (1,20)	0.17 (1,22)	0.10 (1,22)
year× + NPK×–I	0.70 (4,59)	0.74 (4,62)	0.87 (4,60)	0.43 (4,54)	0.15 (4,52)
–V	0.54 (1,23)	0.50 (1,24)	0.23 (1,20)	0.56 (1,22)	1.12 (1,22)
year×–V	1.92 (4,59)	1.62 (4,62)	0.17 (4,60)	0.50 (4,54)	0.82 (4,52)
+ NPK×–V	0.12 (1,23)	0.36 (1,24)	0.02 (1,20)	0.21 (1,22)	0.08 (1,22)
year× + NPK×–V	1.72 (4,59)	0.81 (4,62)	1.23 (4,60)	0.47 (4,54)	0.12 (4,52)
–I×–V	0.07 (1,23)	0.12 (1,24)	0.01 (1,20)	1.41 (1,22)	0.31 (1,22)
year×–I×–V	0.81 (4,59)	1.05 (4,62)	0.51 (4,60)	0.75 (4,54)	1.98 (4,52)
+ NPK×–I×–V	0.17 (1,23)	0.02 (1,24)	0.00 (1,20)	0.07 (1,22)	0.03 (1,22)
year× + NPK×–I×–V	1.48 (4,59)	1.53 (4,62)	1.76 (4,60)	0.37 (4,54)	0.78 (4,52)

treatments with nutrient additions over the five year span of the study (Supplementary material Appendix 3, Table 1).

Community dissimilarity, based on shifts in treatment centroids in MDS space, was not affected by either vertebrate or invertebrate herbivore removals alone, though community composition was significantly different with NPK additions (Fig. 2, Supplementary material Appendix 4). However, dispersion within a treatment was significantly higher in the –I treatment (Fig. 2, Supplementary material Appendix 5). Based on SIMPER analyses, nutrient additions resulted in a doubling in the abundance of one dominant grass (*A. gerardii*), as well as an increase in two subdominant grasses (*Panicum virgatum* and *Eragrostis spectabilis*) and one subdominant forb (*Aster ericoides*; Table 2a). In contrast, invertebrate herbivore removals resulted in an increase in the abundance of several subdominant species, an effect that was particularly apparent in the combined + NPK,–I treatment (Table 2b–c). Notably, the abundance of the dominant grass did not increase as dramatically in the combined + NPK,–I treatment as it did in the + NPK only treatment. The change in community composition with the + NPK,–I treatment was primarily due to an increase in the abundance of two subdominant grass species (*P. virgatum* and *Sporobolus heterolepis*) and two subdominant forb species (*Salvia azurea* and *Asclepias verticillata*) (Table 2c), with each of these species increasing in abundance in some + NPK and –I treatment replicates, but not others, resulting in the increase in dispersion with invertebrate herbivore removals (Supplementary material Appendix 5).
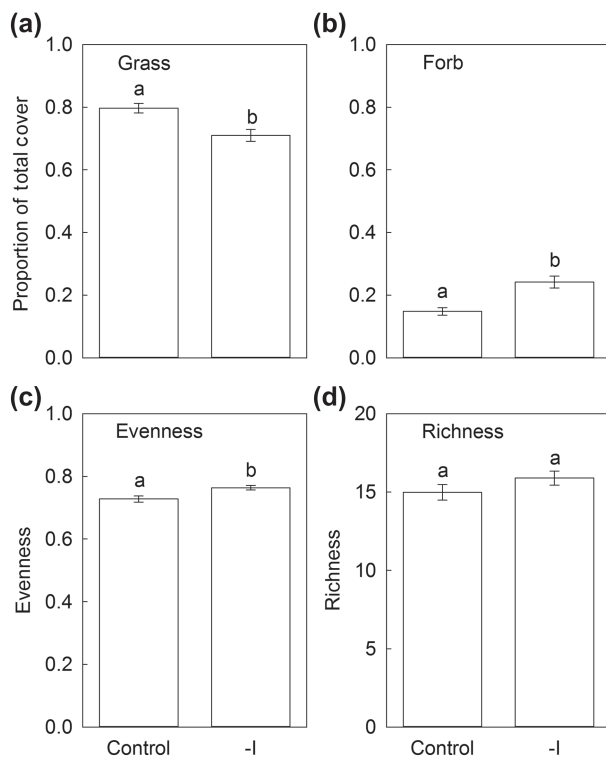

