

# Positive interactions between large herbivores and grasshoppers, and their consequences for grassland plant diversity

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**Abstract.** Although the influence of positive interactions on plant and sessile communities has been well documented, surprisingly little is known about their role in structuring terrestrial animal communities. We evaluated beneficial interactions between two distantly related herbivore taxa, large vertebrate grazers (sheep) and smaller insect grazers (grasshoppers), using a set of field experiments in eastern Eurasian steppe of China. Grazing by large herbivores caused significantly higher grasshopper density, and this pattern persisted until the end of the experiment. Grasshoppers, in turn, increased the foraging time of larger herbivores, but such response occurred only during the peak of growing season (August). These reciprocal interactions were driven by differential herbivore foraging preferences for plant resources; namely, large herbivores preferred *Artemisia* forbs, whereas grasshoppers preferred *Leymus* grass. The enhancement of grasshopper density in areas grazed by large herbivores likely resulted from the selective consumption of *Artemisia* forbs by vertebrate grazers, which may potentially improve the host finding of grasshoppers. Likewise, grasshoppers appeared to benefit large herbivores by decreasing the cover and density of the dominant grass *Leymus chinensis*, which hampers large herbivores' access to palatable forbs. Moreover, we found that large herbivores grazing alone may significantly decrease plant diversity, yet grasshoppers appeared to mediate such negative effects when they grazed with large herbivores. Our results suggest that the positive, reciprocal interactions in terrestrial herbivore communities may be more prevalent and complex than previously thought.

**Key words:** compensatory effects; dietary selectivity; grasshoppers; grassland; indirect interactions between herbivore species; large herbivores; plant-associational defense; plant diversity; positive interactions.

## INTRODUCTION

In studies of herbivores, empirical and theoretical work has mostly emphasized the prevalence of negative, competitive interactions between herbivore species for shared plant resources (Connell 1983, Belovsky 1984, Gómez and González-Megías 2002). Yet, positive interactions between herbivores, like those documented in Serengeti ungulate communities (Vesey-FitzGerald 1960, McNaughton 1976, Jarman and Sinclair 1979), may also be essential for maintaining community structure and ecological functioning (Bruno et al. 2003, Brooker et al. 2007, Gross 2008). Indeed, there is increasing awareness that many interactions among herbivore species may be positive rather than purely negative (Ohgushi 2005, Odadi et al. 2011, Karban et al. 2012). Nevertheless, understanding of the mechanisms leading to such interactions in animal communities remains incomplete.

Positive interactions among herbivores exist between species of similar body sizes, such as between wild ungulates and cattle (Odadi et al. 2011), hares and geese (van der Wal et al. 2000), and moths and beetles (Utsumi and Ohgushi 2008). It still uncertain, however, whether and how positive interactions occur when there are large asymmetries in body size composition of herbivore communities (but see Kuijper et al. 2008, Cease et al. 2012, Barrio et al. 2013). Here, we report on how two groups of herbivores from widely different taxa with vastly different body sizes (sheep and grasshoppers) engaged in positive interactions mediated through their shared plant resources. We also explored the potential consequences of these interactions on grassland plant diversity.

In our study system, the perennial grass *Leymus chinensis* is the dominant plant species (Wang and Ba 2008), and *Artemisia* forbs (*A. scoparia*, *A. mongolica*, and *A. anethifolia*) are particularly abundant. The northeast fine-wool sheep and the generalist grasshoppers *Euchorthippus cheui* and *E. unicolor* are the dominant vertebrate and invertebrate herbivores in this native grassland. We tested the working hypothesis that

Manuscript received 8 June 2013; revised 23 September 2013; accepted 25 September 2013. Corresponding Editor: T. J. Valone.

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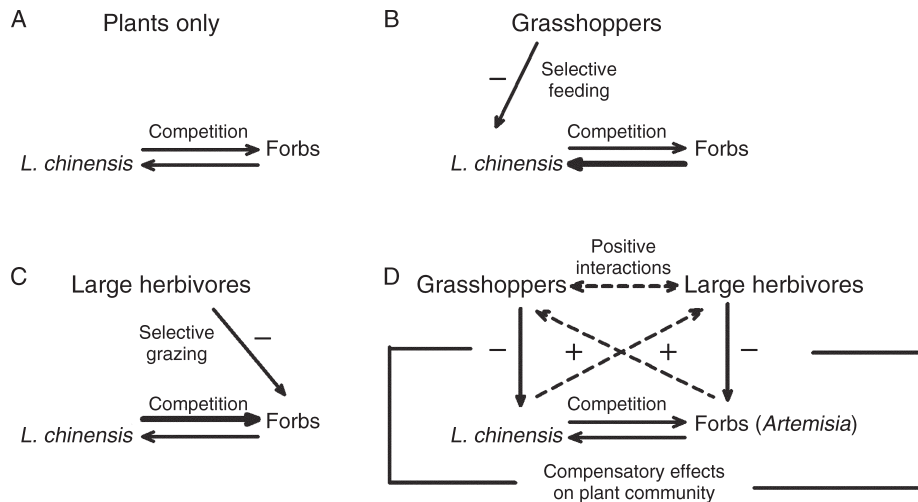


FIG. 1. The hypothesized mechanisms for underlying changes in plant diversity (the grass *Leymus chinensis*, forbs, and *Artemisia* forbs) consequent to the presence of large herbivores and grasshoppers, as well as their reciprocal interactions mediated by shared plant resources. The competitive interactions between plants are denoted by the two arrows (a thick arrow between plants indicates the more competitive plant group), the solid arrows between herbivores and plants indicate direct effects, and the dashed lines indicate indirect effects.

large herbivores might increase grasshopper density (*E. cheui* and *E. unicolor*) through the disruption of plant associational defense, whereas grasshoppers should benefit large herbivores' foraging activities by increasing the resource accessibility.

