



Interactive effects of large herbivores and plant diversity on insect abundance in a meadow steppe in China



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ABSTRACT

The structure and dynamics of insect community in grasslands can be influenced by grazing management via altered characteristics of plant community. However, attempts to better understand the complex relationships among plants, insects, and large herbivores is still hampered largely by the interactive effects of plants, insects, and large grazers on each other. In this study, we test the hypothesis that the effect of large herbivores on insect abundance is grazer species-specific and pre-grazing plant diversity-dependent using an experiment with manipulating four grazing treatments (i.e., control, cattle, goats, and sheep) at low, intermediate, and high plant diversity levels in a meadow steppe at northeast in China. We show that grazing significantly increased the abundance of the entire insect community. The abundance of each insect order responded differently to grazing treatments, with higher abundance of Orthoptera and Homoptera under sheep grazing, enhanced abundance of Coleoptera and Diptera under cattle grazing, and reduced Hemiptera abundance, but greater abundance of Lepidoptera under goats grazing. Thus different treatments profoundly changed insect taxonomic composition. The six most dominant species (*Euchorthippus unicolor*, *Aelia nasuta*, *Trigonotylus ruficornis*, Curculionidae sp., *Coccinula quatuordecimpustulata*, and Cicadellidae sp.) responded differently to grazing by large herbivores, with either increased or decreased its abundance. The effects of grazing on insect abundance were driven by their differential responsive mechanisms for vegetation. More importantly, the effects of grazing on insect abundance at both order- and species-levels potentially depended on plant diversity levels of pre-grazing. Our results suggest that different herbivore species should be used in the background of different plant communities for better conservation of insect community in managed grassland.

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1. Introduction

Insects are a major but often under-appreciated component of terrestrial ecosystems (Belovsky and Slade, 2000; Bronstein et al., 2006; Matt and Charlton, 2006). However, growing evidence shows that insects are also experiencing local/regional species loss or even global extinction (Collinge, 2000), and that the diversity of insects apparently declines even more rapidly than that of vertebrates and plants (Thomas et al., 2004). Therefore, understanding critical factors that determine their diversity and species composition becomes an urgent task facing ecologists and conservation biologists. Although many studies have identified a

range of management factors that could contribute to changes in insect communities in grassland ecosystems (Kruess and Tscharrntke, 2002a; Batáry et al., 2010), there is a need to integrate these knowledge into predictive and adaptive ongoing management options.

Livestock grazing is a key management tool in grasslands, and its widespread prevalence has generated great interest in understanding its ecological effects, especially for insects. Previous studies show that grazing managements in grasslands can lead to either lower (Gonzalez-Megias et al., 2004; Joern, 2005; Littlewood, 2008), or higher insect abundance and richness (Cagnolo et al., 2002; Ryder et al., 2005; Debano, 2006; Rosa-García et al., 2009). Such conflicting results might partly derive from the difference in grazing intensity (Kruess and Tscharrntke, 2002b; Cease et al., 2012), grazing season (Fonderflick et al., 2014), and grazer species (Dolek and Geyer, 2002). Surprisingly, while most

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efforts have been devoted to the effects of grazing intensity on insect community, little attention has been given the effects of grazer species (Järegui et al., 2008). Different large herbivore species may alter vegetation features due to diet selection and body size, potentially influencing insect community, because grazing affect insect community through modifying plant communities (Kruess and Tscharrntke, 2002b; Jonas and Joern, 2007; Zhu et al., 2012; van Klink et al., 2015). Therefore, the resulting 'species-specific' of grazing management difficultly renders the development and application of general management principles for biological conservation.

Food resources for herbivores are heterogeneously distributed in most grasslands (Li and Reynolds, 1995; Wang et al., 2014), the responses of plant community to large herbivores strongly depend on the distribution of plant resources before grazing (Palmer et al., 2003; Bakker et al., 2006). The difference in plant species diversity is a typically important characteristic of heterogeneity, and diverse plant communities can markedly affect foraging strategies of large herbivores and their concomitant impacts on the dynamics of vegetation itself (Bergvall et al., 2006; Miller et al., 2007; Wang et al., 2011). Our previous work found that complex spatial neighborhood of several plant species makes the palatable species for herbivores to less selected, contributing to the maintenance of plant diversity (Wang et al., 2010). This 'plant species diversity disaffinity' may be an important factor influencing subsequent herbivores' impacts on plant community, which, in turn, consequently impacts insect community. Therefore, understanding the effects of grazing by large herbivores on insect community will require explicit knowledge of pre-grazing plant diversity levels.

Our previous experimental efforts at the same grassland mainly focus on the treatment effects on diversity. Zhu et al. (2012) showed that large herbivores strongly affected insect species richness by modifying plant structural heterogeneity, which reversed the positive relationship between plant and insect diversity. Zhong et al. (2014) found that the positive interactions between large herbivores and grasshoppers were driven by differential herbivore foraging preferences for plant resources that break down the associational plant defense between grasses and forbs. In contrast, the main goal of this study is to test the effects of different large herbivore species on insect abundance at different plant diversity levels. Although most extensive research on the effects of livestock grazing on grassland insect abundance has taken place, and has provided valuable insights (Littlewood, 2008; Cease et al., 2012), several important gaps in our knowledge remain. Because not all species are sensitive to grazing, and the use of taxonomic hierarchies such as order and species levels could be advantageous in biodiversity assessments (Williams and Gaston, 1994). Thus, there is a pressing need for studies that examine how insect community responds to livestock grazing at taxonomic composition. In this study, as part of a previous, targeted experiment to identify multiple species interactions (plants, insects, and large herbivores) under large herbivore grazing at different plant diversity levels, we examined that the responses of insect abundance at both order- and species-levels to the interactive effects of large herbivore grazing and plant diversity of pre-grazing. Specifically, we test the hypothesis that the effect of large herbivores on insect abundance is grazer species-specific and pre-grazing plant diversity-dependent using a field experiment in a meadow steppe at northeast in China.

