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Herbivore phenology can predict response to changes in plant quality by livestock grazing

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Research

Herbivore phenology can predict response to changes in plant quality by livestock grazing

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Livestock grazing can have a strong impact on herbivore abundance, distribution and community. However, not all species of herbivores respond the same way to livestock grazing, and we still have a poor understanding of the underlying mechanisms driving these differential responses. Here, we investigate the effect of light intensity cattle grazing on the abundance of two grasshoppers (*Euchorthippus cheui* and *E. unicolor*) that co-occur in the same grasslands and feed on the same food plant (the dominant grass *Leymus chinensis*). The two grasshopper species differ in phenology so that their peak abundances are separated into early- and late-growing seasons. We used an enclosure experiment to monitor grasshopper abundance and food quality in the field under grazed and ungrazed conditions, and performed feeding trials to examine grasshopper preference for grazed or ungrazed food plants in the laboratory. We found that the nitrogen content of *L. chinensis* leaves continuously declined in the ungrazed areas, but was significantly enhanced by cattle grazing over the growing season. Cattle grazing facilitated the early-season grasshopper *E. cheui*, whereas it suppressed the late-season grasshopper *E. unicolor*. Moreover, feeding trials showed that *E. cheui* preferred *L. chinensis* from grazed plots, while *E. unicolor* preferred the leaves from ungrazed plots. We conclude that livestock grazing has opposite effects on the two grasshopper species, and that these effects may be driven by grazing-induced changes in plant nutrient content and the unique nutritional niches of the grasshoppers. These results suggest that insects that belong to the same guild can have opposite nutrient requirements, related to their distinct phenologies, and that this can ultimately affect their response to cattle grazing. Our results show that phenology may link insect physiological needs to local resource availabilities, and should be given more attention in future work on interactions between large herbivores and insects.

Keywords: indirect interactions, insect community, nutrient requirement, species coexistence, temporal pattern, vertebrate grazing



Introduction

There is increasing evidence for rapid biodiversity loss and community collapse at both local and regional scales (Sala et al. 2000). Human land use is often identified as an important driver of these population and diversity declines (Ollerton et al. 2014, Jantz et al. 2015), including livestock grazing, which is the most widespread land use in terrestrial habitats and an important biotic process affecting plant and herbivore communities (Eldridge et al. 2016, Wang et al. 2019). Among all herbivores, insects are by far the most important in terms of biomass, abundance and diversity. The role of livestock grazing, especially moderate and heavy grazing, in driving the richness and composition of insect communities has been well documented (Negro et al. 2011, Hao et al. 2015, Zhu et al. 2017), but the effects of light intensity grazing on insect communities are still relatively poorly understood. By modifying vegetation and habitats (Milchunas and Lauenroth 1993, Teague et al. 2011, Howison et al. 2017), large herbivore grazing has major effects on insect abundance, distribution and community composition (Debano 2006, Spalinger et al. 2012). However, the effects of large herbivores on insects are not consistent among studies, ranging from negative to neutral to positive (Joern 2004, Takagi and Miyashita 2014, van Klink et al. 2015, Li et al. 2018). This poses the question of why co-occurring insects are affected in different ways by large herbivores.

The pathways by which large herbivores affect insect communities are complex and manifold (van Klink et al. 2015, Vandegehuchte et al. 2017). Livestock will have negative effects on insects when they reduce the quantity and quality of shared food plant, and simplify the structural complexity of vegetation (Huntzinger et al. 2008), or positive effects when they increase plant quantity and quality or improve physical conditions for insects (Zhong et al. 2014, Li et al. 2018). The response of insects to livestock-induced changes may be species-specific and dependent on the insects' food and/or microsite preferences, which is determined by the morphology, physiology and ecological characteristics (Schoonhoven et al. 2005). It is known that insects from different feeding guilds differ in food or microhabitat preferences (niche segregation) (Novotny and Basset 2005, Knolhoff and Heckel 2014). However, responses may also differ between closely-related insect species that belong to the same guild. For example, there is evidence that several grasshopper species from the same genus have different nutrient requirements that may lead to niche segregation in the field (Behmer and Joern 2008). Nevertheless, knowledge on species-specific responses of taxonomically close insects to grazing is still scarce, and the mechanisms by which large herbivores drive these species-specific responses are not well understood.