Our hypothesis was based on several independent observations that, when combined, are suggestive of such positive interactions. First, large herbivores mainly forage on *Artemisia* forbs (C. Feng, unpublished data), whereas grasshoppers prefer *L. chinensis* grass (Ren 2002). Second, *L. chinensis* and *Artemisia* often grow intermingled with one another in this grassland. Since *Artemisia* often contain secondary compounds that can act as defenses against insect herbivores (Welch and McArthur 1981, Karban and Myers 1989), we suggest that they might act as a protective neighbor and prevent *L. chinensis* from being fed upon by grasshoppers. The selective grazing of *Artemisia* by large herbivores, however, should disrupt this plant–plant interaction and potentially improve the host-finding efficiency of grasshoppers. Similarly, we predict that the tall and dense *L. chinensis* might hamper the ability of large herbivores to find their preferred food plants. Grasshopper feeding on *L. chinensis* should suppress the spatial cover and density of this grass, and thus, benefit large herbivores' foraging activities (Fig. 1).

As large herbivores and grasshoppers selectively consume different competing plant species, we expected that the herbivore manipulations should generate different effects on plant diversity as well. In the absence of both herbivore species, *L. chinensis* should dominate the field and coexist with other plant species (Fig. 1). In the presence of grasshoppers only, *L. chinensis* should be foraged on heavily, and thus, its abundance suppressed. Whether this will lead to higher plant diversity depends

on the foraging intensity or the density of grasshoppers. In the presence of large herbivores only, forb species should be foraged heavily, and thus, facilitate the dominant species *L. chinensis*, which, in turn, should lead to lower plant diversity. When both herbivore species co-occur, we expected that their vastly different plant preferences should cause their net effects on the plant community to balance, leading to a compensatory effect on plant composition (Fig. 1; Ritchie and Olff 1999).

## MATERIALS AND METHODS

### Study system and background

We conducted our research from July 2011 to September 2012 at the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, China (44°45' N, 123°45' E). This site is characterized by a semiarid continental monsoon, with cold, dry winters and warm, rainy summers. Annual mean temperature ranges from 4.6°C to 6.4°C, and annual precipitation is 280–400 mm with 70% falling in June–August. This native grassland is dominated by the perennial grass *L. chinensis* (Wang and Ba 2008). Other species include grasses such as *Phragmites australis*, *Calamagrostis epigejos*, *Hemarthria sibirica*, and *Chloris virgata*; and forbs such as *A. scoparia*, *A. mongolica*, *Potentilla flagellaris*, and *Carex duriuscula*. The dominant forbs *Artemisia* (*A. scoparia*, *A. mongolica*, and *A. anethifolia*) often grow interspersed with *L. chinensis* grass in this grassland. See Zhu et al. (2012) for details of the site characteristics.

The generalist grasshoppers *Euchorthippus cheui* and *E. unicolor* are the dominant herbivore insects, they have similar body sizes and dietary preferences, and annually

account for >65% of all insect individuals (Ren 2002). Northeast fine-wool sheep are the most important vertebrate herbivores, whose density or grazing pressure is controlled by human agrarian practices. Most of our experiments were conducted within three 25 × 25 m sheep-grazed paddocks. Each year, large-herbivore grazing period began in June and persisted to September in these paddocks; grazing activity lasted for three days in each month.

#### *Experimental design*

The first year of the study (2011) was devoted to assigning plot locations for the experiment and measuring initial conditions within each plot. In July 2011, each of the three 25 × 25 m large-herbivore-grazed paddocks was divided into two 12.5 × 25 m blocks (but with no fences in the middle of the paddocks). One of the blocks was randomly assigned to a “large-herbivore-grazed block” and the other was designated as the “large-herbivore enclosure” (i.e., large herbivores had free access to both types of blocks in 2011, while initial conditions were measured, but were excluded from the large-herbivore enclosure in 2012 to measure treatment effects). We designated two pairs of 2 × 2 m plots both inside and outside of the “enclosure” within each paddock, resulting in eight plots, four inside and four outside of the “enclosure.” We randomly assigned one of the paired plots as a grasshopper exclusion. Thus, we had four treatments in each set of two pairs of plots: (1) large herbivore only (L), (2) large herbivore + grasshopper (L + G), (3) grasshopper only (G), and (4) no herbivore (None). Each treatment was replicated twice in a paddock (see Appendix A: Fig. A1). (Note: we did not conduct any herbivore manipulations in 2011, so large herbivores and grasshoppers had free access to the four types of plots within the paddocks in this year.)

In August 2011, we measured the initial conditions in the None, G, L, and L + G plots. We measured total plant biomass, biomass and density of the dominant plant *L. chinensis*, biomass of other grasses, biomass of forbs, plant species richness, and light penetration within each plot. We used a GLZ-C-G PAR (photosynthetically active radiation) point sensor (Top Instrument, Zhejiang, China) to measure light penetration. We measured plant biomass using a nondestructive method. We first estimated the percentage of the plot area covered by each species, and then we estimated the area covered by 10 random samples of each plant species outside of the paddocks and clipped them, dried these at 60°C for 48 h, and weighed them to estimate plant species biomass per square meter. This mass was multiplied by the plot estimate of percent cover to estimate plant species biomass and total plant biomass in each plot (Schmitz 2008). We estimated plant species richness by counting the total number of plant species within each plot. We measured *L. chinensis* density by using five 0.2 × 0.2 m quadrats randomly located within each plot.