2. Methods and materials

2.1. Study site

This study was conducted at the Grassland Ecological Research Station of Northeast Normal University, Jilin Province, P.R. China

(44°45'N, 123°45'E). The site is in a meadow steppe region where mean annual temperature and precipitation are from 4.6 °C to 6.4 °C, and 280–400 mm, respectively. Monthly mean temperature ranges from –16 °C in January to 25 °C in July, and ninety percent of the total precipitation is distributed from May to October. Annual potential evaporation is approximately three times as much as the mean annual precipitation. Soils are mixed saline and alkaline (pH 8.5–10.0). The dominant plant species in meadow steppe that lies in the eastern region of the Eurasian Steppe Zone is perennial grasses, *Leymus chinensis* (Wang and Ba, 2008; Gao et al., 2008). Other species include perennial and annual grasses such as *Phragmites australis*, *Calamagrostis epigejos* and *Chloris virgata*, legumes such as *Lespedeza davurica*, and forbs such as *Potentilla flagellaris*, and *Artemisia scoparia*.

2.2. Experimental design and grazing treatments

To test the interactive effects of large herbivore species and plant diversity of pre-grazing on insect community, three plant diversity levels and three single grazer species were selected. In this study, 9 blocks (each for 0.3 ha in size) with flat topography and similar soil type were established. The nine blocks investigated were classified into three plant diversity levels: low (4–5 species), intermediate (8–9 species), and high (15–17 species) based on vegetation investigation of pre-grazing, with three blocks (three replicates) for each plant diversity level. Each block for each plant diversity level was one plant community, and the plant community composition (species, and individual number of each plant species) that was similar with other blocks in same plant diversity level, and was different from the blocks in other plant diversity levels. Detailed descriptions of experimental plant diversity treatments of pre-grazing can be found in Zhu et al. (2012). Grazing treatments were nested within the block of each plant diversity treatment, and no grazing and three single grazing treatments with one grazer in each grazed plot were used. That is, each block was composed of four plots (each for 0.05 ha in size) enclosed with barbwire. Neighboring plots within each block were separated by 18–20 m. The four grazing treatments were assigned randomly to the four plots in each of the nine blocks as follows: (1) no livestock grazing (control); (2) grazing by cattle (a hybrid of native and yellow breed), and two cattle (221 ± 5.5 kg) per plot ($= 7.14$ sheep ha^{-1}); (3) grazing by goats (Liaoning Cashmere breed), and eight goats (34 ± 1.6 kg) per plot ($= 7.08$ sheep ha^{-1}); (4) grazing by sheep (small-tail Han breed), and eight sheep (33 ± 1.6 kg) per plot ($= 7.21$ sheep ha^{-1}).

Intermediate grazing intensity (50–65% of the available forage was removed) was used in all grazed plots. The similar grazing pressure (sheep unit per hectare) among cattle, goats, and sheep was gained by calculating the intake and body weight of each individual of the three grazers. The plots were grazed by livestock from the second week of July in 2007 and 2008 when new growth ensured sufficient forage for grazing. Grazing was conducted twice per day: from 06:00 to 08:00 AM and from 16:00 to 18:00 PM during their normal grazing time and was terminated when about 60% of the available forage was removed (about 10–15 d within each month). Large herbivores were not allowed to graze the plots that were visibly wet or following measurable rain (10 mm). Vegetation and insects were assessed after 1.5 years of grazing treatments.

2.3. Vegetation measurements

All plots were sampled in July, August, September, and October in 2008. Plant species richness, the individual number, height, and cover of each species were assessed within ten quadrats (0.25×0.25 m) arranged evenly along each of two 20 m cater-

corner transects in each plot. Structural heterogeneity was estimated as the coefficient of variation (CV) of plant height. Aboveground plant biomass was measured by collecting plant materials from five randomly located 0.5×0.5 m quadrats per plot. Plant samples were clipped to 1 cm above the soil surface with pruning shears. Plants samples were air forced oven-dried for 48 h at 80°C , and weighed. Some vegetation attributes, such as plant species richness, plant cover, plant height, plant structural heterogeneity, and plant biomass in this study were calculated in our previous paper (Zhu et al., 2012), and here we did not show these results. Plant diversity (Shannon–Wiener index) was calculated as $H = -\sum_1^s (P_i \times \ln P_i)$, where P_i is the proportion of individuals represented by species i , and s is the number of plant species.

2.4. Insect sampling and identification

Insect sampling was carried out four times from July to October in 2008. We followed sweep net survey method using a light muslin net (40 cm in diameter) along two 2-m wide and 25-m long parallel transects to estimate insect species richness and abundance (Evans et al., 1983; Schaffers et al., 2008). These transects were at least 2 m away from the plot boundary to minimize edge effect. On each transect 15 sweeps at 1 m interval were performed above ground level through the vegetation. Each sample consisted of 30 sweeps and two samples were carried out in each plot to ensure that those samples were representative of each sampling date. Insect specimens were collected under favorable monitoring conditions (sunny days with minimal cloud cover and calm or no wind), from 09:00 AM to 15:00 PM. In the experimental area, all grazed plots were visited on the same day and in random order in each sampling date. The contents of the sweep net were preserved in bottles containing ethyl acetate.

All collected individuals were sorted into 9 orders: Orthoptera, Hemiptera, Coleoptera, Diptera, Hymenoptera, Homoptera, Lepidoptera, Mantodea, and Neuroptera. And furthermore, all individuals were identified to species, and specimens that could not be identified to species were separated into recognizable taxonomic units. Nymphs were not considered due to problems of species identification (4.72% of overall samplings). Insect abundance, such as total insects, each order, and each species, was recorded as the accumulative abundance (excluding nymphs) throughout sampling periods in a given experimental year. The ratio in abundance of each insect species (all 147 species) was calculated. In this study, *Euchorthippus unicolor* (43.9%), *Cicadellidae* sp. (24.5%), *Curculionidae* sp. (2.4%), *Trigonotylus ruficornis* (1.5%), *Coccinula quatuordecimpustulata* (1.5%), *Aelia nasuta* (0.8%) were relatively dominant species.

2.5. Statistical analyses

Three-way ANOVAS with a split-plot design were carried out to test the main and interactive effects of block, plant diversity, and grazing by large herbivores on the plant Shannon–Wiener index and insect abundance in experimental year. General Linear Model (GLM) with a Tukey test was used to examine the statistical difference in the mean values of the treatments and specific comparisons between different groups of treatments were performed using LSMEANS statement of this procedure. Effects of blocks were tested together with the treatments in all the above analyses, but they were not discussed in this study due to insignificant effects on measured variables. Data were further analyzed the effects of grazing breed using one-way ANOVA within each plant diversity level. The relationships between plant Shannon–Wiener index and the abundance of total insects and

the six dominant species were analyzed by using linear regressions. These analyses were performed using SAS version 6.12 (SAS Institute Inc., 1989). Significant level was set at $P < 0.05$.