Figure 1. The effects of invertebrate herbivore removals (–I) on the proportion of total plant cover made up by grasses and forbs (a and b, respectively) and species evenness and richness (c and d, respectively). Shown are means ± standard errors across all other treatments and years. Letters indicate significant differences at $p \le 0.05$.
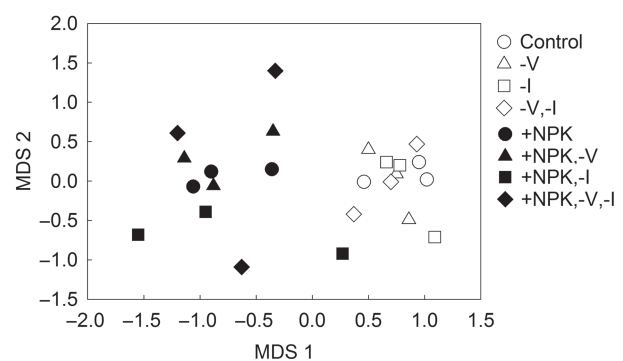

Figure 2. MDS plot of plant community dissimilarity with nutrient additions (+ NPK), vertebrate herbivore removals (–V), and invertebrate herbivore removals (–I) after five years of treatments (2013).

Table 2. The percent contribution (% contrib.) and relative abundances of the species contributing to between treatment dissimilarity in community composition: (a) control versus NPK addition; (b) control versus invertebrate removal; and (c) control versus NPK addition and invertebrate removal combined. Bold indicates the species that increased in abundance in each treatment. Average dissimilarity = 37.24.

Species	Functional type	% Contrib.	Control abund.	+ NPK abund.
(a) Average dissimilarity = 63.43				
Schizachyrium scoparium	C_4 grass	28.93	68.33	1.67
Panicum virgatum	**C_4 grass**	**20.29**	**6.33**	**52.67**
Andropogon gerardii	**C_4 grass**	**14.59**	**33.33**	**66.67**
Ambrosia psilostachya	**C_3 forb**	**6.26**	**1.33**	**15.67**
Sporobolus heterolepis	C_4 grass	3.21	6.67	2.67
Eragrostis spectabilis	**C_3 grass**	**2.18**	**0.00**	**5.00**
Aster ericoides	**C_3 forb**	**2.18**	**1.00**	**4.67**
Salvia azurea	C_3 forb	2.11	5.00	0.00
Sorghastrum nutans	C_4 grass	1.97	7.00	2.67
Muhlenbergia racemosa	C_4 grass	1.95	4.33	0.00
Bouteloua curtipendula	C_4 grass	1.84	11.67	11.67
Aster oblongifolius	C_3 forb	1.69	4.00	0.00
Dichanthelium oligosanthes	C_3 grass	1.64	6.00	5.33
Sporobolus asper	C_4 grass	1.54	2.67	2.33
(b) Average dissimilarity = 37.24				
Sporobolus heterolepis	**C_4 grass**	**13.95**	**6.67**	**16.67**
Schizachyrium scoparium	C_4 grass	13.24	68.33	56.67
Bouteloua curtipendula	**C_4 grass**	**7.83**	**11.67**	**18.33**
Amorpha canescens	**legume**	**7.10**	**5.33**	**13.67**
Andropogon gerardii	C_4 grass	6.97	33.33	32.67
Aster oblongifolius	**C_3 forb**	**5.37**	**4.00**	**6.67**
Aster ericoides	**C_3 forb**	**4.34**	**1.00**	**7.00**
Panicum virgatum	C_4 grass	4.13	6.33	5.00
Sorghastrum nutans	C_4 grass	3.91	7.00	7.00
Salvia azurea	C_3 forb	3.82	5.00	3.33
Carex meadii	**C_3 grass**	**3.80**	**3.33**	**8.00**
Muhlenbergia racemosa	C_4 grass	3.19	4.33	0.00
Sporobolus asper	**C_4 grass**	**2.96**	**2.67**	**3.67**
Solidago missouriensis	**C_3 forb**	**2.52**	**0.33**	**3.67**
Dichanthelium oligosanthes	C_3 grass	2.29	6.00	5.00
Asclepias verticillata	**C_3 forb**	**1.98**	**0.67**	**2.67**
Physalis pumila	**C_3 forb**	**1.92**	**1.33**	**2.33**
Kuhnia eupatorioides	C_3 forb	1.86	2.67	0.00
(C) Average dissimilarity = 66.37				
Schizachyrium scoparium	C_4 grass	21.24	68.33	8.33
Panicum virgatum	**C_4 grass**	**20.61**	**6.33**	**60.67**
Salvia azurea	**C_3 forb**	**11.68**	**5.00**	**36.67**
Asclepias verticillata	**C_3 forb**	**10.43**	**0.67**	**31.33**
Sporobolus heterolepis	**C_4 grass**	**7.11**	**6.67**	**23.33**
Bouteloua curtipendula	**C_4 grass**	**3.78**	**11.67**	**14.00**
Andropogon gerardii	**C_4 grass**	**3.01**	**33.33**	**40.00**
Sorghastrum nutans	C_4 grass	2.30	7.00	0.67
Amorpha canescens	**legume**	**2.25**	**5.33**	**7.33**
Ambrosia psilostachya	**C_3 forb**	**2.19**	**1.33**	**7.67**
Dichanthelium oligosanthes	C_3 grass	1.76	6.00	2.33
Muhlenbergia racemosa	C_4 grass	1.57	4.33	0.00
Physalis pumila	**C_3 forb**	**1.53**	**1.33**	**4.00**
Aster ericoides	**C_3 forb**	**1.49**	**1.00**	**4.00**