In early June 2012, we conducted herbivore manipulations within the paddocks. We first created large-herbivore enclosures by fencing the middle of each paddock, and enclosed plots within blocks with 2 × 2 × 1.5 m cages with aluminum window screen (5 × 5 mm mesh size). Then, we manipulated the presence or absence of grasshoppers in the cages according to their pre-assigned treatments (Appendix A: Fig. A1). For the None plots, we checked the cages weekly and eliminated any emerging grasshoppers within them. For the G plots, we measured grasshopper density weekly within each enclosure block, and then stocked grasshoppers to the cages with the current field density in the corresponding enclosure. For the L plots, we removed the cages from the paddocks during the three-day grazing period each month. After the grazing period, we replaced the cages on the plots and eliminated grasshoppers from the cages immediately. Although these plots were exposed to grasshoppers during the three days of large-herbivore grazing each month, the effects of grasshoppers on plants were minor: Only 3% of the plants exhibited damage from grasshoppers at the end of the experiment (Z. Zhong, unpublished data). We created the L + G plots in the similar way as L plots, and we measured grasshopper density weekly within each large-herbivore-grazed block, and then stocked that density of grasshoppers to the cages.

#### *Diet selection of large herbivores and grasshoppers*

In August 2012, we conducted a set of field experiments to provide information about the dietary preferences of large herbivores and grasshoppers in our grassland. We measured the grazing frequency of different vegetation groups (*L. chinensis*, other grasses, *Artemisia*, and other forbs) by large herbivores at the block scale. We laid out three 25-m linear transects in each grazed block, each transect consisted of 10 0.5 × 0.5 m quadrats spaced 2 m apart. Where vegetation was grazed, we assigned that quadrat a value of one for that vegetation group, otherwise zero. Values assigned for each vegetation group were summed per transect and divided by 10 to obtain a frequency of grazing use ranging from 0% to 100% (Clark et al. 2012).

On a sunny day in August 2012, five cylindrical cages (a diameter of 2.0 m and 1.5 m in height) wrapped with aluminum mesh were randomly installed outside the grazing paddocks, and then we surveyed grasshopper density in the field and stocked the corresponding number of grasshoppers into each cage. We randomly chose one of the grasshoppers and put an identifying red paint mark on its thorax and abdomen to help observers to see and relocate it. Five observers simultaneously monitored the feeding activities of the marked grasshoppers from each cage. We recorded the feeding time of grasshoppers on different plant species using a voice recorder (Lenovo B316+; Lenovo Group, Beijing, China), and all individuals were observed for eight hours continuously from 09:00 to 17:00 h.

*Effects of large herbivores on grasshopper density and plant cover*

From 25 June to 27 August in 2012, we monitored grasshopper density weekly in large-herbivore-grazed and enclosure blocks. In each block, two 25-m transects with 10 rings of 0.1-m<sup>2</sup> area were placed randomly and centered on the transects. Rings were left undisturbed for one day before the grasshopper sampling. We conducted the surveys on sunny days with minimal cloud cover and calm or no wind, we slowly walked along each transect and counted the number of grasshoppers in each ring (Joern 2004). The average density for the two transects at each block was used in statistical analyses.

In August 2012, we measured the cover of *Artemisia* and *L. chinensis* along the two 25-m linear transects in each of the block. The percent cover was estimated visually in the 0.5 × 0.5 m quadrat. The average cover for the two transects at each block was used in statistical analyses.

*Effects of Artemisia forbs on the feeding opportunity of grasshoppers*

In June 2012, 10 pairs of 2 × 2 m plots were placed in the field outside the experimental paddocks. We randomly selected one plot of each pair and removed *Artemisia* using lawn clippers, while the other plot served as the control. In late August 2012, we laid out a transect of 2 × 0.2 m that consisted of 10 subplots to assess the use frequency of grasshoppers on *L. chinensis* in each of the plot. We randomly counted 10 tillers of *L. chinensis* in all subplots of the transect and recorded how many of them had been grazed by grasshoppers (Veen et al. 2012). The average use frequency for the 10 subplots in each plot was used in statistical analyses.

*Effects of grasshoppers on the foraging behavior of large herbivores*

From June to August 2012, we used a hand-held computer to record the total number of visits and total grazing time by large herbivores in the L and L + G plots (Appendix A: Fig. A1). The observations were conducted twice daily (from 06:00 to 08:00 h and from 16:00 to 18:00 h), for a total of four hours each day. The observations lasted for three days in each month.

*Effects of herbivore manipulations on vegetation characteristics and understory light availability*

In August 2012, we evaluated the effects of herbivore manipulations on vegetation characteristics and understory light availability in the None, G, L, and L + G plots. We measured total plant biomass, biomass and density of the dominant plant *L. chinensis*, biomass of other grasses, biomass of forbs, plant species richness, and light penetration within each plot using the methods described in the second paragraph of *Experimental design*.

*Statistical analyses*

All data were assessed for normality, and if needed, normalized by log transformations and analyzed using SAS statistical package (SAS Institute 1997). We conducted Tukey's tests to evaluate the mean difference of large-herbivore grazing frequency on the vegetation groups in the grazed blocks. Using the same method, we evaluated the mean difference of grasshopper feeding time on the five grass species in the field experiments. We used repeated measures ANOVA to test the effects of large-herbivore grazing on grasshopper density in the blocks; results were reported using the Greenhouse-Geisser correction when Mauchly's test of sphericity was violated. We used paired-sample *t* tests to assess the effects of large-herbivore grazing on *L. chinensis* and *Artemisia* cover in the blocks. We also performed paired-sample *t* tests to evaluate the effects of *Artemisia* on the *L. chinensis* utilization by grasshoppers. Finally, we conducted a linear regression analysis to assess the effects of *Artemisia* cover on August grasshopper density in the six blocks.