The relationship between abundance of each insect order and vegetation properties were analyzed by multivariate ordination redundancy analysis (RDA) using the program CANOCO 4.5 (ter Braak and Šmilauer, 1998). The RDA was applied considering treatment plots as samples, the abundance of insect orders as species, and plant species richness, the cover and height of plant community, variation in plant height, plant biomass (these vegetation data resulted from our previous results, see Zhu et al., 2012), and plant Shannon–Wiener index, as environmental variables. Forward selection with Monte Carlo permutations (999 unrestricted permutations) was used to assess the plant variables showing significant (inflation factor < 20 , $P < 0.05$) on the variation within the insect order abundance data. Only significant variables were included in the model, which was tested using an unrestricted Monte Carlo permutation test (999 permutations). Insect abundance data were log-transformed prior to analysis.

3. Results

3.1. Effects on plant Shannon–Wiener index

There were significant interactive effects of plant diversity levels of pre-grazing and large herbivores on the plant Shannon–Wiener index ($F_{6, 22} = 3.895$, $P = 0.007$), as well as plant diversity levels of pre-grazing ($F_{2, 22} = 123.71$, $P < 0.0001$) and grazing treatments ($F_{3, 22} = 25.783$, $P < 0.0001$). Plant Shannon–Wiener index was higher in grazed plots by cattle and sheep than that in control and grazed plots by goat at low plant diversity level. It was also higher in all grazed plots than in control plots at both intermediate and high plant diversity levels (Fig. 1).

3.2. Effects on insect abundance

Significant interactive effects of plant diversity and large herbivore species on total insect abundance were detected (Table 1). At low plant diversity level, total insect abundance was higher in grazed plots by cattle and sheep than that of control plots, and was higher in grazed plots by goats than that of other plots at intermediate plant diversity level, was higher in grazed plots by goats and sheep than that of non-grazed and cattle grazed plots at high plant diversity level (Table 1).

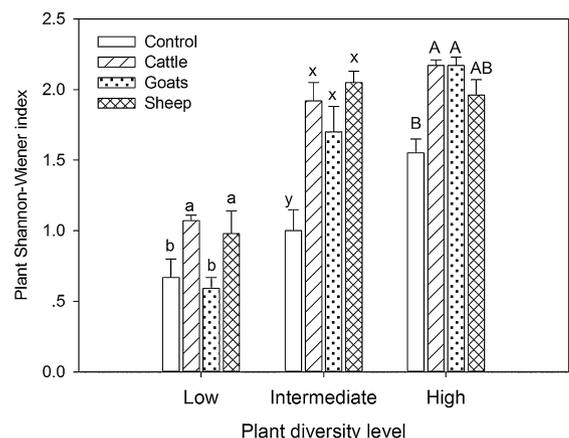


Fig. 1. The effects of grazing treatments (control, cattle, goats, and sheep) on plant Shannon–Wiener index at low, intermediate, and high plant diversity levels. Values shown are means \pm S.E. Letters (a, b, x, y; A, AB, B) indicated significant differences in plant Shannon–Wiener index among different grazing treatments at low, intermediate, and high plant diversity levels, respectively ($P < 0.05$).

Table 1
The effects of block, plant diversity, grazing breed, and their interaction on insect abundance based on the following categories: total insects, Orthoptera, Hemiptera, Coleoptera, Diptera, Hymenoptera, Lepidoptera, Homoptera, Mantodea, and Neuroptera.

Insect taxa	Plant diversity level	Grazing breed treatment				Effects of			
		Control	Cattle	Goats	Sheep	Block	Plant diversity (PD)	Grazing breed (GB)	PD*GB
Total insects	Low	354.7 ± 12.8c	566.7 ± 22.3b	477.3 ± 23.8bc	722.3 ± 44.2a	NS	***	***	**
	Intermediate	680 ± 67.1b	679.3 ± 31.4b	1093.3 ± 42.7a	796 ± 63.5b				
	High	739 ± 53.8b	790.7 ± 45.8b	1116 ± 65.51a	1131 ± 91.4a				
Orthoptera	Low	211 ± 7.8b	321.7 ± 21.1a	218.7 ± 18.3b	327 ± 2.6a	NS	***	**	**
	Intermediate	538 ± 68.9a	287.7 ± 19.1b	339 ± 20.6ab	401.3 ± 51.8ab				
	High	623.7 ± 49.4ab	516.7 ± 48.3b	775 ± 69.7ab	842 ± 103.5a				
Hemiptera	Low	21 ± 7.9b	53.7 ± 6.9a	39.7 ± 6ab	28.7 ± 2.9ab	NS	***	*	**
	Intermediate	15 ± 2.7a	20 ± 3.5a	23 ± 3.6a	29 ± 6.3a				
	High	16.7 ± 3.3a	7.7 ± 1.5a	15 ± 2.1a	7.3 ± 1.2a				
Coleoptera	Low	35 ± 4.6b	37.7 ± 7.7b	71.7 ± 8.4a	34.3 ± 4.8b	NS	***	**	**
	Intermediate	45 ± 6.7b	32.7 ± 12.1b	55.3 ± 12.4ab	95.7 ± 12.3a				
	High	22 ± 3.6a	21 ± 4.4a	29.3 ± 5.4a	24 ± 0.6a				
Diptera	Low	40.7 ± 5.2b	58 ± 6.5ab	48 ± 3.6ab	64 ± 3.5a	NS	***	***	***
	Intermediate	21.3 ± 1.5b	66 ± 9.3a	25 ± 8.7b	70.3 ± 3.8a				
	High	29.3 ± 3.7a	20.3 ± 4.5a	29.7 ± 6.3a	18.3 ± 2.4a				
Hymenoptera	Low	2 ± 0.6a	3.7 ± 0.3a	2.7 ± 1.4a	4.3 ± 0.9a	NS	NS	NS	NS
	Intermediate	5.7 ± 1.8a	2.3 ± 0.3a	4.3 ± 0.7a	3 ± 0a				
	High	5.7 ± 1.2a	2.3 ± 1.2a	5.7 ± 0.3a	3 ± 1.1a				
Homoptera	Low	35.3 ± 0.3b	88.3 ± 8.8b	91.7 ± 20.8b	260 ± 48.6a	NS	***	***	***
	Intermediate	34.3 ± 11.9d	262.7 ± 12.8b	642 ± 11.9a	190 ± 9.4c				
	High	17.7 ± 4.7c	214.3 ± 3.7b	252.3 ± 2.7a	229 ± 14.8ab				
Lepidoptera	Low	7.7 ± 1.2a	2.3 ± 1.3b	2.7 ± 0.9ab	3 ± 1.1ab	NS	**	**	NS
	Intermediate	5.7 ± 1.7a	4.3 ± 2.2a	4 ± 3.1a	4 ± 2.1a				
	High	22 ± 6.1a	6.7 ± 2.1b	6.7 ± 1.4b	6 ± 1.5b				
Mantodea	Low	1 ± 0.3a	0.7 ± 0.3a	0.7 ± 0.3a	1 ± 0.3a	NS	NS	NS	NS
	Intermediate	1.3 ± 0.9a	0a	0a	0a				
	High	0.3 ± 0.1a	0a	0a	0.3 ± 0.1a				
Neuroptera	Low	1 ± 0.6a	0.7 ± 0.3a	1.7 ± 0.9a	0a	NS	NS	NS	NS
	Intermediate	13.7 ± 7.8a	3.7 ± 1.2a	0.7 ± 0.3a	2.7 ± 1.4a				
	High	1.7 ± 0.9a	1.7 ± 0.9a	2.3 ± 1.4a	1 ± 0.3a				