Little is known about if the effects of livestock grazing can be impacted by the temporal dynamics of plant–insect interactions. The effects of large herbivores on insect species could vary within a season because phenological differences among

species can lead to variation in community composition over time (van Asch and Visser 2007, Venner et al. 2011, Fei et al. 2014). Furthermore, plant quality is dynamic and often changes over the season (Schoonhoven et al. 2005, Barton and Koricheva 2010). For instance, concentrations of plant foliar nitrogen decrease throughout the course of a growing season in North American forests (Zehnder et al. 2009) and grasslands (Oedekoven and Joern 2000). Therefore, large herbivores may have different effects on plant resources as well as on associated insects over time. It is well-known that grazing-induced changes in host food plant nutrients can have important consequences for insect herbivores. Yet most studies have focused on these interactions within a single time frame (e.g. peak of the growing season) (Martinsen et al. 1998, Cease et al. 2012, Zhu et al. 2019).

The aim of this study was to explore the effects of light intensity of livestock grazing on two closely related species of insect herbivores that differ in phenology. This study builds on previous work showing that domestic cattle *Bos taurus* grazing may affect the abundance of grasshoppers in a semi-arid grassland of northeastern China (Zhu et al. 2019). The species studied previously, *Euchorthippus unicolor* (Acrididae: *Arcypterinae*), peaks in abundance in August, whereas its sister species *E. cheui* is instead most abundant in July. Both grasshoppers mainly feed on the dominant grass species, *Leymus chinensis*, and share this food source with cattle. Because grass quality commonly changes over the season, different species of grasshoppers feeding at different times may therefore encounter foods of different quality (Schoonhoven et al. 2005). In this scenario, the effect of cattle may depend on how grazing affects the nutritional composition of grass and how grasshoppers respond to this variation. Here, we test three hypotheses: 1) livestock grazing affects the phenology of two co-occurring grasshoppers, 2) the abundance of grasshoppers with different phenology responds differently to grazing-induced changes in food characteristics and 3) if food is a key driver, these different responses to grazing lead to differential feeding preferences for plants originating from grazed and ungrazed plots.

To test these hypotheses, we first conducted a livestock grazing experiment in the semi-arid grassland to examine how domestic cattle affect plant quality, plant community composition and microclimate. We then examined how these factors alter the population density and phenological patterns of the two grasshoppers *Euchorthippus cheui* and *E. unicolor* in sites with and without livestock grazing. Finally, we used results from the livestock grazing experiment to develop feeding trials that tested whether grasshoppers prefer grass leaves from grazed or ungrazed plots.

Material and methods

Study area and species

Our study was conducted in an eastern region of the Eurasian steppe in the Jilin Province of northeast China

(44°45'N, 123°45'E). The study site has a continental climate with mean annual temperature and growing season temperature (1 May–30 September) of 5.7°C and 20.1°C, respectively. Annual precipitation is 280–400 mm with 70% falling during the growing season, especially between June and August. The meadow steppe in the area is dominated by the perennial grass *Leymus chinensis*, which accounts for 50–80% of the total aboveground vegetation biomass (Zhong et al. 2017). Experiments conducted at the same site have demonstrated that *L. chinensis* is a grazing-resistant species with strong compensatory regrowth after livestock activity (Wang et al. 2017). Other common plant species include grasses such as *Phragmites australis*, *Calamagrostis epigejos* and *Setaria viridis*; and forbs such as *Artemisia scoparia* and *Messerschmidia sibirica* (Liu et al. 2015). There are more than 13 common grasshopper species in the area, mainly coming from four genera, *Euchorthippus*, *Epacromius*, *Oedaleus* and *Calliptamus*. *Euchorthippus cheui* and *E. unicolor* are the two most numerous species in our study regions, together accounting for more than 65% of the total grasshopper abundance (Zhu et al. 2017).

Experimental design and livestock management

Historically, cattle and horses grazed at these areas, but from the 1970s to 2011, the study area (about 7.5 ha) was fenced to exclude livestock and human residents for grassland management and conservation purposes. In 2012, domestic cattle *B. taurus* were reintroduced into the study site. Domestic cattle feed predominantly on the grass *L. chinensis*, which accounts for 50% of their diet. Detailed descriptions of herbivore diet selection experiment can be found in Zhu et al. (2019). From 2012 to 2015, the study site was seasonally grazed by *B. taurus* from June to October at a stocking density of 0.61 cattle ha⁻¹, which represents a light intensity of grazing for this region. We established five 50 × 50 m fenced livestock enclosures ('no livestock' plots) that preclude livestock grazing. Plots were paired with five 50 × 50 m nonfenced plots with livestock access ('livestock' plots). All grasshopper species were permitted to move freely in and out of the plots.

