

# Effects of large herbivore grazing on grasshopper behaviour and abundance in a meadow steppe

HUI ZHU,<sup>1,†</sup> VENUSTE NKURUNZIZA,<sup>1,†</sup> JINGTING WANG,<sup>1</sup> QINFENG GUO,<sup>2</sup> HANG RUAN<sup>1</sup> and DELI WANG<sup>1,3</sup> <sup>1</sup>School of Life Sciences/Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University, Changchun, China, <sup>2</sup>USDA – Southern Research Station, Cornwallis Road, Research Triangle Park, North Carolina, U.S.A. and <sup>3</sup>School of Environment, Northeast Normal University, Changchun, China

**Abstract.** 1. Adaptive phenotypic plasticity has been a major subject in evolutionary ecology, but how a species' behaviour may respond to certain environmental change is still not clear. In grasslands worldwide, large herbivores are increasingly used as a tool for grazing management, and occur to interact with grasshoppers that dominate grassland insect communities. Previous studies have been well-documented about grazing effects on diversity and abundance of grasshoppers. Yet, how grazing may alter grasshopper behaviour, and potential effects on their abundance remains elusive.

2. We conducted a field experiment by manipulating grazing using sheep, cattle, and their mix to examine the behavioural responses and abundance of the grasshoppers (*Euchorthippus unicolor*) to grazing in a *Leymus chinensis*-dominated grassland.

3. Results showed that the grasshoppers spent less time on feeding and resting on grasses, but more time on switching and resting on forbs under cattle grazing and mixed grazing with cattle and sheep. In contrast, the grasshoppers spent more time on feeding but less time on switching and resting on forbs under sheep grazing. The behavioural changes were also potentially linked to grasshopper abundance in the context of grazing management.

4. The responses of grasshopper behaviour and abundance to grazing may be largely triggered by altered vegetation and microclimates. Such behavioural flexibility of grasshoppers must be considered when large herbivores are recognised as a management tool for influencing grasshopper abundance, and grazer species should be paid more attention both individually and jointly for better grassland conservation.

**Key words.** Behavioural activity, grasshoppers, grassland, grazing, large herbivore assemblage.

## Introduction

With ongoing climate change and human disturbance, an important issue in evolutionary ecology is to understand how species adapt to rapidly changing environments (Wong & Candolin, 2015; Van Baaren & Candolin, 2018). Adaptive phenotypic plasticity allows individuals and genotypes to adjust traits to local conditions in order to cope with novel and changing environments (Snell-Rood, 2013; Sgró, Terblanche,

& Hoffmann, 2016). Such plasticity has been seen for a large array of traits including behaviour, physiology, or morphology of species (West-Eberhard, 2003; Sih *et al.*, 2010; Wong *et al.*, 2019). However, most related studies have focused on physiological and morphological phenotypes (Dingemanse & Wolf, 2013; Rosenblatt *et al.*, 2019), and behavioural plasticity has received little attention. Yet, mounting evidence shows that the development and expression of behaviour is remarkably sensitive to environmental conditions (Stahlschmidt *et al.*, 2016; van Baaren & Candolin, 2018). Indeed, behavioural modification not only potentially buffers against starvation due to resource reduction or against deteriorating habitat conditions (Knolhoff & Heckel, 2014) but also reduces predation risk (Hermann & Thaler, 2018). The behavioural changes could then be

Correspondence: Deli Wang, School of Life Sciences/Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University, Changchun, Jilin 130024, China. E-mail: wangd@nenu.edu.cn

<sup>†</sup>These authors contributed equally to this work.

linked to species abundance through immigration and migration (Shi, 2013; Poyet *et al.*, 2015). Therefore, behavioural responses of species to changing environments have become an important research subject for linking adaptative behaviour with abundance, an important factor in species conservation (Phillips & Suarez, 2012; Pol *et al.*, 2017).

Insects are important components of terrestrial systems, and an alteration in their behaviour is very often the first and important response to changing surrounding conditions (Huey *et al.*, 2003). For example, dung beetles look for shady places at higher temperatures (Macagno *et al.*, 2018). Mosquitoes can show strong anti-predator responses to increase survival (Andrade *et al.*, 2017). The altered behaviour could be the outcome of changes in microclimate and resource availability, or both. Recently, some studies have shown that species interactions may mediate behavioural changes of insects directly (e.g. predator–prey, Hawlena *et al.*, 2011a) or indirectly (Poelman & Dicke, 2014). In natural ecosystems, species interactions between insects and other species happen everywhere (Kumar *et al.*, 2016; Zhong *et al.*, 2017), and insects could show more complex behavioural activity when participating in species interactions. Thus, behavioural changes of insects should be paid more attention within species interactions, particularly in the context of the correlations between behavioural activity and abundance (Fisher *et al.*, 2015; Jandt & Gordon, 2016).