Since each plot type (None, G, L, and L + G) was replicated two times in each paddock, we averaged all the variables of these two plots to obtain a single value for that plot type in each paddock, resulting in *n* = 3 for further analysis. For the foraging behavior of large herbivores (total number of visits per plot and total grazing time per plot) and the plant characteristics (cover and density of *L. chinensis*) in the L and L + G plots, we used paired-sample *t* tests to examine the effects of grasshoppers on these variables. We also performed linear regression analyses to assess the effects of *L. chinensis* cover and density on the total grazing time per plot by large herbivores.

We used a general linear mixed model procedure to test for effects of large herbivore, grasshopper, and large herbivore × grasshopper interaction on plant species richness and light penetration in the plots. For these two variables, we further conducted Tukey's tests to evaluate how treatment means differed.

RESULTS

*Diet selection of grasshoppers and large herbivores*

Grasshoppers only fed on five grass species: *L. chinensis*, *P. australis*, *C. epigeios*, *H. sibirica*, and *C. virgata*. The average feeding time of grasshoppers on the five grass species differed significantly ( $F_{4,20} = 20.429$ ,  $P < 0.001$ ), and grasshoppers particularly preferred the dominant grass *L. chinensis* (Fig. 2A).

In large-herbivore-grazed blocks, large herbivores appeared to selectively consume the dominant forbs *Artemisia*. The average grazing frequency of large herbivores on *L. chinensis*, other grasses, *Artemisia*, and other forbs was 3%, 10%, 37%, and 16%, respectively (Fig. 2B).

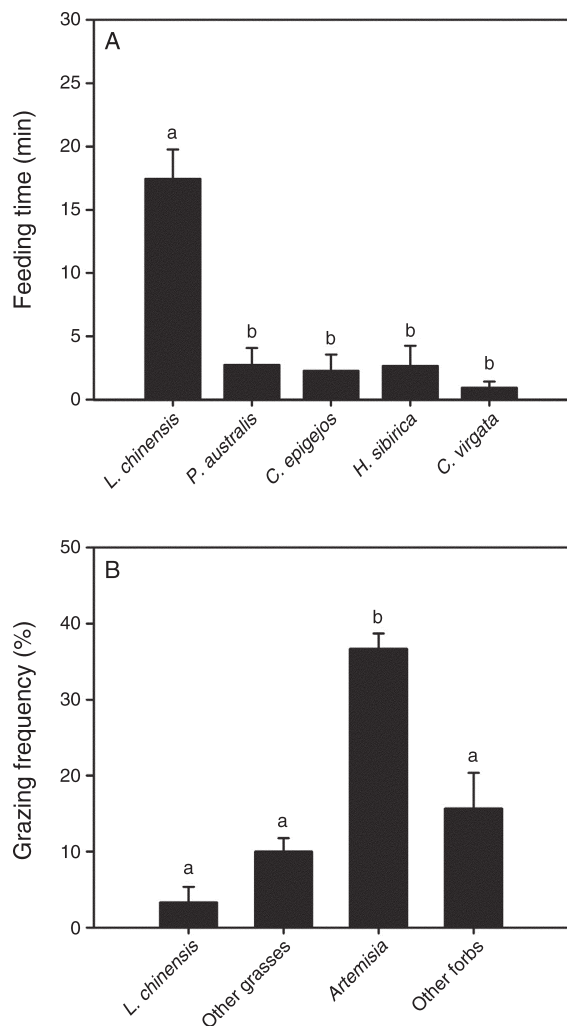


FIG. 2. Dietary preferences for grasshoppers (*Euchorthippus cheui* and *E. unicolor*) and large herbivores (northeast fine-wool sheep) in a set of field experiments in eastern Eurasian steppe of China. (A) The feeding time grasshoppers spent on different plant species during eight hours of field observation. Species include the grasses *Leymus chinensis*, *Phragmites australis*, *Calamagrostis epigejos*, *Hemarthria sibirica*, and *Chloris virgata*. (B) Grazing frequency by large herbivores on each vegetation group in the large-herbivore-grazed blocks. See *Diet selection of large herbivores and grasshoppers* for clarification of grazing frequency.

#### Effects of large herbivores on grasshopper density and plant cover

Grasshopper density was significantly affected by time (repeated measures ANOVA;  $F_{2,895,11.578} = 44.324$ ,  $P < 0.001$ , corrected using Greenhouse-Geisser estimates of sphericity) and time  $\times$  large herbivore grazing interaction (repeated measures ANOVA;  $F_{2,895,11.578} = 8.097$ ,  $P = 0.004$ , corrected using Greenhouse-Geisser estimates of sphericity). Large-herbivore-grazed blocks had significantly higher grasshopper density compared to large-herbivore exclosures (repeated measures ANOVA, test for between subject effects;  $F_{1,4} = 92.024$ ,  $P = 0.001$ ).

Grasshopper density showed a clear pattern over time, with a strong increase in the large-herbivore-grazed blocks, and a weak increase in the large-herbivore exclosures (Fig. 3).

*Artemisia* cover decreased significantly in large-herbivore-grazed blocks ( $t_2 = 5.000$ ,  $P = 0.038$ ), whereas there was no detectable difference in cover of *L. chinensis* grass between grazed and exclosure blocks (Fig. 4A). Additionally, a negative relationship was found between August grasshopper density and *Artemisia* cover in the six blocks ( $R^2 = 0.829$ ,  $F_{1,5} = 19.418$ ,  $P = 0.012$ ; Fig. 4B).