Grasshopper population monitoring

In order to detect the effect of cattle grazing on phenology and peak population densities of the two grasshoppers, we monitored their density nine times from June to October of 2015. Grasshopper densities were sampled within each plot using standardized sweep netting, using a light muslin net of 30.5 cm diameter with a 150 cm handle, along two parallel 30 m long transects that were separated by 10 m. Transects were placed at least 5 m from the plot boundary to minimize edge effects. In each transect, 10 sweeps at 2-m intervals were performed in the vegetation (10 cm above the ground) and the samples from the two transects were pooled per plot. Previous studies suggest that sweep netting provides accurate estimates of grasshopper species richness and abundance (Joern 2004, Spalinger et al. 2012). All samples were

taken between 09:00 and 15:00 h on sunny, calm days. To minimize potential movements of grasshopper between plots during sampling, each pair (exclosure and adjacent control) was visited simultaneously and sampling order within pairs was random on every given sampling date. For each plot, we recorded only adults of *E. cheui* and *E. unicolor*. Nymphs of the two species were excluded from the sampling, as they are difficult to be distinguished in the field (especially from first to third instars). We then calculated the relative abundance of adult *E. cheui* and *E. unicolor* across the total sampling period.

Vegetation and microclimate measurements

We measured vegetation composition during time periods in August 2015 when live biomass was near peak levels. Plant cover was assessed in 14 quadrats (0.5 × 0.5 m) along the same transects used to monitor grasshopper populations. All plant species were identified and the percentage cover of each species was visually estimated. Vegetation was divided into three functional groups: *L. chinensis* (dominant grass), other grasses and forbs. Total cover can be less than 100% if the plot contained bare soil. The average cover of each plant group for the 14 quadrats per plot was used in statistical analyses.

We measured microclimatic conditions within each plot using the method described previously (Zhu et al. 2019). Understorey light availability, air temperature and humidity at 5 cm and 35 cm above the ground (grasshopper active zones) were measured within the same quadrats used for vegetation surveys. The average microclimatic parameters for the two transects in each plot was used in statistical analyses.

In order to test whether cattle grazing induced alterations in host plant quality, we measured a series of leaf attributes of *L. chinensis* between June and September 2015: carbon (C), nitrogen (N), water, cellulose, total phenol content and toughness. These attributes were selected because previous studies in other systems have suggested that they determine the palatability of plants for herbivores (Ibanez et al. 2013, Grutters et al. 2017). Leaf C and N content was measured seven times, whereas other variables were only measured three times for logistic reasons. At each sampling, twenty live *L. chinensis* individuals were collected randomly. Collected leaves were processed (sheaths and stems were removed), and fresh- and dry-weight measured to determine water content, then ground using a ball-mill and analysed for leaf N and C concentration using an elemental analyzer. Leaf toughness was measured using a modified penetrometer, which can record the force needed to penetrate a leaf with a thin rod (Siska et al. 2002). Cellulose was measured using the anthrone colorimetry method (Abidi et al. 2010), where dry ground samples were first mixed with 60% sulphuric acid for 30 min, filtered and diluted. The product was then allowed to react with 2% anthrone reagent and concentrated H₂SO₄, and the absorbance was thereafter measured at 620 nm on a spectrophotometer. Total phenols of *L. chinensis* were measured using the Folin-Ciocalteu method (Massey et al. 2005). Ground leaf samples were extracted in 70% acetone,

reacted with Folin–Ciocalteu reagent and saturated Na_2CO_3 for 30 min. The absorbance at 725 nm was measured on the spectrophotometer.

Grasshopper feeding trials

We conducted paired-choice feeding trials examining grasshopper consumption of plants originating from grazed and ungrazed plots to test the hypothesis that grazing-induced changes in host plant attributes affected feeding preferences. The feeding trials were conducted under a 16:8 light: dark photo cycle in an environmentally controlled room ($28 \pm 2^\circ\text{C}$ and 30% relative humidity). Female *E. cheui* and *E. unicolor* were originally collected at 5th instar (final nymphal instar) from field populations in the ungrazed plots. Nymphs were kept in a 1 m^3 cage on undamaged *L. chinensis* collected from the field until they moulted to adults. After two days of reaching the adult stage, *E. cheui* and *E. unicolor* were individually housed in cages of 0.15 m diameter and 0.3 m height and were kept overnight with sufficient water but no food (22 replicates per species). Fresh, undamaged *L. chinensis* leaves were then collected from the ‘livestock’ and ‘no livestock’ plots and each grasshopper was offered a choice between these leaves. Leaves were placed into water-filled petri dishes immediately upon collection through two slits on the lid within each mesh cage (Supplementary material Appendix Plate 1), with each petri dish receiving two leaves of equal size (8 cm long). Grasshoppers were left undisturbed until they fed substantially on at least one leaf (~40% removal) or up to a maximum of 30 h. Grasshoppers that either did not eat or ate both samples completely were excluded from the analyses because of lack of effective information (four replicates were removed in total). Original dry mass of each *L. chinensis* sample was calculated by multiplying the initial wet mass by the dry:wet mass ratio of *L. chinensis* leaves. The dry:wet mass ratio of *L. chinensis* was calculated from additional leaves that were treated similarly as in our selection experiment, but without subjecting them to herbivory. Dry leaf consumption was further calculated by subtracting the dry mass of the leaves after consumption from the calculated original dry mass for each plant sample. There were several slightly negative consumption values in the data set, possibly due to the use of calculated original dry mass, which is subject to a slight estimation error associated with the gravimetric method applied (Grutters et al. 2017). As no consumption was observed in these trials, the mean consumption of grass with negative values was set to zero in further analyses. For each species the feeding trials were conducted within the week corresponding to the species’ peak abundance in the field (*E. cheui* peaked in mid-July and *E. unicolor* in late August). Individual grasshoppers were used only once.