In many grasslands worldwide, grazing by large herbivores is one of the most important tools for grassland management (Wang *et al.*, 2019), and thus large herbivores often occur to interact with other components such as grasshoppers that often dominate grassland insect community (Zhong *et al.*, 2014; Zhu *et al.*, 2020). Although previous studies mainly focused on the effects of grazing on grasshopper species richness and abundance (Zhu *et al.*, 2017; Le Gall *et al.*, 2019), behavioural response of grasshoppers to grazing is largely unexplored. This gap may further hamper the understanding of mediated effects of large herbivores on grasshoppers. Due to the presence of grazers and the grazing process, physical habitat for grasshoppers, i.e. vegetation structure and microclimates are affected by grazing (Zhong *et al.*, 2017; Zhu *et al.*, 2017). In responses, grasshoppers usually move away from grazers to avoid physical disturbances. Moreover, grazing could alter grasshoppers' food resources, and thereby further modifying their foraging behaviour due to competing food or helping to find food (Branson & Haferkamp, 2014; Zhong *et al.*, 2014) because some studies have shown that grasshoppers reared in single-forage environments and mixed-resource environments make different alternative feeding strategies that vary with the possibility of using behavioural resource selection (Hawlena *et al.*, 2011a; McMahon *et al.*, 2018). Additionally, grazing may mediate the presence/absence and abundance of certain grasshopper predators. When encountering predators, grasshoppers adopt changing foraging strategies, or enhanced escaping performance (such as adjustments in jumping technique) that eliminate predation risk (Barton, 2011; Hawlena *et al.*, 2011b). Thus, in a grazed grassland, large herbivores may modify grasshoppers' behaviour through the above pathways, and behavioural changes in grasshoppers are likely related to their abundance

through reproduction and migration. To date, few studies yet have examined the effects of grazing on behavioural activity and abundance of grassland grasshoppers.

Here, we conduct a field experiment to investigate how grasshoppers respond in their behaviour to large herbivore grazing which may thus link to their abundance in a grassland of northeastern China. The grassland ecosystem is dominated by *Leymus chinensis* and included two common grazers, domestic sheep (*Ovis aries subsp.*), and cattle (*Bovine subsp.*), and a major insect species, grasshopper (*Euchorthippus unicolor*). Cattle and sheep have markedly different foraging preferences due to their different physiological and digestive characteristics. Cattle prefer the dominant grass, *L. chinensis*, but sheep prefer to the forbs (Liu *et al.*, 2015), consequently, these two grazer species differently influence habitat and food resources for grasshoppers. Thus, we hypothesise that cattle grazing and mixed grazing by both cattle and sheep may have negative effects on behavioural activities, but sheep grazing may have positive effects, and therein grasshopper abundance. To better understand the possible mechanisms behind the possible behavioural responses of grasshoppers, we also investigated the effects of grazing on characteristics of vegetation (i.e. biomass, cover, and height) and microclimates (solar radiation and air temperature).

## Materials and methods

### Study site

The experiment 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 has a semiarid continental monsoon weather, with a cold and dry winter, and a warm and rainy summer. Mean annual temperature is from 4.6 °C to 6.4 °C, and annual rainfall is around 280–400 mm during 2005–2016. The vegetation belongs to the eastern region of the Eurasian Steppe Zone where main vegetation type is meadow steppe. The most dominant species is *Leymus chinensis* (Wang *et al.*, 2017), followed by *Phragmites australis*, *Calamagrostis epigejos*, *Artemisia scoparia*, and *Kalimeris integrifolia* (Zhu *et al.*, 2014). The soil is mixed saline and alkaline (pH 8.5–10.0).

### Experimental design and grazing treatments

Three blocks with 12 experimental plots were established and enclosed in August 2008, and similar plant species composition of each plot was recorded at the same time (detailed descriptions of vegetation attributes can be found in Liu *et al.*, 2015). Each block was randomly assigned for four grazing treatments: ungrazed (NG), cattle grazing (CG), sheep grazing (SG), and mixed grazing by cattle and sheep (MG), with three replicates for each treatment (Fig. S1). Plots for NG, CG, and SG were 25 m × 25 m in size, while plots for MG were 25 m × 50 m. The MG plots were twice as large as other plots to ensure equal grazing intensity between CG, SG, and MG treatments (Liu *et al.*, 2015). Neighbouring plots were separated by 18–20 m.

Two-year-old northeast fine-wool sheep (body weight  $32.0 \pm 1.8$  kg), and adult Simmental cattle (body weight  $300.0 \pm 7.5$  kg) were used in this experiment. To ensure a moderate grazing intensity (about 50–55% aboveground plant biomass was removed), 16 sheep were used in each SG treatment, and four cattle were used in each CG treatment based on the intake and body weight of each individual grazer, and the recent 5 years plant production in this experimental site. Grazing occurred from early June – early August in 2009. Sheep and cattle grazing were rotated across the nine plots that were randomly assigned among the grazing treatments (3 large herbivore assemblages  $\times$  3 replicates). During each month, each plot was grazed for 2 days, for a total of 12 days of grazing over 3 months (simultaneous sheep and cattle grazing, and then followed by mixed grazing). Grazing took place from 06.00 to 08.00 and from 16.00 to 18.00 each grazing day, which was in accordance with local grazing habit.