#### Effects of *Artemisia* forbs on the feeding opportunity of grasshoppers

The use frequency of *L. chinensis* by grasshoppers was twofold higher in the plots where *Artemisia* was removed ( $t_9 = -5.496$ ,  $P < 0.001$ ; Fig. 4C).

#### Effects of grasshoppers on the foraging behavior of large herbivores

In June, July, and August, the total number of visits per plot by large herbivores was not affected by the presence of grasshoppers (Fig. 5A). However, the total time large herbivores spent grazing significantly decreased in the plots without grasshoppers in August ( $t_2 = -5.303$ ,  $P = 0.034$ ); there was no evidence that grasshopper exclusion affected the total grazing time per plot in June and July (Fig. 5B).

#### Effects of herbivore manipulations on vegetation characteristics and understory light availability

In August 2011, initial conditions (vegetation characteristics and understory light availability) were not significantly different among the None, G, L, L + G plots (see Appendix B: Table B1). In August 2012, the cover and density of *L. chinensis* were significantly higher in the L than the L + G plots ( $t_2 = 37.000$ ,  $P =$

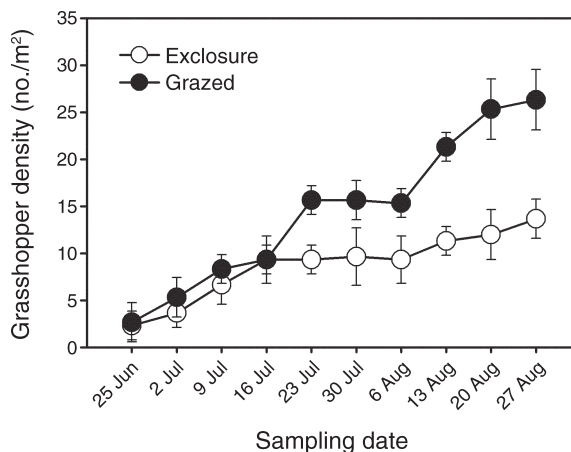


FIG. 3. Grasshopper density in the blocks with (grazed) and without (exclosures) large herbivores during the study. Error bars represent  $\pm$  SE.

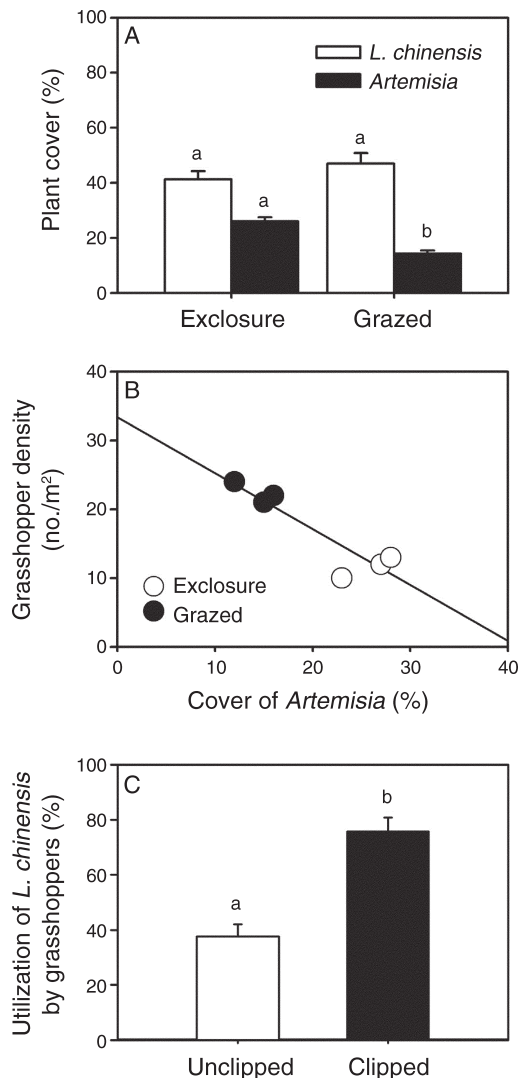


FIG. 4. (A) Percent cover of *L. chinensis* and *Artemisia* in the large-herbivore-grazed and exclosure blocks. (B) The relationship between August grasshopper density and *Artemisia* cover in the six  $12.5 \times 25$  blocks (three large-herbivore-grazed blocks and three large-herbivore exclosures), and (C) the utilization of dominant grass *L. chinensis* by grasshoppers in the plots where *Artemisia* was either clipped or unclipped. Different letters above the bars indicate significant differences at  $P < 0.05$ . Error bars represent  $\pm$  SE.

0.001 and  $t_2 = 5.000$ ,  $P = 0.038$ ; Fig. 6A, B), and the cover and density of *L. chinensis* were negatively related to the total grazing time per plot in August ( $R^2 = 0.806$ ,  $F_{1,5} = 16.590$ ,  $P = 0.015$  and  $R^2 = 0.724$ ,  $F_{1,5} = 10.469$ ,  $P = 0.032$ ; Fig. 6C, D). Plant species richness was significantly affected by large herbivore and large herbivore  $\times$  grasshopper interaction ( $F_{1,8} = 14.063$ ,  $P = 0.006$  and  $F_{1,8} = 10.562$ ,  $P = 0.012$ ), and the L plots had significantly lower plant species richness than the other three plot types ( $F_{3,8} = 9.896$ ,  $P = 0.005$ ; Fig. 7).