Total aboveground biomass

Total aboveground biomass increased with nutrient additions in all years except 2012 (Fig. 3a, Table 3). A significant year × + NPK × –I effect was observed for forb biomass (Fig. 3b, Table 3), with the combined nutrient addition and invertebrate herbivore removal treatment resulting in an increase in forb biomass in the final three years of the experiment (2011–2013). A significant year by invertebrate removal effect on graminoid biomass was observed (Fig. 3c, Table 3), with graminoid biomass decreasing with invertebrate herbivore removals in 2013 only. Finally, a significant year by nutrient addition effect on graminoid biomass was observed, with graminoid biomass increasing with nutrient additions in all years except 2012 (Fig. 3c, Table 3). In no case did the removal of small vertebrate herbivores alone affect aboveground biomass (Fig. 3, Table 3).
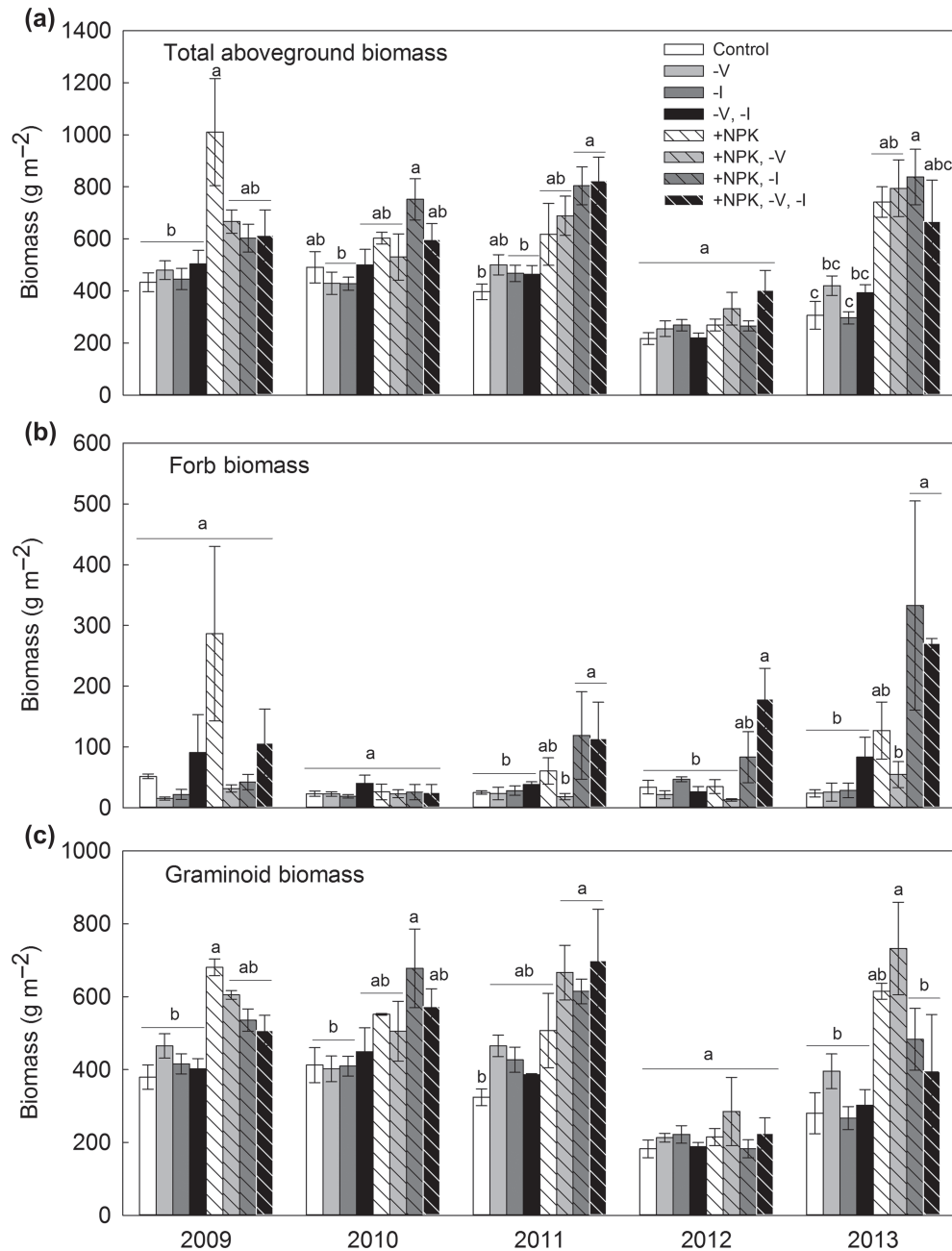
Figure 3. Interactive effects of nutrient additions (+ NPK), small vertebrate herbivore removals (−V), and invertebrate herbivore removals (−I) on (a) total aboveground biomass, (b) forb biomass, and (c) graminoid biomass over five years (2009–2013) in a tallgrass prairie ecosystem. Shown are means ± standard errors. Letters indicate significant differences among treatments within each year (p ≤ 0.05). Note the difference in scale among the three panels.

Discussion