#### Behavioural observation of grasshoppers

After grazing, these large herbivores were removed from the plots. To examine grasshopper *E. unicolor*' behavioural responses to large herbivores (CG, SG, and MG), microcosm experiments were conducted on August 15–28, 2009 when all grazing activities were stopped 5–7 days. Two cylindrical cages (2.0 m in diameter, 1.5 m in height, and wrapped with aluminium mesh of 5 mm  $\times$  5 mm in size) were installed on each plot, for a total of 24 cages. The bottoms of these cages were placed in the ground (approximately 10 cm in depth) to prevent grasshoppers from escape. Twelve adult grasshoppers *E. unicolor*, based on their crossvein, were selected from the surrounding area for behavioural observation, and each grasshopper was marked with a dot of red paint on the thorax and abdomen for tracking. The 12 grasshoppers were then randomly divided into three groups of four individuals. These groups were then randomly allocated to three cages.

Observations were conducted in sunny (no or little cloud cover) and no wind days. Before observation, invertebrates were removed from each cage using a vacuum cleaner. Grasshoppers were then added to these cages and left to settle for 30 min. The behaviour of the grasshoppers were observed for 8 h from 09.00 to 17.00 and recorded simultaneously by 12 observers, with 1 tracking the activity of one grasshopper. To minimise possible disturbance, the observer was at least 2–3 m from the experimental cage. Behavioural activities were recorded using a voice recorder (Lenovo B316+) for the following events: (i) Feeding time, quantified by time spent on consuming leaves or stems; (ii) Switching time, quantified by time spent on walking, flying, or jumping, and (iii) Resting time, quantified by times spent on resting on leaves or stems of plant species, including grasses and forbs with no contact between grasshopper mouthparts and plant tissue. After observation, grasshoppers were captured and placed in a cage (20 cm in diameter, 15 cm in height), secured with muslin, and left overnight with no supplementary food to be used the next day. Dead grasshoppers were replaced by new individuals. These

observations were finished within 8 days, with three cages per observation day (Fig. S2). The following variables were analysed to test the differences in grasshopper behaviour among the four grazing treatments: time spent feeding total all plants and *L. chinensis* only, time spent locomoting, time spent resting, and time spent resting on stems and leaves of plants, grasses, and forbs. One cage with four grasshoppers was used as one replicate.

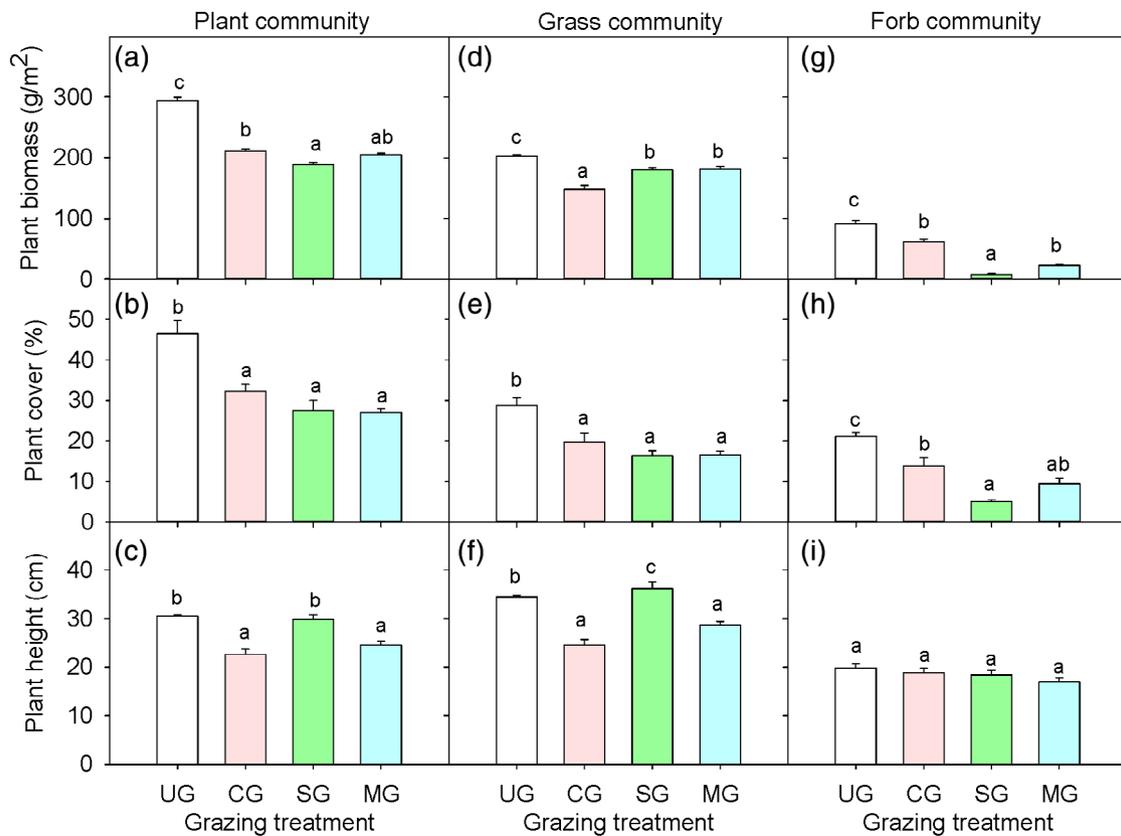
#### Vegetation and microclimate measurements

Vegetation and microclimate in cages were measured before the behavioural observation of grasshoppers. Within each cage, plant species richness, and the height, and cover of each species were assessed in three quadrats ( $0.5 \times 0.5$  m<sup>2</sup>/a quadrat). Plant heights were measured to the nearest centimetre using a ruled rod. Species cover was estimated visually as the percentage of surface area covered by each plant species (with a maximum of 100% total cover). Aboveground plant biomass was sampled from one randomly located  $0.5 \times 0.5$  m<sup>2</sup> quadrats per plot, and dried for 48 h at 80 °C, and weighed. Plants were divided into two functional groups: grasses and forbs. Grasses are considered as food sources for grasshoppers, and plant communities are regarded as their physical habitat (Zhu *et al.*, 2017). We therefore tested the treatment-effects on their entire plant communities (we used plant biomass cover, and height represents the biomass cover, and height of whole plant community), grass, and forb communities, respectively.