Note: value was mean ± SE; different small letters indicate significant difference among grazing treatments; Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS: not significant ($P > 0.05$).

Insect abundance and vegetation relationships were examined. Total insect abundance was positively related to plant Shannon–

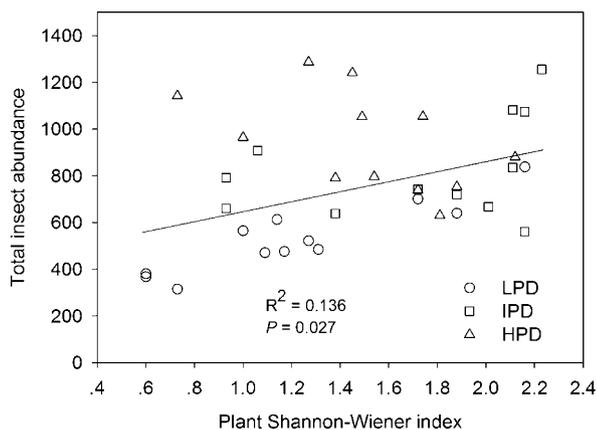


Fig. 2. Relationships between plant Shannon–Wiener index and total insect abundance in grazing treatments with low, intermediate, and high plant diversity levels. For each insect species, twelve points in grazing treatment at each plant diversity level, resulting in $n = 36$ in three plant diversity levels for linear regressions analysis ($y = 190.551x + 484.76$).

Wiener index (Fig. 2). RDA on insect abundance constrained by vegetation was performed to quantify the effects of vegetation on variation in abundance of each insect order (Fig. 3 and Table 2).

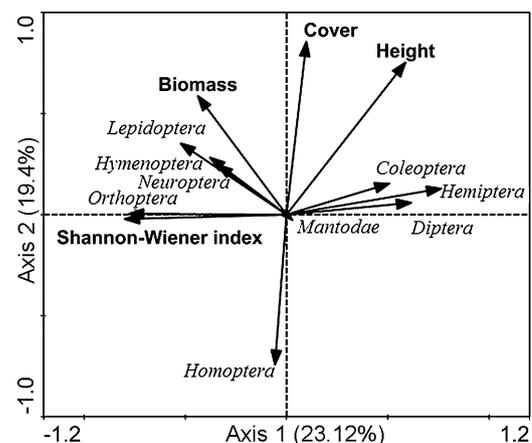


Fig. 3. Insect taxonomic composition (orders) in relation to grazing and vegetation environmental variables based on RDA. Significant vegetation variables are shown (inflation factor < 20 , $P < 0.05$).

Table 2

Eigenvalues cumulative variance (%) of species data and species–environment correlation coefficients for the first two axes obtained by RDA using a reduced set of environmental variables procedure. Both value and significance (after 999 Monte Carlo permutations) for the models are given.

Variables	Axis 1	Axis 2	Axis 3	Axis 4	F	P
Eigenvalues	0.2312	0.1939	0.0349	0.0138	8.16	0.001
Species–environment correlations	0.7793	0.8814	0.5903	0.4725		
Cumulative percentage variance of species data	23.12	42.52	46.01	47.39		
Cumulative percentage variance of species–environment relation	47.89	88.05	95.28	98.15		

Axis 1 explained most of the variation in insect abundance. The first axis of the RDA was positively correlated with height ($R^2=0.69$, $F_{1, 34}=12.451$, $P=0.001$), but negatively related with plant Shannon–Wiener index ($R^2=0.496$, $F_{1, 34}=26.457$, $P<0.0001$), and biomass ($R^2=0.149$, $F_{1, 34}=5.941$, $P=0.02$). The RDA second axis was positively correlated with plant cover ($R^2=0.415$, $F_{1, 34}=24.099$, $P<0.0001$), height ($R^2=0.322$, $F_{1, 34}=16.121$, $P<0.0001$), and biomass ($R^2=0.195$, $F_{1, 34}=8.241$, $P=0.007$). All the vegetation data together could explain 48.3% of the variation in abundance of insect orders ($F=9.218$, $df=6$, $P<0.0001$). Variable explaining the largest statistically significant amount of variation was plant Shannon–Wiener index ($F=10.352$, $df=6$, $P<0.0001$). Plant Shannon–Wiener index could explain the largest statistically significant amount of the variance in insect orders abundance (18.6%, $F=114.583$, $df=6$, $P<0.0001$).

At the order level, the interactive effects of plant diversity and large herbivore grazing were found on Orthoptera, Hemiptera, Coleoptera, Diptera, and Homoptera, but not on Lepidoptera, Hymenoptera, Mantodea, and Neuroptera. At low plant diversity, the abundance of Orthoptera and Diptera was higher under cattle and goat grazing than sheep grazing and control, Hemiptera had higher abundance under cattle and sheep grazing than goat grazing and control, Coleoptera abundance was lower in the control, cattle and goats grazed plots than in sheep grazed plots, Homoptera was more abundant in the plots grazed by cattle, goats, and sheep compared to control, and Lepidoptera showed the opposite patterns (Table 1). At intermediate plant diversity level, Orthoptera abundance in the control was higher than that of other grazed plots, Coleoptera abundance was higher in goat grazed plots than that of other grazed plots, Diptera abundance was lower in grazed plots by cattle and goats than that of control and sheep, and Homoptera abundance was lower in sheep grazed plots than that of other grazed plots (Table 1). At high plant diversity level, Orthoptera abundance reduced in cattle grazed plots, but increased in goats and sheep grazed plots, relative to the control, the abundance of Hemiptera and Lepidoptera were lower under grazing by cattle, goats, and sheep, but that of Homoptera was higher compared to control (Table 1).