Data analysis

We used generalized linear mixed models with Poisson error structure to detect time partitioning between grasshoppers (glmer function in R lme4 package). In this analysis,

we considered the grasshopper species and the sampling time to be the fixed factors and time nested within replicate plot as random factors. We tested whether the interaction between the grasshopper species and the sampling date significantly explained the grasshopper abundance in the field (Venner et al. 2011). We used a similar generalized linear mixed model with grazing treatment and grasshopper species as fixed factors and the time nested within plot as random factors to test for opposite effects of livestock grazing on the abundance of two grasshopper species. The effects of livestock grazing on the phenological pattern of grasshoppers were analysed with a full model including grazing treatment, species and sampling time as fixed factors and plot as random factors. We used paired t-tests to assess the impact of livestock grazing on the phenological overlap between grasshopper species and phenological length of each species. Paired t-tests were also performed to evaluate the percentage of each species in the total grasshopper abundance over the growing season in livestock and no livestock plots; data for percentage of *E. cheui* and *E. unicolor* were arcsine transformed before analysis. Using the same method, we evaluated the impacts of livestock grazing on air temperature, air humidity and light penetration. Wilcoxon signed-rank tests were used to assess the effects of livestock grazing on plant cover for each plant group (*L. chinensis*, other grasses, forbs). Repeated measures ANOVA (ezANOVA function in ez package) with time as the repeated factor and grazing treatment as a fixed factor were used to assess the impacts of livestock grazing on the leaf nitrogen, carbon, toughness, cellulose and total phenols content in the host plant, the grass *L. chinensis*, during the growing season. Results were reported using the Greenhouse–Geisser correction when Mauchly’s test of sphericity was violated. Finally, we performed paired t-tests to examine the dry mass consumption of the two grasshoppers on *L. chinensis* leaves from both livestock and no livestock plots in the laboratory feeding trials. The homogeneity of the variances was tested for all response variables using a Levene’s test. The residuals were tested for normality using the Shapiro–Wilk test. For paired t-tests the normality of the difference between each pair of values was tested. Untransformed data are presented in the figures. All statistical analyses were performed in the open source software R ver. 3.1.0 (<www.r-project.org>).

Results

Effects of livestock grazing on grasshopper

As previously observed, the two grasshopper species had different peak emergence times (species \times time: $\chi^2 = 131.68$, $p < 0.001$; Fig. 1a). *Euchorthippus cheui* occurred early in the season, and hatched in mid-June with adults appearing in early to late July, whereas *E. unicolor* occurred later in the season and emerged in late July with adults appearing in mid-August to early September. Contrary to our first hypothesis, livestock grazing did not change the phenological pattern of the two grasshopper species, as indicated by a non-significant