Solar radiation was measured using light illuminometer (TES-1339, TaiShi Instrument, Ltd., TaiWan, accuracy  $\pm 0.1$  Lux), and air temperature was measured using Thermo-Hydro recorder (TES-1360A, TaiShi Instrument, Ltd., Taiwan, accuracy  $\pm 0.01$  °C) at the height of 30 cm aboveground the ground during the behavioural observation of grasshoppers in each cage. Each measurement was conducted with five points per cage at the times of 9.00, 12.00, and 15.00. Microclimatic variables for each cage were calculated as the average of measurements from five points at three sampling times on the sampling date prior to analysis.

#### Grasshoppers sampling

To determine the effects of grazing treatments on grasshopper abundance, *E. unicolor* were sampled using standardised sweep netting along four 25 m transects spaced 6 m apart in each plot when grazers were removed for 4 days each sampling time (Zhu *et al.*, 2017). Each sampling was composed of 20 sweeps and two samplings were carried out on each plot to ensure that those samples were representative of each sampling date. The contents of the sweep nets were preserved in bottles containing ethyl acetate. The samples were collected between 09.30 and 15.30 h under sunny and light or no wind conditions. Grasshoppers were sampled at each plot three times during late July and late August in 2009. All plots were visited on the same day and in a random order on each sampling date. The accumulative abundance of grasshoppers (both nymphs and adults were included) of



**Fig. 1.** The effects of large herbivore assemblages (UG: Ungrazed, CG: cattle grazing, SG: sheep grazing, and MG: mixed grazing) on vegetation characteristics, including plant biomass (a), plant height (b), plant cover (c), grass biomass (d), grass height (e), grass cover (f), forb biomass (g), forb height (h), and forb cover (i). Values are means  $\pm$  SE. Different lowercase letters indicate significant difference among different grazing treatments ( $P < 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

each plot was calculated throughout three sampling dates for analysing.

#### Data analysis

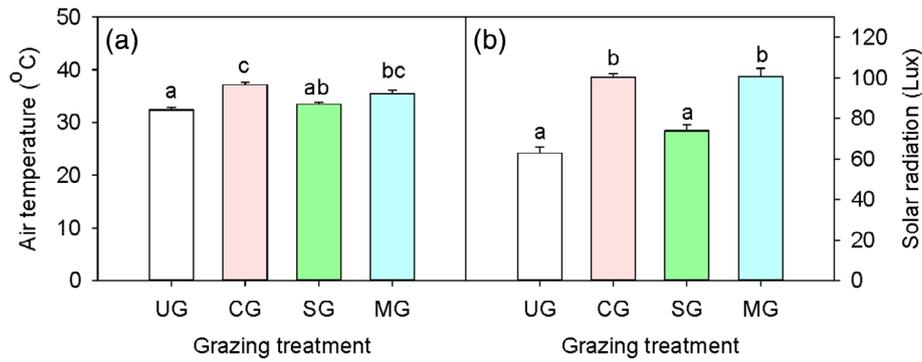
To test whether combinations of grazing and other environmental variables affect grasshopper behaviour, we used linear mixed models (LMMs) with behavioural variables as dependent variables, and grazing treatments and environmental variables as independent variables with the `lm`-function of the `vegan` package. We first checked for collinearity among environmental variables based on variance inflation factors (VIF) with the `lm`-function. We only accepted models where VIF of all terms were less than 10 by a stepwise backward procedure. This leads to removal of height of grasses and forbs, cover of forbs, plant biomass and forb biomass, air temperature, and solar radiation due to their high correlations ( $R^2 > 0.8$ ,  $VIF > 10$ ),  $R^2$  values among remaining environmental variables were mostly  $< 0.76$  ( $VIF = 1.7\text{--}2.6$ ). Thus, we used the model with grazing treatments, plant height, plant cover, grass cover, and grass biomass only to estimate grazing effects on behavioural variables of grasshoppers. To examine the potential interactive

effects of grazing and environmental variables on grasshopper behaviour, we used linear mixed models (LMMs) with `lme`-function of `nlme` package. We also analysed the effects of grazing on vegetation, microclimate, and behaviour, and abundance of grasshoppers among grazing treatments with the `lm`-function of the `vegan` package. The block and cage were included in the above three data analysis as random factors in order to take in account possible nested effects. We compared the variable means using Tukey tests with `multcomp` package, and results were considered significant at  $P \leq 0.05$ . All statistical analyses were performed using R version 3.2.4 (R Development Core Team 2016).