At the species level, significant effects of plant diversity, grazing breed, and their interactions were found on the abundance of five dominant insect species, including *E. unicolor*, *T. ruficornis*, *A. nasuta*, Curculionidae sp., Cicadellidae sp., but not for *C. quatuordecimpustulata*, excluding for plant diversity-effects (Table 3). The abundance of *E. unicolor* was higher in the control

than that in grazed plots by cattle and sheep at low plant diversity level, and was lower than that of grazed plots by sheep at intermediate plant diversity level and by goats in high plant diversity level (Fig. 4a). *T. ruficornis* decreased in abundance in the grazed plots by goats and sheep at low and by cattle and sheep intermediate plant diversity levels than that of the control (Fig. 4b). The abundance of *A. nasuta* was lower in grazed plots at low plant diversity, and was higher in grazed plots at intermediate plant diversity level except for the grazed plots by cattle (Fig. 4c). *C. quatuordecimpustulata* was more abundant in grazed plots than that of the control at intermediate plant diversity level, but was less in grazed plots by goats and sheep at high plant diversity level (Fig. 4d). Curculionidae sp. had lower abundance in the control than that of grazed plots (Fig. 4e). The abundance of Cicadellidae sp. was higher in grazed plots by goats and was lower in grazed plots by sheep at low plant diversity level, and was higher in grazed plots by cattle and was lower in grazed plots by sheep and sheep at intermediate plant diversity level, and was higher in grazed plots by cattle and was lower in grazed plots by sheep at high plant diversity level (Fig. 4f).

The abundance of *E. unicolor* and Cicadellidae sp. significantly increased with increasing plant Shannon–Wiener index (Fig. 4g and m). The abundance of *T. ruficornis* and *A. nasuta* significantly decreased with increasing plant Shannon–Wiener index (Fig. 4h and i). However, the abundance of Curculionidae sp. and *C. quatuordecimpustulata* was not correlated to plant Shannon–Wiener index (Fig. 4j and k).

4. Discussion

Although it may be intuitive to assume that the effects of different large herbivore species on vegetation characteristics could also be seen at higher trophic levels (Jáuregui et al., 2008), such cascading effect has rarely been well-quantified. Our results show that the effects of large herbivores on insect community are grazer species-specific; and more importantly, depending on plant diversity of pre-grazing (Table 1 and 3). Consequently, despite our accumulated knowledge about the role of individual factors such as grazing management or plant diversity, the lack of clear understanding on their interactive effects (e.g., plant diversity \times large herbivore species) may have led to coarse predictions about insect community in the past (Sjödin et al., 2008; Zhu et al., 2012).

Our previous study showed detailed response of overall insect species richness to large herbivore grazing at different plant

Table 3

Results of three-way ANOVA for the effects of Block, plant diversity, and grazing breed and their interactions on the abundance of six dominant insect species: *Euchorthippus unicolor*, *Trigonotylus ruficornis*, *Aelia nasuta*, *Coccinula quatuordecimpustulata*, Curculionidae sp., Cicadellidae sp.

	d.f.	<i>E. unicolor</i>		<i>T. ruficornis</i>		<i>A. nasuta</i>		Curculionidae sp.		<i>C. quatuordecimpustulata</i>		Cicadellidae sp.	
		F	P	F	P	F	P	F	P	F	P	F	P
Block	2,24	0.643	0.381	1.251	0.302	1.485	0.246	0.457	0.623	1.482	0.243	0.086	0.87
Plant diversity (PD)	2,224	44.964	<0.0001	27.635	<0.0001	40.836	<0.0001	71.943	<0.0001	5.317	0.012	244.9	<0.0001
Breed	3,324	7.222	0.001	6.566	0.002	3.134	0.044	6.555	0.002	1.377	0.274	411.63	<0.0001
PD \times breed	6,24	4.348	0.004	6.638	<0.0001	6.476	<0.0001	5.078	0.002	2.339	0.064	192.45	<0.0001