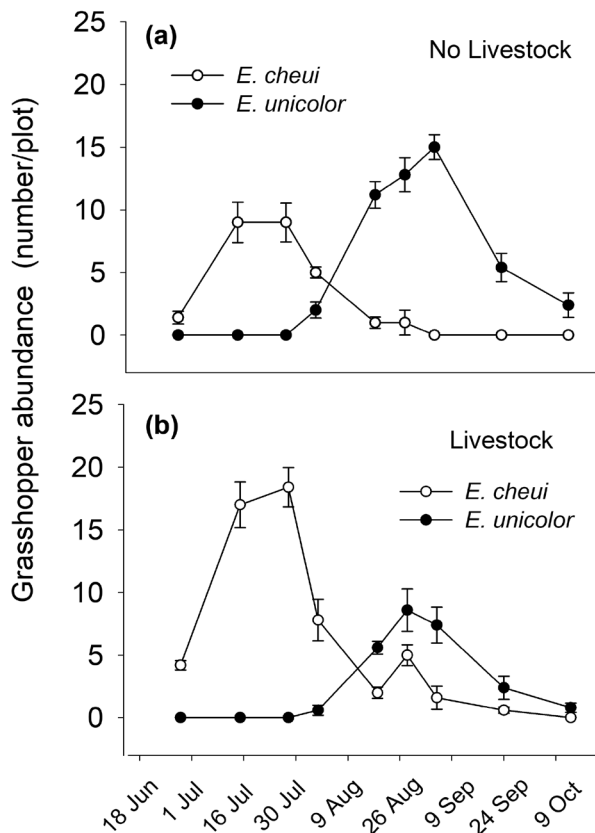


Figure 1. Population dynamics of the two grasshopper species (a) without and (b) with livestock grazing. Abundance of grasshopper *Euchorthippus cheui* and *E. unicolor* (number of grasshoppers in 20 sweeps per plot) were monitored nine times during the whole growing season. Values are means \pm SE.

interaction effect between grazing treatment, species and sampling time ($\chi^2 = 1.00$, $p = 0.32$; Fig. 1b).

Consistent with our second hypothesis, however, the abundance of the two grasshopper species was affected in opposite directions by livestock grazing, as was indicated by an interaction between grazing treatment and grasshopper species ($\chi^2 = 88.66$, $p < 0.001$; Fig. 1). The relative abundance of *E. cheui* was 34% higher in plots grazed by livestock than in plots without livestock grazing ($t_4 = -3.81$, $p = 0.02$; Supplementary material Appendix 1 Fig. A1a), while the relative abundance of *E. unicolor* was 46% lower in plots with livestock grazing compared to plots left ungrazed ($t_4 = 4.24$, $p = 0.01$; Supplementary material Appendix 1 Fig. A1b).

Effects of livestock grazing on vegetation and microclimate

Livestock grazing did not affect plant cover for any plant functional group (Supplementary material Appendix 1 Table A1). In particular, *Leymus chinensis*, the main food plant for the grasshoppers, was equally abundant in grazed and ungrazed plots (with 31% absolute cover and 59% of total plant cover). Livestock grazing did not affect air temperature,

relative humidity or understory light penetration at either ground surface or 35 cm above the ground (Supplementary material Appendix 1 Table A2).

Livestock grazing increased the N content of *L. chinensis* leaves (grazing: $F_{1,8} = 406.18$, $p < 0.001$; Table 1, Fig. 2). Moreover, the N content of *L. chinensis* gradually decreased over the growing season in plots without livestock, whereas it was stable in livestock-grazed plots (time: $F_{6,48} = 20.34$, $p < 0.001$). The effect of grazing on leaf N content was therefore stronger late in the growing season (grazing \times time: $F_{6,48} = 11.32$, $p < 0.001$). The C content of *L. chinensis* leaves changed over time ($F_{6,48} = 5.60$, $p < 0.001$; Table 1, Supplementary material Appendix 1 Fig. A3) and there was a significant interaction effect between grazing treatment and sampling time ($F_{6,48} = 3.69$, $p = 0.004$). Livestock grazing also increased the total phenolic content in *L. chinensis* (grazing: $F_{1,8} = 14.04$, $p = 0.006$; Table 1, Supplementary material Appendix 1 Fig. A2b), but this effect did not vary over the growing season (grazing \times time: $F_{2,16} = 0.61$, $p = 0.32$, corrected using Greenhouse–Geisser estimates of sphericity). Finally, livestock grazing did not change leaf toughness and water content (Table 1), whereas a significant interaction effect between grazing treatment and time on the cellulose content of *L. chinensis* could be detected ($F_{2,16} = 6.26$, $p = 0.01$; Table 1, Supplementary material Appendix 1 Fig. A2c). Grazing increased the cellulose content of *L. chinensis* in July, whereas the cellulose content was similar in grazed and ungrazed plots in August and September (Supplementary material Appendix 1 Fig. A2c).

Table 1. Repeated measures ANOVA for the effects of livestock grazing on the quality of *Leymus chinensis* leaf tissue during the growing season. Leaf nitrogen (N) and carbon (C) were sampled seven times from June to September; leaf water content, toughness, cellulose and total phenolic content were sampled three times from July to September in 2015. Grazing is the effect of large herbivore grazing and time is the effect of sampling date. Bold numbers indicate a significance of $p < 0.05$.