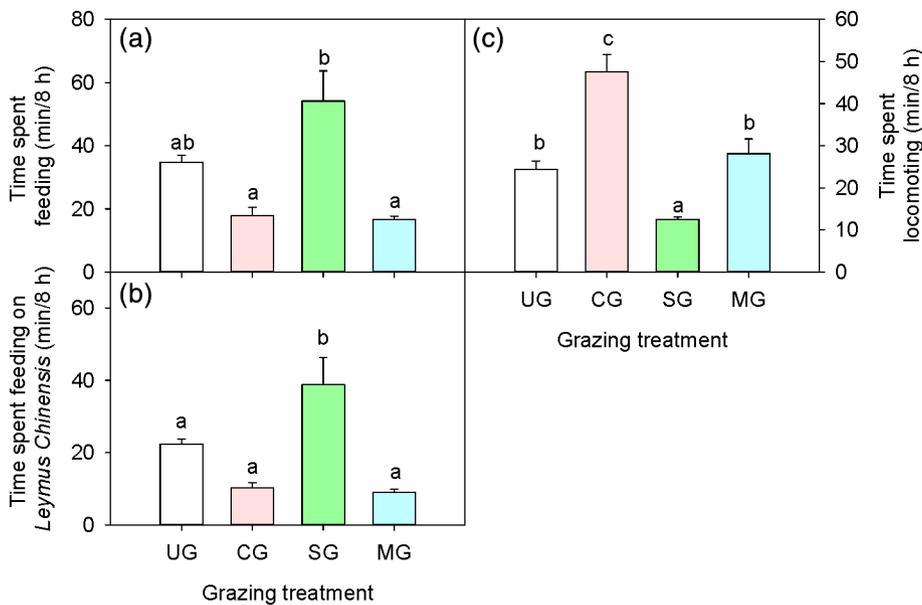
## Results

### Grazing effects on plants and microclimates

Grazing treatments had significant effects on biomass, cover, and plant height in entire plant community, or in the grass and forb communities, respectively. Taking plant communities as a whole, biomass in grazed plots was lower than that of control plots ( $F_{3,12} = 33.032$ ,  $P < 0.0001$ , Fig. 1a), plant height in grazed plots by cattle, and cattle and sheep together were lower than



**Fig. 2.** The effects of large herbivore assemblages (UG: Ungrazed, CG: cattle grazing, SG: sheep grazing, and MG: mixed grazing) on microclimate characteristics, including air temperature (a), and solar radiation (b). Values are means  $\pm$  SE. Different lowercase letters indicate significant difference among different grazing treatments ( $P < 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



**Fig. 3.** The effects of large herbivore assemblages (UG: Ungrazed, CG: cattle grazing, SG: sheep grazing, and MG: mixed grazing) on time spent feeding, including time spent feeding on plants (a), time spent feeding on *Leymus chinensis* (b), and time spent locomoting (c). Values are means  $\pm$  SE. Different lowercase letters indicate significant difference among different grazing treatments ( $P < 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

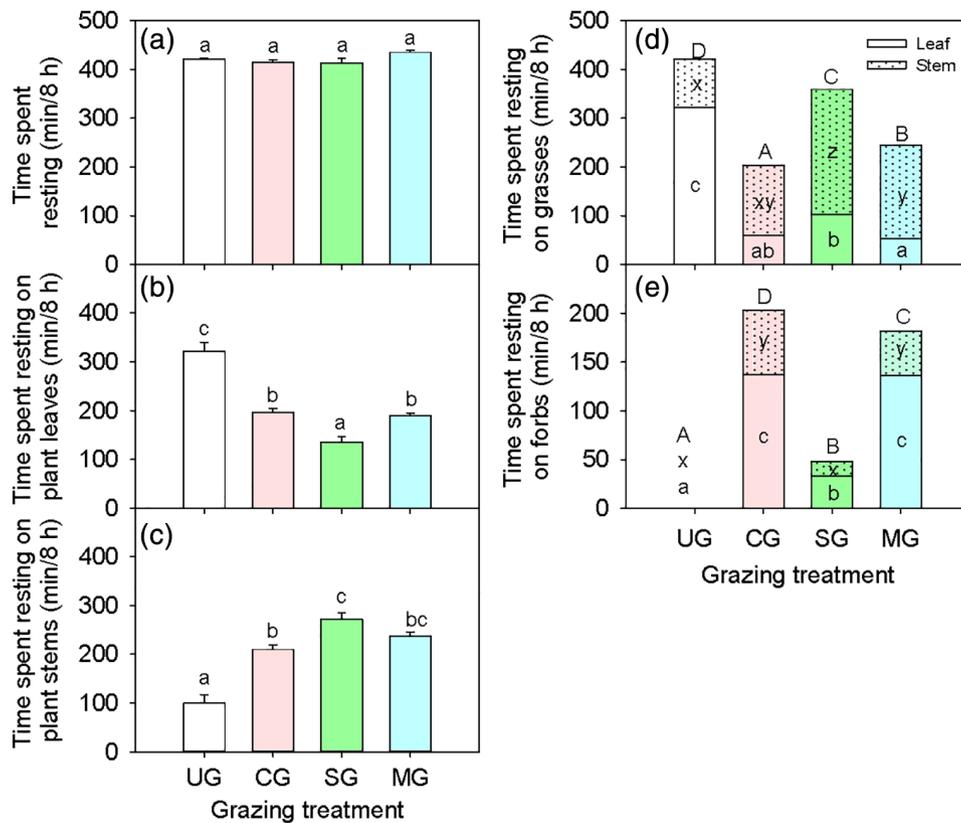
that of ungrazed plots ( $F_{3,12} = 21.541$ ,  $P < 0.0001$ , Fig. 1b), and plant cover in grazed plots by cattle and sheep was lower than that of ungrazed and other grazed plots ( $F_{3,12} = 15.226$ ,  $P < 0.0001$ , Fig. 1c). In grass communities, biomass, height and cover in grazed plots by cattle, and cattle and sheep were lower than that of other plots ( $F_{3,12} = 119.83$ ,  $P < 0.0001$  for biomass,  $F_{3,12} = 27.902$ ,  $P < 0.0001$  for height,  $F_{3,12} = 13.201$ ,  $P < 0.0001$  for cover, Fig. 1d–f). In forb communities, biomass in ungrazed plots was higher than that of other grazed plots ( $F_{3,12} = 129.99$ ,  $P < 0.0001$ , Fig. 1g), and cover in grazed plots by sheep was lower than that of other plots ( $F_{3,12} = 28.34$ ,  $P < 0.0001$ , Fig. 1i).