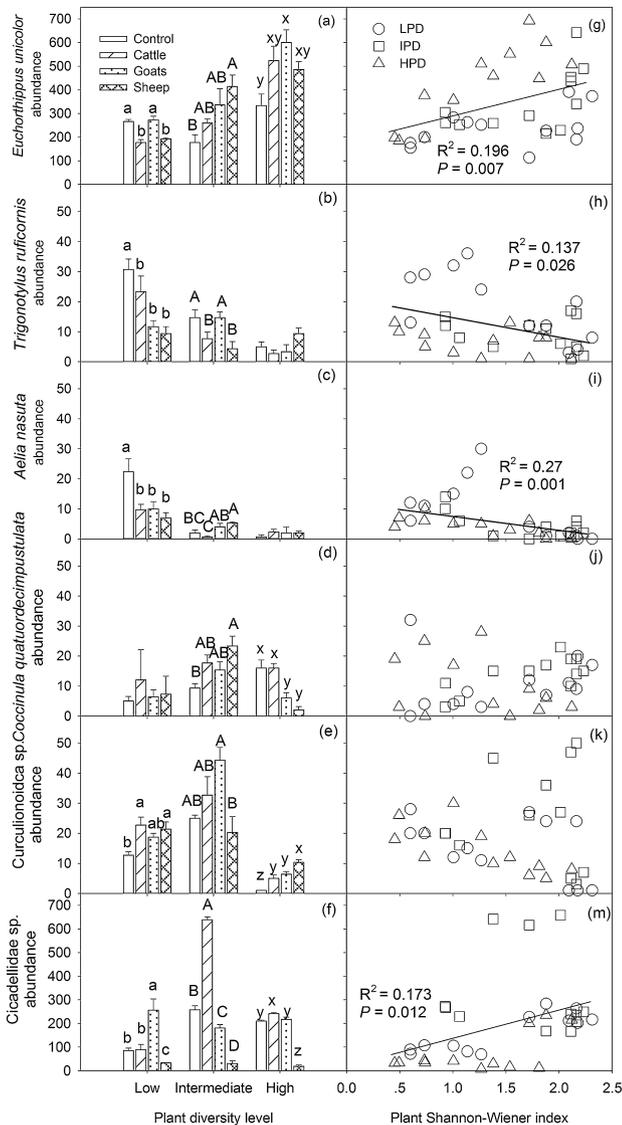


Fig. 4. The effects of grazing treatments (control, cattle, goats, and sheep) on insect abundance at low, intermediate, and high plant diversity levels, and the relationships between the abundance of each insect species and plant Shannon–Wiener index in grazing treatments with the three plant diversity levels. (a, g) *Euchorthippus unicolor*, (b, h) *Trigonotylus ruficornis*, (c, i) *Aelia nasuta*, (d, j) *Coccinula quatuordecimpustulata*, (e, k) Curculionidae sp., (f, m) Cicadellidae sp. Values shown are means \pm S.E. Small letters (a, ab, b, c; A, AB, B, C, D; x, xy, y, z) indicated significant differences in insect abundance among different grazing treatments at low, intermediate, and high plant diversity levels respectively ($P < 0.05$). For each insect species, twelve points in grazing treatment at each plant diversity level, resulting in $n = 36$ in three plant diversity levels for linear regressions analysis (*E. unicolor*: $y = 110.49x + 172.4$, *T. ruficornis*: $y = -5.62x + 19.74$, *A. nasuta*: $y = -5.68x + 14.1$, *C. quatuordecimpustulata*: $y = 1.806x + 8.677$, Curculionidae sp.: $y = -3.13x + 23.009$, Cicadellidae sp.: $y = 114.9x + 17.2$).

diversity levels (Zhu et al., 2012). In comparison, this study shows that grazing by large herbivores greatly changed insect abundance (Table 1), which are consistent with the observations in a semi-arid grassland (Debano, 2006) and a Montane grassland (Cagnolo et al., 2002). Generally, large herbivores affect insect community by modifying vegetation characteristics, especially species richness (Joern, 2005), structural complexity (Pöyry et al., 2006; Körösi et al., 2012), and productivity (Perner et al., 2005). Whether grazing alters insect community depends on how herbivores may influence these vegetation variables. Grazing could reduce plant cover, height, structural heterogeneity, and biomass (see Zhu et al., 2012), but these altered vegetation characteristics cannot explain

the variation of insect abundance. This observation differs from previous studies, where difference in insect abundance is attributed to alterations in plant productivity and plant structure (Perner et al., 2005; Pöyry et al., 2006; Woodcock et al., 2009). We therefore propose that our empirical evidence points to plant Shannon–Wiener index for the increased insect abundance following grazing by large herbivores. In our experiment, grazing by large herbivores increased plant Shannon–Wiener index, which was perfectly matched by changes in insect abundance (Table 1 and Fig. 1), when a positive relationship between insect abundance and plant Shannon–Wiener index was found (Fig. 2). This is further evidenced in our RDA analysis (Table 2 and Fig. 3). Plant Shannon–Wiener index includes both species richness and evenness information, and is closely related to spatial heterogeneity of vegetation (Wang et al., 2014). Higher plant Shannon–Wiener index induced by grazing may support more insects due to higher spatial heterogeneity that provide habitat and food resources for insects.

At the order-level, different insect orders exhibited various responses to each grazing treatment, which was similar with other cases where higher numbers of beetles and bugs, and unchanged abundance of flies and caterpillars in the less-grazing treatments were found (Dennis et al., 2008), lower numbers of bees and butterflies were detected (Sjödin et al., 2008). In this study, grazing had a positive impact on the abundance of Orthoptera, and Homoptera, and a negative impact on Lepidoptera abundance (Table 1). Clearly, both insect habits and surrounding environmental features could affect their responses to grazing (Dennis et al., 1998; Specht et al., 2008). RDA analysis indicated that Orthoptera may be affected by changes in plant species richness, and Lepidoptera and Hymenoptera may be closely correlated with plant biomass, while other groups including Coleoptera, Diptera, and Hemiptera seemed to respond mainly to plant structural characteristics (Fig. 3). Most Orthoptera are herbivores, food quality may be more important for their development (Berner et al., 2005), which likely lead to plant Shannon–Wiener might be more important than plant biomass. In contrast, Lepidoptera and Hymenoptera were more affected by plant biomass due to their need for food quantity and insect lifestyles (their larva need abundant foods for development toward adulthood). However, for other insect orders, plant community structure may be important owing to habitat selection, e.g., for oviposition or refuges (Morris, 2000). More evidence of insect responses to grazing is lacking, and needs to be explored in the future.

At the species-level, grazing strongly affected the abundance of each insect species, and the effects were grazer-specific (Table 3). For example, the abundance of *E. unicolor* and Cicadellidae sp. increased in grazed treatments (Fig. 4a and f), and show a positive relationship along with plant Shannon–Wiener index (Fig. 4g and m). The abundance of *T. ruficornis* and *A. nasuta*, two species of Hemiptera, decreased in grazed plots (Fig. 4b and c), indicating that the insects would be unable to tolerate such conditions. The decline in abundance of the two Hemiptera species may be due to structural variations induced by grazing, but not plant Shannon–Wiener index, because the coverage and height of plant community significantly decreased in grazing treatments (Zhu et al., 2012). The explanations that the abundance of dominant species of Orthoptera and Hemiptera were affected by grazing through modifying plant community were consistent with RDA analysis (Fig. 3). The changes in abundance of Curculionidae sp. and *C. quatuordecimpustulata* were not consistent across grazed plots (Fig. 4d and e), but unfortunately we were unable to identify the mechanisms. Nevertheless, our results show that not all insect species are susceptible to grazing by large herbivores, implying that sensitive insect species would be conserved in grazed grasslands.

Results from the study showed that changes in insect abundance of not all orders may be largely attributed to altered abundance of dominant species in response to grazing by large herbivores, rather than the contributions of altered abundance of each species at the order, such observations indicate that alterations at order and species levels would be considered for better conservation of insect diversity in grazed grasslands. Additionally, we recognize that some caution must be taken with respect to interpreting our data due to our sweep sampling, because highly mobile insects such as grasshoppers influence the abundance at the time of sweeping. While sweep netting does not sample all insects in the community, community measures obtained from sweep netting have been shown to be highly correlated with insects sampled by other methods at grasslands (Siemann 1998; Haddad et al. 2009), particularly suction sampling at this experimental site (H. Zhu et al., unpublished data). Despite the fact that we cannot ascertain the accurate responses of insects to grazing by large herbivores due to their movements, our data seem to support the conclusion that grazing treatments have significant effects on insect abundance over the experimental periods.