Light penetration was strongly affected by both large herbivores and grasshoppers and their interaction ( $F_{1,8} =$

32.794,  $P < 0.001$ ;  $F_{1,8} = 41.043$ ,  $P < 0.001$ ; and  $F_{1,8} = 18.167$ ,  $P = 0.003$  for the effects of large herbivore, grasshopper, and large herbivore  $\times$  grasshopper interaction, respectively). Additionally, Tukey's tests revealed that the L + G plots had dramatically higher light penetration than the other three plot types ( $F_{3,8} = 30.668$ ,  $P < 0.001$ ).

## DISCUSSION

Our study showed that large herbivores and herbivorous insects exerted a positive effect on each other mediated by shared plants. Only four weeks after the start of the experiment, grazing by large herbivores caused significantly higher grasshopper density, and this pattern persisted until the end of the experiment. The much smaller grasshoppers, in turn, increased the foraging time of larger herbivores, but such a response occurred only during the peak of the growing season (August). Moreover, we found that large herbivores grazed alone could significantly decrease plant diversity, but grasshoppers appeared to mediate such negative effects when they grazed with large herbivores.

### *The positive interactions between vertebrate and invertebrate herbivores*

Herbivore species may interact positively with each other in a variety of ways. One herbivore species can act

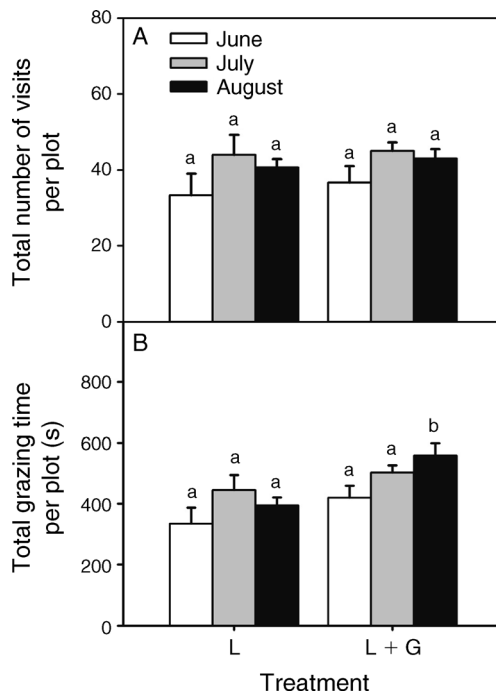


FIG. 5. The foraging behavior of large herbivores in June, July, and August. (A) The total number of visits per plot and (B) the total grazing time per plot by large herbivores in the large herbivore only (L) and the large herbivore + grasshopper (L + G) plots. Different letters above the bars indicate significant differences at  $P < 0.05$ . Error bars represent  $\pm$  SE.

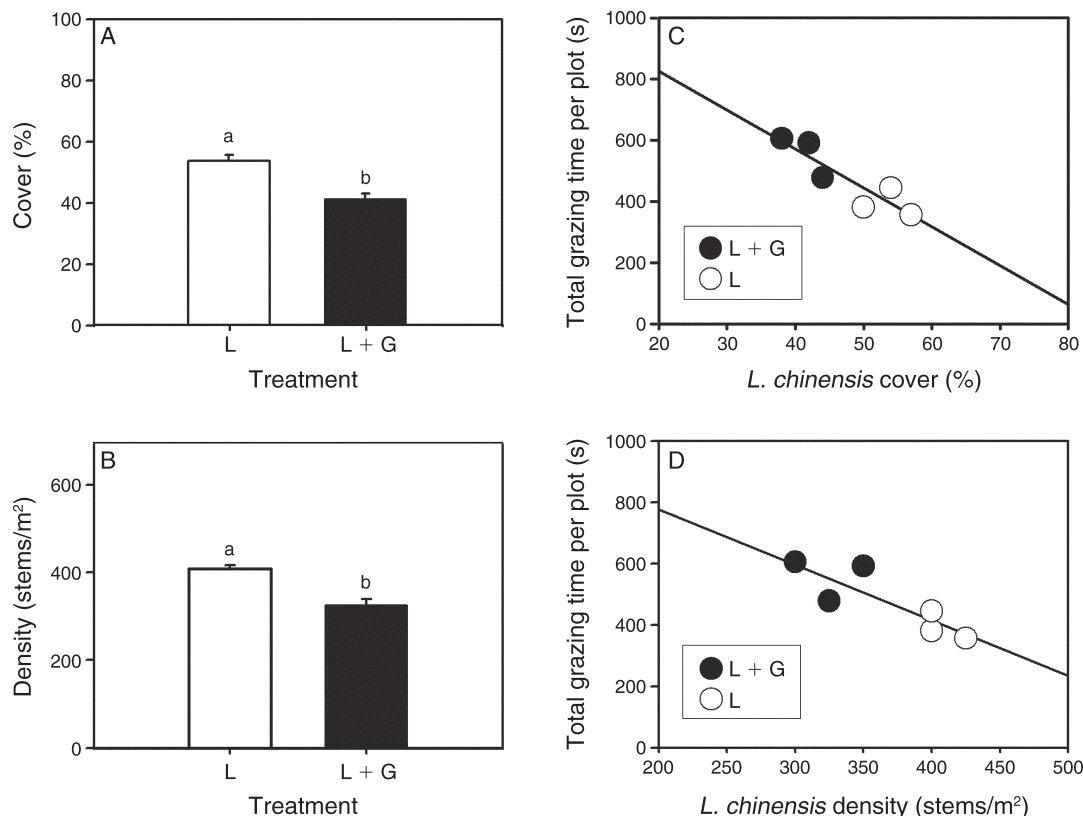


FIG. 6. The (A) cover and (B) density of *L. chinensis* in the L and L + G plots, and the relationships between (C) cover and (D) density of *L. chinensis* and total grazing time per plot in August by large herbivores. Different letters above the bars indicate significant differences at  $P < 0.05$ . Error bars represent  $\pm$  SE.