Air temperature in grazed plots by cattle and mixed grazing was higher than that of ungrazed plots (Fig. 2a), and solar radiation in grazed plots by cattle and mixed grazing was higher than that of ungrazed and sheep grazing plots (Fig. 2b).

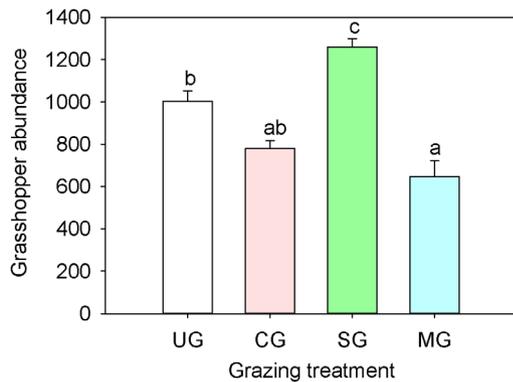
#### Grazing effects on behavioural activity of grasshoppers

Grazing had a remarkable effect on the time spent on feeding by grasshoppers ( $F_{8,15} = 9.547$ ,  $P = 0.0008$  for feeding time,  $F_{8,15} = 4.564$ ,  $P = 0.006$  for time feeding on *L. chinensis*,  $F_{8,15} = 9.851$ ,  $P < 0.0001$  for locomoting time) by the model analysis. Time spent on feeding all plants and *L. chinensis* by grasshopper *E. unicolor* significantly decreased in cattle, and mixed grazing treatments, but increased in sheep grazing treatment (Fig. 3a,b). Time spent on switching by *E. unicolor* in ungrazed plots was shorter than that of grazed plots by cattle and mixed grazing, but was longer than that of grazed plots by sheep (Fig. 3c).

Resting time, including resting time on plant leaves by *E. unicolor* ( $F_{8,15} = 63.943$ ,  $P < 0.0001$ ), plant stems ( $F_{8,15} = 45.06$ ,



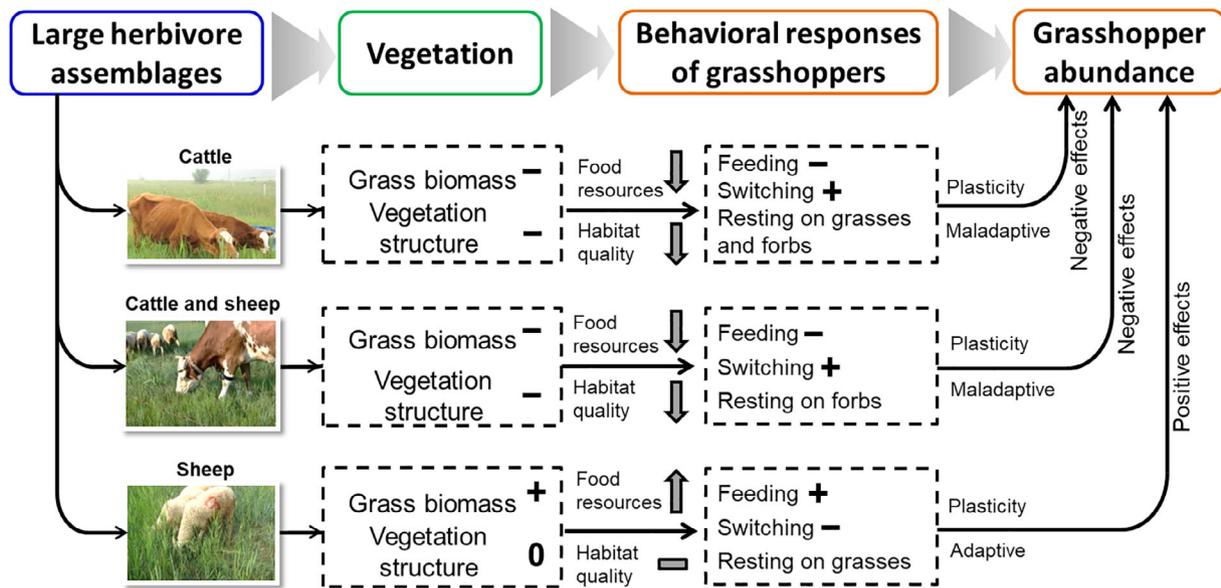
**Fig. 4.** The effects of large herbivore assemblages (UG: Ungrazed, CG: cattle grazing, SG: sheep grazing, and MG: mixed grazing) on the time spent resting, including time spent resting on plants (a), plant leaves (b), plant stems (c), leaf and stem of grasses (d), and leaf and stem of forbs (e). Values are mean  $\pm$  SE. Different letters indicate significant difference among different grazing treatments ( $P < 0.05$ ), lowercase letters a, b, and c was for plant leaves, plant stem, grass leaves, and for b leaves, lowercase letters x, y, and z was for stems of grasses and forbs, and capital letters A, B, C, and D was for grasses and forbs. [Colour figure can be viewed at [wileyonlinelibrary.com](#)].