Another finding in this study is that the effects of grazing by large herbivores on insect abundance depend on plant diversity of pre-grazing (Table 1 and 3). At low plant diversity level, cattle and sheep grazing have larger effect than goats, but this trend is not apparent at intermediate and high plant diversity levels. Due to differences in diet and body size among cattle, goats, and sheep, their foraging and trampling have different effects on plant community (Fig. 1, Zhu et al., 2012), and then could cascade to insect community (Table 1). The changes in plant communities under grazing by large herbivores strongly depend on plant species richness of pre-grazing (Table 1, and see Wang et al., 2011). Thus, it is clear that the responses of insect community in grazed grassland ecosystems strongly depend on two major factors: i.e., large herbivore species and plant diversity of pre-grazing. The mechanisms underlying the variation in insect community, however, require further detailed examination and evaluation. Our results highlight the need for further examining different taxa in order to obtain a more complete picture of how grazing impacts invertebrate community, though simultaneous focus on multi-trophic levels would be helpful (Hartley et al., 2003; Potts et al., 2009).

5. Conclusions

Our results clearly show interactive effects of large herbivores (as ecosystem engineers) and plants on insect abundance at both order and species levels, and indicate an advantage of using taxonomic hierarchies as well as species richness in assessing the effects of grazing on invertebrate communities. As our understanding of species interactions at multiple trophic levels in grassland ecosystems (Carvell, 2002) is still weak, our study offers new insights regarding the important cascading effects involving three major species groups. For example, the outcome of grazing by large herbivores on insects clearly depends on the plant diversity of pre-grazing. In our grassland system, plant resources are highly heterogeneous, and the responses of insect community are significantly different across diverse plant communities and under different managements, especially of grazing by different large herbivore species. Better understanding the complex relationships among plants, insects, and large herbivores will facilitate future efforts to effectively balance the trade-offs between conservation of plants and insects and animal production to maintain sustainability in grasslands.

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References

- Bakker, E., Ritchie, M., Olf, H., Milchunas, D., Knops, J., 2006. Herbivore impact on grassland plant diversity depends on habitat productivity and herbivore size. *Ecol. Lett.* 9, 780–788.
- Batáry, P., Báldi, A., Sárospataki, M., Kohler, F., Verhulst, J., Knop, E., Herzog, F., Kleijn, D., 2010. Effect of conservation management on bees and insect-pollinated grassland plant communities in three European countries. *Agric. Ecosyst. Environ.* 136, 35–39.
- Belovsky, G.E., Slade, J.B., 2000. Insect herbivory accelerates nutrient cycling and increases plant production. *Proc. Natl. Acad. Sci. U. S. A.* 97, 14412–14417.
- Bergvall, U.A., Rautio, P., Kesti, K., Tuomi, J., Leimar, O., 2006. Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. *Oecologia* 147, 253–260.
- Berner, D., Blanckenhorn, W.U., Körner, C., 2005. Grasshopper cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos* 111, 525–533.
- Bronstein, J.L., Alarcón, R., Geber, M., 2006. The evolution of plant–insect mutualisms. *New Phytol.* 172, 412–428.
- Cagnolo, L., Molina, S.I., Valladares, G.R., 2002. Diversity and guild structure of insect assemblages under grazing and exclusion regimes in a Montane grassland from Central Argentina. *Biodivers. Conserv.* 11, 407–420.
- Carvell, C., 2002. Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biol. Conserv.* 103, 33–49.
- Cease, A.J., Elser, J.J., Ford, C.F., Hao, S.G., Kang, L., Harrison, J.F., 2012. Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. *Science* 335, 467–469.
- Collinge, S.K., 2000. Effects of grassland fragmentation on insect species loss, colonization, and movement patterns. *Ecology* 81, 2211–2226.
- Debano, S.J., 2006. Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodivers. Conserv.* 15, 2547–2564.
- Dennis, P., Yong, M.R., Gordon, I.J., 1998. Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed indigenous grasslands. *Ecol. Entomol.* 23, 253–264.
- Dennis, P., Skartveit, J., McCracken, D.L., Pakeman, R.J., Beaton, K., Kunaver, A., Evans, D.M., 2008. The effects of livestock grazing on foliar arthropods associated with bird diet in upland grasslands of Scotland. *J. Appl. Ecol.* 45, 279–287.
- Dolek, M., Geyer, A., 2002. Conserving biodiversity on calcareous grasslands in the Franconian Jura by grazing: a comprehensive approach. *Biol. Conserv.* 104, 351–360.
- Evans, E.W., Rogers, R.A., Opfermann, D.J., 1983. Sampling grasshoppers (Orthoptera: Acrididae) on burned and unburned tallgrass prairie: night trapping vs. sweeping. *Environ. Entomol.* 12, 1449–1454.
- Fonderflick, J., Besnard, A., Beuret, A., Dalmais, M., Schatz, B., 2014. The impact of grazing management on Orthoptera abundance varies over the season in Mediterranean steppe-like grasslands. *Acta Oecol.* 60, 7–16.
- Gao, Y., Wang, D.L., Ba, L., Bai, Y.G., Liu, B., 2008. Interactions between herbivory and resource availability on grazing tolerance of *Leymus chinensis*. *Environ. Exp. Bot.* 63, 113–122.
- Gonzalez-Megias, A., Gomez, J.M., Sanches-Pinero, F., 2004. Effects of ungulates on epigeal arthropods in Sierra Nevada National Park (Southeast Spain). *Biodivers. Conserv.* 13, 733–752.
- Haddad, N.M., Crutsinger, G.M., Kevin, G., Haarstad, J., Knops, J.M.H., Tilman, D., 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecol. Lett.* 12, 1029–1039.
- Hartley, S.E., Gardener, S.M., Mitchell, R.J., 2003. Indirect effects of grazing and nutrient addition on the hemipteran community of heather moorlands. *J. Appl. Ecol.* 40, 793–803.
- Jáuregui, B.M., Rosa-García, R., García, U., WallisDeVries, M.F., Osoro, K., Celaya, R., 2008. Effects of stocking density and breed of goats on vegetation and grasshopper occurrence in heathlands. *Agric. Ecosyst. Environ.* 123, 219–224.
- Joern, A., 2005. Disturbance by fire frequency and bison grazing modulate grasshopper assemblages in tallgrass prairie. *Ecology* 86, 861–873.
- Jonas, J.L., Joern, A., 2007. Grasshopper (Orthoptera: Acrididae) communities respond to fire, bison grazing and weather in North American tallgrass prairie: a long-term study. *Oecologia* 153, 699–711.
- Körösi, Á., Batáry, P., Orosz, Á., Rédei, D., Báldi, Á., 2012. Effects of grazing, vegetation structure and landscape complexity on grassland leafhoppers (Hemiptera:

- Auchenorrhyncha) and true bugs (Hemiptera: Heteroptera) in Hungary. *Insect Conserv. Divers.* 5, 57–66.
- Kruess, A., Tscharntke, T., 2002a. Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conserv. Biol.* 16, 1570–1580.
- Kruess, A., Tscharntke, T., 2002b. Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biol. Conserv.* 106, 293–302.
- Li, H., Reynolds, J.F., 1995. On definition and quantification of heterogeneity. *Oikos* 73, 280–284.
- Littlewood, N.A., 2008. Grazing impacts on moth diversity and abundance on a Scottish upland estate. *Insect Conserv. Divers.* 1, 151–160.
- Matt, R.W., Charlton, R.E., 2006. The ecological significance of tallgrass prairie arthropods. *Annu. Rev. Entomol.* 51, 387–412.
- Miller, A.M., McArthur, C., Smethurst, P.J., 2007. Effects of within-patch characteristics on the vulnerability of a plant to herbivory. *Oikos* 116, 41–52.
- Morris, M.G., 2000. The effects of structure and its dynamics on the ecology and conservation of arthropods in British grasslands. *Biol. Conserv.* 95, 129–142.
- Palmer, S.C.F., Hester, A.J., Elston, D.A., Gordon, I.J., Hartley, S.E., 2003. The perils of having tasty neighbors: grazing impacts of large herbivores at vegetation boundaries. *Ecology* 84, 2877–2890.
- Perner, J., Wytrykush, C., Kahmen, A., 2005. Effects of plant diversity, plant productivity and habitat parameters on arthropod abundance in montane European grasslands. *Ecography* 28, 429–442.
- Potts, S.G., Woodcock, B.A., Roberts, S.P.M., Tscheulin, T., Pilgrim, E.S., Brown, V.K., Tallwin, J.R., 2009. Enhancing pollinator biodiversity in intensive grasslands. *J. Appl. Ecol.* 46, 369–379.
- Pöyry, J., Luota, M., Paukkunen, J., Pykälä, J., Raatikainen, K., Kuussaari, M., 2006. Different responses of plants and herbivore insects to a gradient of vegetation height: an indicator of the vertebrate grazing intensity and successional age. *Oikos* 115, 401–412.
- Rosa-García, R., Jáuregui, B.M., García, U., Osoro, K., Celaya, R., 2009. Effects of livestock breed and grazing pressure on ground-dwelling arthropods in Cantabrian heathlands. *Ecol. Entomol.* 34, 466–475.
- Ryder, C., Moran, J., Mc Donnell, R., Gormally, M., 2005. Conservation implications of grazing practices on the plant and dipteran communities of a turlough in Co. Mayo, Ireland. *Biodivers. Conserv.* 14, 187–204.
- Schaffers, A., Raemakers, I.P., Sýkora, K.V., ter Braak, C.J.F., 2008. Arthropod assemblages are best predicted by plant species composition. *Ecology* 89, 782–794.
- Siemann, E., 1998. Experimental tests of the effects of plant productivity and plant diversity on grassland arthropod diversity. *Ecology* 79, 2057–2070.
- Sjödin, N.E., Bengtsson, J., Ekblom, B., 2008. The influences of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *J. Appl. Ecol.* 45, 763–772.
- Specht, J., Scherber, C., Unsicker, S.B., Köhler, G., Weisser, W.W., 2008. Diversity and beyond: plant functional identity determines herbivore performance. *J. Anim. Ecol.* 77, 1047–1055.
- ter Braak, C.J.F., Šmilauer, P., 1998. *CANOCO Reference Manual and User's Guide to Canoco for Windows. Software for Canonical Community Ordination Version 4.* Microcomputer Power, Ithaca, NY, USA.
- Thomas, J.A., Telfer, M.G., Preston, D.B., Greenwood, J.J.D., Asher, J., Fox, R., Clarke, R. T., Lawton, J.H., 2004. Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science* 303, 1879–1881.
- van Klink, R., van der Plas, F., van Noordwijk, C.G.E., WallisDeVries, M.F., Olff, H., 2015. Effects of large herbivores on grassland arthropod diversity. *Biol. Rev.* 90, 347–366.
- Wang, D.L., Ba, L., 2008. Ecology of meadow steppe in northeast China. *Rangel. J.* 30, 247–254.
- Wang, L., Wang, D.L., He, Z.B., Liu, G.F., Hodgkinson, K.C., 2010. Spatially complex neighboring relationships among grassland plant species as an effective mechanism of defense against herbivory. *Oecologia* 164, 193–200.
- Wang, L., Wang, D.L., Liu, J.S., Huang, Y., Hodgkinson, K.C., 2011. Diet selection variation of a large herbivore in a feeding experiment with increasing species numbers and different plant functional group combinations. *Acta Oecol.* 37, 263–268.
- Wang, L., Liu, C., Alves, D.G., Frank, D.A., Wang, D.L., 2014. Plant diversity is associated with the amount and spatial structure of soil heterogeneity in meadow steppe of China. *Landsc. Ecol.* doi:http://dx.doi.org/10.1007/s10980-013-9955-0.
- Williams, P., Gaston, K., 1994. Measuring more of biodiversity can higher-taxon richness predict wholesale species richness? *Biol. Conserv.* 67, 211–217.
- Woodcock, B.A., Potts, S.G., Tscheulin, T., Pilgrim, E., Ramsey, A.J., Harrison-Cripps, J., Brown, V.K., Tallwin, J.R., 2009. Responses of invertebrate trophic level, feeding guild and body size to the management of improved grassland field margins. *J. Appl. Ecol.* 46, 920–929.
- Zhong, Z.W., Wang, D.L., Zhu, H., Wang, L., Feng, C., Wang, Z.N., 2014. Positive interactions between large herbivores and grasshoppers, and their consequences for grassland plant diversity. *Ecology* 95, 105–1064.
- Zhu, H., Wang, D.L., Wang, L., Ba, Y.G., Fang, J., Liu, J., 2012. The effects of large herbivore grazing on meadow steppe plant and insect diversity. *J. Appl. Ecol.* 49, 1075–1083.