The effects of nutrient additions on grassland community composition and aboveground biomass may be altered by interactions with herbivores (Hillebrand et al. 2007, Gruner et al. 2008). Additionally, vertebrate and invertebrate herbivores may differentially interact with the effects of nutrient additions due to differences in their physiology, behavior and spatial distribution (Huntly 1991, Throop and Lerdau 2004, Schmitz 2008b, Bakker et al. 2009, Hopcraft et al. 2010, Allan and Crawley 2011). Here we addressed these expecta-

tions by examining the independent effects of vertebrate and invertebrate herbivore removals and their interactions with nutrient additions in tallgrass prairie.

Invertebrate herbivore removals affected plant community composition in the tallgrass prairie system studied here. In particular, forbs increased in relative abundance with invertebrate herbivore removals, resulting in a decrease in the grass:forb ratio. Although it is well known that forb abundances in grasslands are limited by competition with grasses (Haag et al. 2004, Harpole and Tilman 2006), the decrease in the grass:forb ratio with invertebrate herbivore

Table 3. F-values from a repeated measures analysis of variance (rmANOVA) examining the effects of nutrient additions (+ NPK), invertebrate herbivore removal (–I), and vertebrate herbivore removal (–V) and their interactions on total aboveground biomass, graminoid biomass, and forb biomass. Degrees of freedom are shown in parentheses. Bold text indicates a significant effect (p < 0.05).