	Factors	df	F	p
N content (%)	Grazing	1,8	406.18	<0.001
	Time	6,48	20.34	<0.001
	Grazing \times Time	6,48	11.32	<0.001
C content (%)	Grazing	1,8	1.87	0.21
	Time	6,48	5.60	<0.001
	Grazing \times Time	6,48	3.69	0.004
Water content (%)	Grazing	1,8	4.30	0.07
	Time	2,16	7.32	0.006
	Grazing \times Time	2,16	0.06	0.94
Toughness (N)	Grazing	1,8	1.14	0.32
	Time	2,16	11.01	<0.001
	Grazing \times Time	2,16	0.34	0.72
Cellulose (%)	Grazing	1,8	1.15	0.31
	Time	2,16	0.76	0.48
	Grazing \times Time	2,16	6.26	0.01
Total phenols (%)	Grazing	1,8	14.04	0.006
	Time	2,16	0.61	0.44
	Grazing \times Time	2,16	0.61	0.32

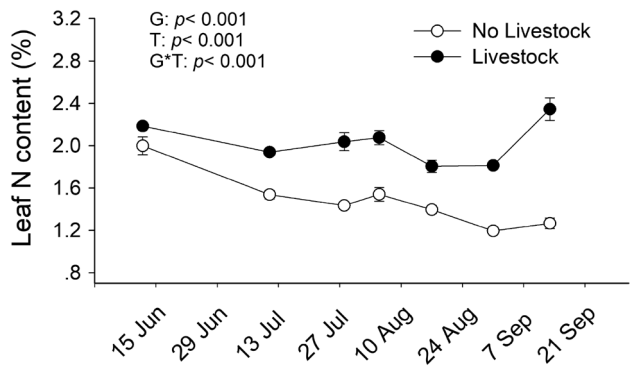


Figure 2. Concentration of leaf nitrogen (percentage dry mass) in the grass *Leymus chinensis* with and without livestock grazing during the growing season. *Leymus chinensis* is the primary host plant of *Euchorthippus cheui* and *E. unicolor* in our grassland. G is the effect of livestock grazing and T is the effect of sampling date (time). Each data point represents five replicate plots. Values are means \pm SE.

Grasshopper feeding selection

Consistent with our third hypothesis, the food selection experiment revealed that *E. cheui* preferred leaves of *L. chinensis* from plots where livestock had grazed ($t_{19} = -3.46$, $p = 0.003$; Fig. 3). In contrast, *E. unicolor* preferred leaves from plots in which livestock had been excluded ($t_{19} = 2.63$, $p = 0.02$).

Discussion

Livestock grazing is a key determinant of insect abundance, distribution and community composition in grasslands (Joern 2004, Takagi and Miyashita 2014, van Klink et al. 2015). However, if and how the temporal dynamics of plant–insect interactions can predict the effects of livestock grazing

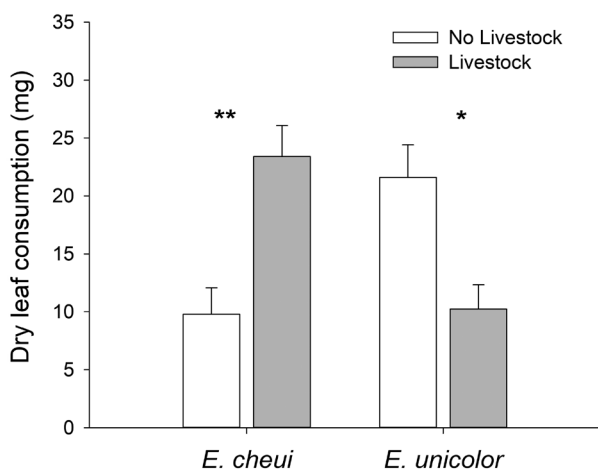


Figure 3. Dry plant consumption over a 30 h period by 5th instar female *Euchorthippus cheui* and *E. unicolor* on *Leymus chinensis* leaves from livestock and no livestock plots. Values are means \pm SE. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

has often been ignored. Our study demonstrates that livestock grazing produces contrasting effects on two grasshopper species differing in phenology, because the two species respond differently to the increased nitrogen (N) content in their food plant following livestock grazing (Fig. 4). As we argue below, evidence points to the early-season species, *Euchorthippus cheui*, shows higher population abundance in livestock-grazed plots because this species prefer grass with a higher N content. In contrast, the second species, *E. unicolor*, is negatively impacted by grazing, because it prefers grass with lower N content. Our results suggest that more attention is needed to integrate temporal and spatial variation (in the case of grazed and ungrazed patches) in plant quality to explain shifts in the structure and composition of insect communities at the landscape scale. Furthermore, our results emphasize that aside from the spatio–temporal patterns of plant resources, phenology may link insects’ physiological needs to their surrounding resource conditions and should be taken into consideration in the study of plant–herbivore interactions.