**Fig. 5.** The effects of large herbivore assemblages (UG: Ungrazed, CG: cattle grazing, SG: sheep grazing, and MG: mixed grazing) on grasshopper abundance. Values are mean  $\pm$  SE. Different letters indicate significant differences among different grazing treatments ( $P < 0.05$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](#)].

$P < 0.0001$ ), grasses ( $F_{8,15} = 202.972$ ,  $P < 0.0001$ ), grass leaves ( $F_{8,15} = 139.291$ ,  $P < 0.0001$ ), grass stems ( $F_{8,15} = 36.135$ ,  $P < 0.0001$ ), forbs ( $F_{8,15} = 815.646$ ,  $P < 0.0001$ ), forb leaves

( $F_{8,15} = 74.399$ ,  $P < 0.0001$ ), and forb stems ( $F_{8,15} = 21.576$ ,  $P < 0.0001$ ), was significantly affected by grazing by the model analysis. Moreover, there were interactive effects of grazing and plant height on time spent resting on forbs ( $F_{1,3} = 7.123$ ,  $P = 0.0063$ ), interactive effects of grazing and plant cover on time spent resting on grass leaves ( $F_{1,3} = 4.427$ ,  $P = 0.028$ ), interactive effects of grazing and grass cover on time spent resting on grass leaves ( $F_{1,3} = 4.801$ ,  $P = 0.023$ ), grass stems ( $F_{1,3} = 3.978$ ,  $P = 0.038$ ), and forbs ( $F_{1,3} = 6.667$ ,  $P = 0.008$ ), and interactive effects of grazing and grass biomass on time spent resting on forbs ( $F_{1,3} = 5.897$ ,  $P = 0.012$ , Table S1). Compared to ungrazed plots, grazing was not significantly affected by total time spent resting by *E. unicolor*, but decreased time spent resting on stems and significantly increased time spent resting on leaves (Fig. 4). Moreover, grazing by cattle, and cattle and sheep significantly decreased *E. unicolor*' time spent resting on grasses, and increased time spent resting on forbs. Compared with ungrazed plots, *E. unicolor* spent less time resting on grass leaves in grazed plots, but spent more time resting on grass stems (Fig. 4d). Time spent resting on both forb leaves and stems significantly increased in grazed plots (Fig. 4e).



**Fig. 6.** An underlying framework of how grazing by large herbivore assemblages may affect the behavioural responses of grasshoppers through food resources and habitat availability, and consequently influencing their abundance. “+”, “-”, and “0” indicate that measurable variables either increased, decreased, no change. ↓, ↓, and ↓ indicate negative, positive, and no effect. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

#### Grazing effects on grasshopper abundance

Grasshopper *E. unicolor* abundance in grazed plots by cattle, and cattle and sheep was significantly lower, but was higher in grazed plot by sheep, compared to ungrazed plots (Fig. 5).

#### Discussion

In this study, grasshoppers clearly exhibited strong behavioural responses to grazing by large herbivores, and these responses appeared to have direct links to the variations in abundance. The behavioural responses by grasshoppers may stem from variation in food availability, perceived and/or actual risk of predation, and/or a combination of the two due to grazing (Rothley *et al.*, 1997; Lhomme *et al.*, 2018). *E. unicolor* mainly prefers grasses as food resource (Zhong *et al.*, 2014). However, cattle fed almost the same grass species, causing reduced grass biomass (especially that of *L. chinensis*) in grazed plots by cattle, and the mixed (Fig. 1). This leads to a decline in food resource and made *E. unicolor* to spend less time on feeding, and more time on switching for finding other host plants. Notably, sheep grazing preferably select forbs for food, and *E. unicolor* spend more time on feeding, especially feeding on *L. chinensis* after sheep grazing because it is likely that finding grasses is easier. High selectivity of grasses by grasshopper *E. unicolor* under lower or more food availability is one type of context-dependent foraging behaviour, which has been detected in other insects, such as ants (Pol *et al.*, 2017). Grasshoppers may alter their foraging behaviour by adjusting foraging efficiency based on food availability.