	Total aboveground biomass	Graminoid biomass	Forb biomass
year	**27.29 (4,46)**	**38.40 (4,53)**	**6.29 (4,43)**
+ NPK	**123.26 (1,28)**	**54.61 (1,25)**	**10.79 (1,13)**
year× + NPK	**5.74 (4,46)**	**5.30 (4,53)**	**4.20 (4,43)**
–I	0.16 (1,28)	1.38 (1,25)	3.98 (1,13)
year×–I	2.26 (4,46)	**3.83 (4,53)**	**2.99 (4,43)**
+ NPK×–I	0.02 (1,28)	0.90 (1,25)	1.42 (1,13)
year× + NPK×–I	2.33 (4,46)	1.64 (3,53)	**2.63 (4,43)**
–V	0.00 (1,28)	1.17 (1,25)	0.31 (1,13)
year×–V	1.18 (4,46)	1.28 (3,53)	0.61 (3,43)
+ NPK×–V	3.60 (1,28)	0.24 (1,25)	0.99 (1,13)
year× + NPK×–V	1.72 (4,46)	0.90 (3,53)	1.70 (3,43)
–I×–V	0.00 (1,28)	2.46 (1,25)	2.93 (1,13)
year×–I×–V	1.40 (4,46)	0.61 (3,53)	2.26 (3,43)
+ NPK×–I×–V	0.11 (1,28)	0.02 (1,25)	0.60 (1,13)
year× + NPK×–I×–V	1.36 (4,46)	0.64 (3,53)	1.12 (3,43)

removals observed here suggests that high levels of invertebrate herbivory may be suppressing forb abundances as well. It is interesting to note that the plant community responses to invertebrate herbivore removals we observed are opposite the responses to large ungulate herbivore exclusion observed in the same system (Knapp et al. 1998, Koerner et al. 2014). The higher level of invertebrate herbivory on forbs relative to grasses is interesting and deserves further study. One possible explanation for the high level of invertebrate herbivory on forbs includes the higher nutritional quality or lower structural defenses (i.e. lower leaf toughness) of forb tissue. Indeed, the common forb species at the study site do have lower C:N ratios and leaf toughness than the common grass species (La Pierre unpubl.). Alternatively, grasses may be better able to tolerate herbivory or compensate for tissue lost to herbivores than forbs (Belsky 1986, Georgiadis et al. 1989, Noy-Meir 1993, Tscharntke and Greiler 1995). Additionally, differences in the abundances of specialist forb-feeding invertebrates (as opposed to grass-feeding or generalist invertebrates) that are able to tolerate or sequester the defensive chemicals produced by many forb species may have driven the observed effects of invertebrate herbivore removals on plant biomass and community composition (Pieper and Beck 1980, Tscharntke and Greiler 1995, Craine et al. 2002, Throop and Lerdau 2004).

The decrease in the grass:forb ratio in response to invertebrate herbivore removal shown here resulted in an increase in plant species evenness. This shift in plant species evenness in response to invertebrate herbivore removals subsequently affected grass and forb biomass. Invertebrate herbivore removals resulted in a decrease in graminoid biomass and an increase in forb biomass in the fifth year of treatments (2013). However, invertebrate herbivore removals did not have an effect on total aboveground biomass in this study. This lack of a total biomass response may have been due to a tradeoff between decreased graminoid biomass and increased forb biomass with invertebrate herbivore removals in 2013,

as evidenced by shifts in the relative abundances of each of these functional types. Thus, invertebrate herbivores do not appear to influence total aboveground biomass, a key ecosystem function. However, invertebrate herbivores may affect other important aspects of biomass production, such as plant tissue quality, by altering the functional composition of tallgrass communities. Effects of invertebrate herbivores on plant nutritional composition, due to differences in the C:N:P and structural and chemical defenses between grasses and forbs, may have important consequences for rangeland forage quality (Pieper and Beck 1980, Whiles and Charlton 2006, Augustine et al. 2010).

As in previous nutrient addition studies (Suding et al. 2005), the + NPK treatment here resulted in a shift in the plant community, primarily driven by an increase in the abundance of the dominant grass species (*Andropogon gerardii*), and a corresponding decrease in plant species richness. However, invertebrate herbivores appear to have played a strong role in determining this response to nutrient additions. In the absence of invertebrate herbivores, *A. gerardii* did not greatly change in abundance with nutrient additions. Rather, a variety of subdominant grasses and forbs increased in their relative abundances with the combined + NPK,–I treatment. Thus, it appears that the decrease in richness with nutrient additions may be due, in part, to the suppression of fast-growing, subdominant species by invertebrate herbivores. In the absence of invertebrate herbivores, these subdominant species are able to outcompete the dominant grasses and increase in abundance with nutrient additions.