Livestock grazing had only limited effects on the phenology of the two co-occurring grasshoppers, allowing us to reject our first hypothesis. Grazing did not affect the timing of emergence and peak abundance of each grasshopper species. We suggest that these results may be attributed to the light grazing regimes (stocking density of 0.61 cattle ha^{-1}), which did not affect the environmental cues (i.e. temperature, food availability) that may trigger phenology shifts of grasshoppers. Livestock grazing pressure imposed in our system was not high enough to decrease the proportion of *Leymus chinensis*, the host plant for the two grasshoppers, nor did it significantly change microhabitat conditions for grasshoppers.

In contrast to the slight effects on phenological patterns due to grazing, livestock did strongly alter the actual abundance of the grasshopper species. However, they did so in different ways. This result is consistent with our second hypothesis. Zhu et al. (2017) found species-specific responses of *E. cheui* and *E. unicolor* to sheep grazing under altered precipitation, but the underlying mechanisms have not been explored. In the present study, light-intensity livestock grazing increased plant nutrient content, but affected neither the plant community composition nor the microclimate conditions. Therefore, we suggest that the differential response of *E. cheui* and *E. unicolor* is mainly driven by livestock-induced changes in plant quality. Many lines of inference predict that herbivorous insects could respond directly to the spatially variable distributions of nutrient content in host plants (Behmer 2009, Loaiza et al. 2011). We found that livestock grazing significantly promotes high leaf N content of *L. chinensis* leaves during the whole growing season, and *E. cheui* was more abundant in plots with livestock, while *E. unicolor* preferred plots without livestock. Our previous experiments have documented that enriching plant N has a negative effect on *E. unicolor* abundance and performance (survival and body size) (Zhu et al. 2019). Cease et al. (2012) similarly showed that N fertilization and an increase in the protein:

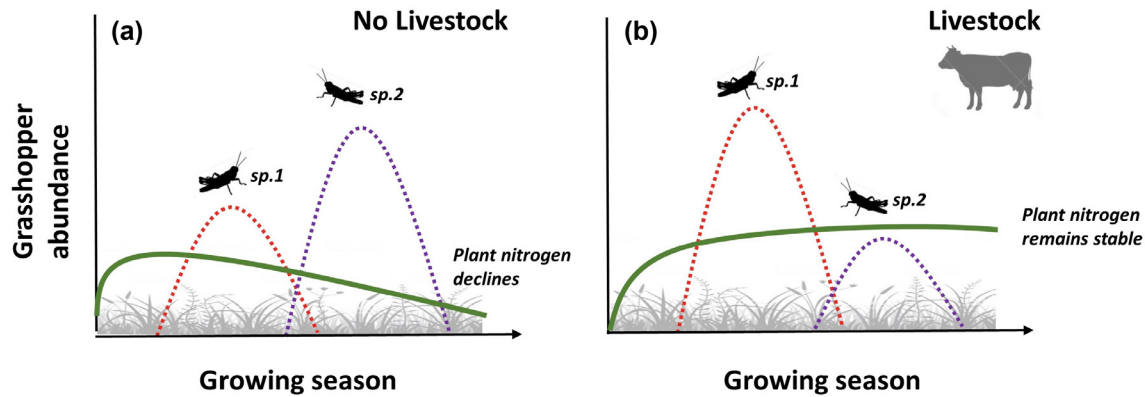


Figure 4. Population abundances of two grasshopper species with different phenology and temporal dynamics of nutrient concentration of host plant (a) without and (b) with livestock grazing along the growing season. The solid line represents grass nitrogen content and the dashed line represents grasshopper abundance; sp. 1 and sp. 2 indicate grasshopper *Euchorthippus cheui* and *E. unicolor*, respectively.

carbohydrate ratio of artificial food negatively affected the performance of grasshopper *Oedaleus asiaticus* in the Eurasian steppe. These studies indicate that responses of insect herbivores to variation in nutritional quality are complex, and cannot be explained fully by the traditional view that high N content is always beneficial for insect herbivores (Tao et al. 2014, Simpson et al. 2015, Ibanez et al. 2017). Moreover, although it may be intuitive to assume that nutrient preference of closely-related species should be similar, our results show that congeners can still have distinct nutrient preferences and respond in contrasting ways to environmental changes, supporting similar observations in other related insect species (Lee et al. 2006, Behmer and Joern 2008). However, it should be noted that the effect of livestock on plant N content may vary based on the grazing intensity (Briske 1996) and the present study of the interactions between livestock and insect herbivores only looked at a relatively light grazing intensity. Whether or not these drastically different responses of closely-related insects with different nutritional niches will be sustained or changed as grazing intensities increases, deserves further attention.