Grasshoppers take most of the time for resting, dependent on grazing. Generally, choosing microhabitats for resting depend

on food availability, thermal conditions, and predation risk (Merrill *et al.*, 2008). Although the total time spent resting did not change, *E. unicolor* prefers plant stems as their resting habitat after grazing. Possible reasons are as follows. Grasshoppers living in ambient temperatures outside of their preferred body temperature range behaviourally thermoregulate to achieve more preferred body temperature (Willott & Hassall, 1998; Ahnesjö & Forsman, 2006). Due to the leaf interception of direct solar radiation, grasshoppers select plant leaves as resting habitat. When grazing by large herbivores occurs, plant cover and height decreased (Fig. 1), and solar radiation and air temperature increased (Fig. 2), which force grasshoppers to rest on plant stems to achieve optimal body temperatures. Meanwhile, simplified vegetation structure resulted from grazing may enhance predation risk for grasshoppers (Woodcock *et al.*, 2007), and predators evidently make grasshoppers to use plant stems (i.e. lower position) for resting.

If grasshoppers were selecting resting habitats based on plant digestibility, they should select grasses, because grasshoppers prefer grasses as food (Zhong *et al.*, 2014); and if grasshoppers selected resting habitats based on optimal air temperature and predation risk, they would select plant stems, because lower positions in the canopy can provide optimal thermal and safe habitats (Pitt, 1999). After grazing, grasshopper *E. unicolor* have a behavioural mediation on resting, with shifting grasses toward forbs as their resting habitats in grazed plots by cattle, and cattle and sheep together, but still mainly selected grasses in grazed plots by sheep. Moreover, *E. unicolor* spent more time on resting on forb stems in cattle and mixed grazing treatments, decreased resting time on grass leaves. This selection is likely attributed to altered vegetation structure induced by grazing because the interactive effects of grazing and plant height, grass cover, and grass biomass

were significant (Table S1). Although we did not examine direct effects of food resource, predation, and microclimate, we indeed found that the behavioural responses of grasshoppers to grazing are plastic, with modification of foraging and habitat selection.

Generally, insect abundance at a given environmental condition depends on present abundance, births, deaths, immigration, and emigration of insects that to some extent closely link to behavioural flexibility of insects. Insects usually alter their behaviour to respond to different habitats when encountering major environmental changes (Long *et al.*, 2017). Thus, such behavioural activity of insects would likely relate to their abundance (van Baaren & Candolin, 2018) through survival and migration. When insects cannot find their suitable host plants or habitats, they may firstly adjust their behaviour rapidly to adapt to changing habitat (Snell-Rood, 2013). In this study, the results found that grasshopper *E. unicolor* altered the time spent feeding and resting to respond to large herbivore grazing, such changes in feeding behaviour and habitat selection may likely correlate with their survival and migration (Franzke *et al.*, 2010). In the plots by cattle and mixed grazing, *E. unicolor* spent less time on feeding plants, especially for their food plants, insufficient food ingestion could suppress survival and development of grasshoppers (Zhu *et al.*, 2020). Meanwhile, *E. unicolor* selected grass stems and forb leaves as resting habitat, but these habitats are unfavourable for their feeding, which could likely lead to more susceptible to predation when they look for food resources. Grasshoppers have to emigrate the grazed plots by cattle and the mixture of cattle and sheep, and consequently inducing a decrease in grasshopper abundance. Conversely, grazing by sheep has opposite effects on feeding behaviour and habitat selection, more food ingestion (i.e. *L. chinensis*), and suitable habitat make more grasshoppers immigrate toward the grazed plot, further enhancing grasshopper abundance. Additionally, grazing could affect grasshoppers within two periods, one is during the grazing process (i.e. immediate response), and the other is after the grazing activity (i.e. adaptive response). In this study, the responses of grasshopper behaviour after grazing were only focused because the immigration or emigration of grasshoppers in a given habitat was mainly derived from adaptation of the behaviour to the habitat. Therefore, behavioural plasticity may respond adaptively to changing environment or not, and maladaptive behaviour make species abundance decline, but adaptive behaviour can improve fitness (van Baaren & Candolin, 2018). These findings from this study indicated that grasshoppers showed adaptive or maladaptive behavioural responses to different large herbivore assemblages (Fig. 6), these behavioural activities may link to grasshopper abundance though no direct evidence is provided through survival and migration in this study.

## Conclusion

Our results demonstrate that grazing by large herbivores affects behavioural activities of grasshoppers, which may subsequently be related with grasshopper abundance in grassland ecosystems. More importantly, such effects depend on large herbivore

assemblages. These findings further indicate that behavioural responses of grasshoppers to grazing are driven by top-down effects of large herbivores on vegetation and microclimate. Clearly, behavioural changes of grasshoppers and potential consequences on abundance must be considered when large herbivores are used for grazing as a management tool. Particularly, careful selection of different herbivore species and/or their combinations should be practiced for achieving better conservation results in managed grasslands.

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## Author contribution

HZ, VN, and DW designed the study; HZ, VN, and JW collected data, HZ and HR analysed data, HZ and QG wrote the draft, and all authors contributed substantially to revisions.

## Data availability statement

The data that support the findings of this study are openly available in the Figshare repository [http://doi.org/\[doi\]](http://doi.org/[doi]), reference number [reference number].

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Interactive effects of grazing and environmental variables on behavioural activities of grasshoppers.

**Fig. S1.** Experimental setup in this study.

**Fig. S2.** Time arrangement on behavioural observation of grasshoppers in each plot.

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