as an “ecological engineer” (Jones et al. 1997), changing the habitat structure for a second species by feeding or trampling activities. Herbivore grazing may also induce plant compensatory regrowth, enhancing the food quality or quantity for another species that shared the same resources (McNaughton 1976, Danell and Huss-Danell 1985, Olofsson and Stenbom 2000). Indeed, Zhu et al. (2012) found that large-herbivore grazing led to higher abundance of herbivorous insects in this grassland and attributed this positive effect to improved habitat conditions for grasshoppers and the compensatory regrowth of food plants following grazing. Yet, given the high plant diversity, as well as the moderate intensity of large-herbivore grazing in our study site, vertebrate grazers did not appear to change the habitat structure significantly (Z. Zhong, *field observations*). Experimentation showed that large-herbivore grazing caused compensatory regrowth of *L. chinensis* (Gao et al. 2008, Liu et al. 2012), the main food plant for grasshoppers. However, large herbivores seem unlikely to have large direct effects on *L. chinensis*, as they consumed only 3% of this grass (Fig. 2B). Therefore, large herbivores might not be able to induce compensatory regrowth of *L. chinensis* widely and further benefit grasshoppers in this grassland. Because our results do not support the “ecological engineers” or

compensatory regrowth hypotheses, we propose that our empirical evidence points to a new mechanism to explain the enhancement of grasshopper density in the presence of large herbivores.

We suggest that large herbivores may indirectly increase grasshopper density by breaking down the associational plant defense between grasses and forbs. The “repellent-plant hypothesis” states that a plant gains protection if associated with unpalatable plants, as herbivores avoid the generally low quality of vegetation (Atsatt and O’Dowd 1976). In our system, manipulative removal of *Artemisia* dramatically increased the likelihood that *L. chinensis* would be attacked by grasshoppers (Fig. 4C), indicating that an associational plant defense appears to emerge between them. We suggest that the relatively low grasshopper density in the blocks without large herbivores may be attributed to the high cover of *Artemisia* forbs (Fig. 4A). However, selective grazing of *Artemisia* by large herbivores indirectly disrupted this associational plant defense, making *L. chinensis* more susceptible to herbivory by grasshoppers, and thus boosted grasshopper density (Fig. 3). The negative relationship between grasshopper density and *Artemisia* cover within the blocks further supports our assertions (Fig. 4B).

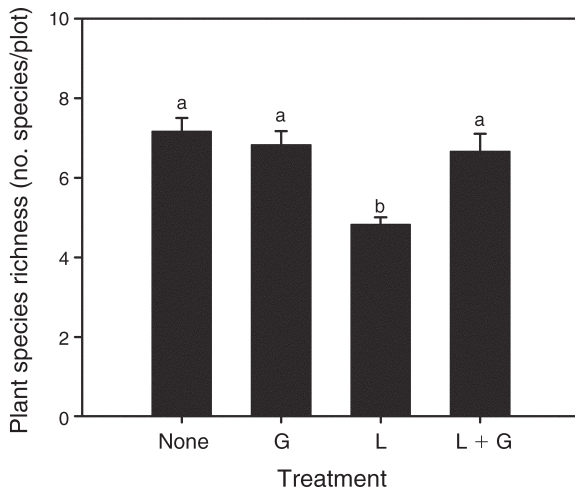


FIG. 7. Effects of different treatments on plant species richness in the  $2 \times 2$  m plots. Herbivory treatments were: large herbivore only (L), large herbivore + grasshopper (L + G), grasshopper only (G), and no herbivore (None). Letters show significantly different means between treatments, significant level was set at  $P < 0.05$ . Error bars represent  $\pm$  SE.

The effectiveness of plant associational defense has been well documented in a number of ecosystems (McNaughton 1978, Hjältén et al. 1993, Olf et al. 1999, Callaway et al. 2005, Barbosa et al. 2009, Wang et al. 2010a). But, plant associational defense in these systems involves only one herbivore species. In natural ecosystems, varieties of herbivore species often coexist with each other and share the same plant resources (Jarman and Sinclair 1979). Our study suggests how the associational defense ideas could be extended to multiple herbivore species in ways that could lead to interesting positive interactions. That is, the effectiveness of plant associational defense for one herbivore species may be modified indirectly by the selective grazing of another herbivore species, and thus facilitating the activities of the former one (also see Kuijper et al. 2008).

It is true that large mammals may alter foraging behavior in response to other ungulates (Bailey et al. 1996), as well as disturbances from other fauna and their impacts on vegetation (e.g., prairies dogs; Davidson et al. 2010), yet whether and how large herbivores alter foraging behavior in response to insect activity is still poorly understood (Clark et al. 2012). We found that herbivorous insects did influence the foraging behavior of large herbivores: Sheep tended to spend more time on grazing during the peak of the growing season (August) when they grazed with grasshoppers (Fig. 5B). This positive interaction is likely due to the selective feeding of *L. chinensis* by grasshoppers, which significantly decreased the cover and density of this dominant grass (Fig. 6A, B), and potentially facilitating large herbivores' ability to access the high-quality food items under grass canopy. In African savanna ecosystems, it is well known that foraging by one ungulate species may benefit

another through facilitating access to food resources (Arsenault and Owen-Smith 2002). For instance, Odadi et al. (2011) suggested that grazing by zebras decreased the cover of standing dead grass stems, indirectly making the nutritious food plants more accessible to cattle in a savanna ecosystem in Kenya. In our grassland, grasshoppers appeared to have a similar role to zebras, despite their small body size. We also noted that grasshoppers did not affect the foraging of large herbivores in the early growing season (June and July; Fig. 5B), which implies that the indirect effects of herbivorous insects on large herbivores may be density dependent.