Interestingly, plant community dispersion increased with invertebrate herbivore removals, primarily due to a differential increase in the relative abundance of four species (*P. virgatum*, *S. heterolepus*, *S. azurea* and *A. verticillata*) among replicate plots where invertebrate herbivores were removed. This effect was especially strong in the combined nutrient addition and invertebrate herbivore removal plots, and appeared to have been driven by slight differences in the initial abundances of these rare species within each plot at the beginning of the experiment. These results suggest that invertebrate herbivores are at least partially responsible for the relative homogeneity and high levels of dominance within tallgrass prairie.

Although invertebrate herbivore removals did not play a strong role in determining aboveground biomass under ambient nutrient conditions, invertebrate herbivore removal did appear as a factor in determining forb biomass in tallgrass prairie when nutrients were added, an effect that emerged after several years of nutrient additions. Nutrient additions are known to alter forage quality and quantity, thereby resulting in shifts in invertebrate feeding pressure (Throop and Lerdau 2004, Schmitz 2008b). This type of selective feeding by invertebrate herbivores (Behmer and Joern 1993, Coupe and Cahill 2003, Schmitz 2008a) may have limited the increase in forb biomass in nutrient addition plots in this experiment. However, with the release from herbivory with invertebrate herbivore removals, forb species dramatically increased in biomass after two years of nutrient additions (2011–2013). Interestingly, these changes in forb biomass with the combined nutrient addition and invertebrate herbivore removal treatments did not affect total aboveground biomass, again due to a tradeoff between graminoid biomass

decreases and forb biomass increases. The two year lag in the response of forb biomass to the combined effects of invertebrate removals and nutrient additions may have been due to the time necessary for the long-lived plant species that dominate under ambient conditions to decrease in abundance enough to allow the subdominant forb species to increase in biomass. Similar lagged responses of primary production and community composition to shifts in an environmental driver have been observed in other systems as well (Jobbágy and Sala 2000, Howe et al. 2006, Olofsson et al. 2007, Gruner et al. 2008).

Small vertebrate herbivore removals alone had no effect on plant community composition or aboveground biomass. Because this study was conducted in an area where deer are the largest vertebrate herbivore and are present in low abundances, the vertebrate removal treatment targeted primarily small rodents. Although previous studies have shown an effect of large vertebrate herbivores on productivity and community composition (Knapp et al. 1998, Borer et al. 2005, Bakker et al. 2006), other studies have shown that small vertebrate herbivores have minimal effects in tallgrass prairie (Gibson et al. 1990, Kaufman and Bixler 1995, Whiles and Charlton 2006; but see Howe et al. 2002, 2006) as well as in other grassland systems (Blue et al. 2011). Small vertebrate herbivores are relatively common in this study system, and their abundances do no vary significantly between burned and unburned years (Kaufman and Kaufman 1997, Kaufman et al. 1998). However, the lack of an effect of small vertebrate herbivores on the plant community in tallgrass prairie may, in part, be due to the patchy spatial distribution of small vertebrate herbivores in this system, which likely exceeds the scale at which this study was conducted (4 m² exclosures; Gibson et al. 1990).

In summary, this study suggests that invertebrate herbivores can play a large role in determining plant community composition and grass:forb ratios under natural and altered nutrient conditions. As global change drivers, including eutrophication of natural systems, increase in intensity in the future, the role that invertebrate herbivores play in driving community and ecosystem processes in tallgrass prairie will likely strengthen. As a result, it is important to continue to examine the role that invertebrate herbivores may play in many ecosystems, as well as to identify global change drivers that may alter both nutrient availability and invertebrate herbivore abundance and behavior in tandem, thus resulting in shifts in plant community composition and productivity.

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Supplementary material (available online as Appendix oik.01869 at < www.oikosjournal.org/readers/appendix >). Appendix 1–5.