In line with our third hypothesis, we found that grasshoppers had differential preferences for leaves from grazed and ungrazed plots. *E. cheui* preferred leaves from plots grazed by livestock, which had higher N content. In contrast, *E. unicolor* was specialized for coping with less N-rich food, consistent with its peak abundance in the late season when plant N content is lower than early in the growing season. Previous work suggests that differences in nutrient preference exhibited by the insects may reflect the nature of the environment they typically encountered (Cease et al. 2012, Ohgushi 2016). We documented that the leaf N content of *L. chinensis* decreased steadily along the growing season. As such, grasshoppers with different phenological patterns may encounter quite different nutritional environments, i.e. early-season grasshoppers cope with high-N food available early in the season and vice versa. Therefore, different nutritional environments may exert different selection pressures such that species can deal efficiently with the resources that are available (Simpson et al. 2015).

In spite of this, to what extent post-ingestive regulation of two grasshoppers can contribute to their nutrient acquisition from dietary nutrient variability is still unclear. Our results are in line with work showing that spatio-temporal patterns of plant quality can explain local-scale, spatial and/or temporal distributions of insect herbivores, especially grasshoppers (Loaiza et al. 2011, Masloski et al. 2014).

We conclude that plant N content is a major driver of livestock effects on grasshopper abundance and food preference, still other plant attributes may also play a role. For example, cattle grazing significantly increased total phenols of *L. chinensis* grass. Phenolic compounds are often seen as feeding deterrents, especially in grasses, which generally lack N-rich alkaloids or other allelochemicals against herbivory (Grutters et al. 2017). Although the amount of total phenols was significantly different between grazed and ungrazed treatments, the magnitude of difference was only four percent. There is evidence that some other graminivorous grasshoppers, for instance *Ageneotettix deorum* and *Phoetaliotes nebrascensis*, are not sensitive to variations in plant phenolic content, even at a four-fold level difference in concentration (Mole and Joern 1994). In addition, other attributes, such as silica content, may also act as physical defenses against herbivores (Clissold et al. 2018). We did not directly measure silica in *L. chinensis* leaves, but leaf toughness (which is positively correlated with silica in plants, Massey et al. 2007) was not affected by cattle grazing, implying that plant physical defense was unlikely an important factor driving the responses of the grasshoppers. It should be noted that, however, we could not exclude the role of cellulose and total carbon content of *L. chinensis* in regulating grasshopper population dynamics, because we detected significant interaction effect between grazing treatment and sampling time on these two variables. Dietary phosphorous content may also be important for insect herbivore performance (Ibanez et al. 2017). Furthermore, the geometric framework emphasizes that the balance of protein and carbohydrate is more important to herbivores than the absolute availability of single nutrients or elemental proxies that include other non-nutritive molecules

(Raubenheimer and Simpson 1993). Hence, future work should consider measuring the ratio of plant digestible protein and nonstructural carbohydrate to explain the grasshopper distribution. Since we were unable to fully disentangle all the underlying mechanisms contributing to feeding preferences by the two grasshopper species, future studies in which plant traits are experimentally manipulated would be required.

Our study examined the effects of temporal variation on multitrophic interactions, a phenomenon that has not been well studied in natural systems. The phenology within different guilds of herbivores (mammals, birds, insects) is important because it captures many life history traits of a species, and it is a useful proxy reflecting adaptation of herbivores to changing environments (Schoonhoven et al. 2005). Phenology therefore could be an important indicator of the vulnerability of species and communities to human-induced environmental change. As we have shown here, even a small phenological difference between closely-related species can translate into large and ecologically significant effects. These differences can potentially predict the effects of altered environments and provide further insight into anthropogenically driven impacts on species interactions and ecosystem processes. By affecting the abundance of herbivores differing in phenology, livestock can also have a potential cascading effect on other trophic levels, such as the plant community (Carson and Root 2000, La Pierre et al. 2015). In our study, livestock grazing resulted in a higher abundance of the early-phenology species and a lower abundance of the late-phenology species, leading to a strong shift in the proportion of the two species within the grasshopper community. As a result, the plant community would probably suffer a larger impact of herbivory, because early-season plants are generally more vulnerable to insect herbivory (Waterton and Cleland 2016). However, understanding the cascading effect of livestock grazing on the vegetation will require future studies that use a community approach and that give attention to the identities of the insect and plant species, not only their temporal pattern.

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.866t1g1mv>> (Zhu et al. 2020).

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Author contributions – YZ, DW, LW and ZZ designed experiments; YZ, QM, HL and DP performed the experiments; YZ, GV, XL and EB analyzed the data; YZ, GV, LW, ZZ and EB drafted the manuscript; YZ, ZZ, GV and EB revised the manuscript.

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Supplementary material (available online as Appendix oik-07008 at <www.oikosjournal.org/appendix/oik-07008>). Appendix 1.