The abundance of herbivorous insects often varies greatly both spatially and temporally in grassland ecosystems (Joern 2004), and they rapidly influence the vegetation characteristics only when their densities are relative high (Zhang et al. 2011). Likewise, foraging by large herbivores is sensitive to multiple factors such as plant quality (Stephen and Krebs 1986), plant diversity (Wang et al. 2010c), plant community composition (Callaway et al. 2005), and even plant spatial distributions (Wang et al. 2010b). To examine the indirect effects of herbivorous insects on large herbivores, we need to consider all these factors through careful manipulative field experiments. This may explain why so few studies have previously reported the behavior response of vertebrate herbivores to herbivorous insects (but see Strauss 1991, Barrio et al. 2013). Here, our study suggests that feeding by herbivorous insects may be an overlooked, but potentially important, factor influencing the foraging behavior of large herbivores.

#### *The effects of herbivore manipulations on plant diversity*

Herbivores can affect plant diversity by altering competitive interactions between species: They can promote diversity by feeding on dominant plants, or reduce diversity by consuming rarer species (see Fig. 1; Ritchie and Olf 1999, Allan and Crawley 2011). Yet, it should also be noted that the effects of herbivores on plant diversity may depend on the type of herbivore species (Olf and Ritchie 1998). Grazing by large mammals is recognized as an important factor in regulating the plant diversity of grasslands (Collins et al. 1998, Bakker et al. 2006), whereas insect herbivores are often thought to be less important (Crawley 1983). Indeed, our manipulative experiments show that large herbivores alone may significantly decrease the number of plant species. In this grassland, large herbivores generally consumed the dominant *Artemisia* forbs (Fig. 2B), and also particularly preferred the nitrogen-rich forb species such as *Cynanchum chinense*, *Melilotus officinalis*, and *Sonchus arvensis* in the understory (C. Feng, unpublished data). These forb species are relatively rare in the study sites, and they often recover slowly from large-herbivore damage (Z. Zhong and C. Feng, field observations). Given the considerably high food intake of large herbivores, the selective consumption by



vertebrate grazers appeared to be strong enough to suppress the abundance of these understory forb species, and further led to a lower number of plant species. However, our results show that grasshoppers failed to affect plant diversity significantly when they grazed alone. This was probably because grasshopper mainly preferred *L. chinensis* grass, the dominant plant species in the grassland. The high host plant biomass may potentially dilute grasshoppers' effects on the plant community, making their effects on plant diversity not obvious in the short term.

Interestingly, although large herbivores grazed alone may significantly decrease plant diversity, we found that grasshoppers appeared to alleviate such negative effects when they grazed with large herbivores. In an early review, Ritchie and Olff (1999) proposed that, if herbivore species feed on different plant functional groups, their effects on the plant community may balance each other and finally lead to a compensating effect on plant composition. Since grasshoppers selectively consumed the dominant grass *L. chinensis*, whereas large herbivores preferred the forb species in our system, feeding by grasshoppers appeared to balance the negative effects of large herbivores on plant diversity. Indeed, one may argue that the effects of smaller invertebrate insects on plant communities were not as strong as these vertebrate grazers, and hard to produce such buffering effects. Yet, the dramatically increased grasshopper density in the large-herbivore-grazed blocks appeared to strengthen their impacts on plant communities. The heavy feeding on the tall, dominant *L. chinensis* by these grasshoppers might allow more light to penetrate the soil surface and benefit the growth and expansion of understory species. Thus, feeding by grasshoppers has the potential to mitigate the negative effects of large herbivores, and finally lead to little or no net effect on the plant community.

### Conclusions

Our study suggests that the positive, reciprocal interactions in terrestrial herbivore communities may be more prevalent and complex than previously thought. The differential dietary preferences among herbivore species did not simply lessen competition, but actually caused positive interactions between them, implying dietary selectivity may be a potential mechanism to explain the species interactions in terrestrial herbivore communities. We also note that the interactions among herbivore species can modify their impacts on plant diversity, making their combined effects unpredictable from their individual effects. The present study of the interactions between herbivores and their impacts on plant communities only looked at a short timescale. Whether or not these positive interactions will be sustained and further lead to facilitation between these two herbivore species (such as increasing grasshopper performance and/or the mass gain of sheep) at a longer timescale deserves further attention.

### ACKNOWLEDGMENTS

We thank Y. Zhang and X. Li for help with the field work, O. J. Schmitz for revising the whole manuscript, and J. Miller and R. Buchkowski for suggestions on the draft of the manuscript. We also thank two anonymous reviewers for their helpful comments on the manuscript. This project was supported by National Natural Science Foundation of China (Numbers 31230012, 31070294, 31072070) and the State Agricultural Commonwealth Project (201003019).

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## SUPPLEMENTAL MATERIAL

### Appendix A

A figure showing the paddock arrangement and experimental layout ([Ecological Archives E095-089-A1](#)).

### Appendix B

A table showing the initial vegetation characteristics and understory light availability among the four types of plots in August 2011 ([Ecological Archives E095-089-A2](#)).