

## RESEARCH ARTICLE

# Negative effects of vertebrate on invertebrate herbivores mediated by enhanced plant nitrogen content

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## Abstract

1. Classic theory holds that the main interaction within the herbivore guild is competition, based on research focused on co-occurring, similarly sized species that reduce the quantity of shared plant resources. However, plant quality may also be crucial in mediating herbivore interspecific interactions. This is especially true when competition occurs between distantly related herbivore species, given that small terrestrial herbivores (e.g., insect herbivores) appear to be more sensitive to alterations of plant quality than plant quantity.
2. In this study, we first tested in the field whether large vertebrate herbivores (cattle *Bos taurus*) exerted a negative effect on smaller insect herbivores (grasshopper *Euchorthippus unicolor*) through their overlapping foraging preferences for a dominant grass *Leymus chinensis*. We measured changes in grass quantity, grass quality, and microclimatic conditions in response to vertebrate grazing and conducted additional manipulative studies in the field and the laboratory to identify potential mechanisms underlying the interaction.
3. Our results showed that grazing by large herbivores caused a significant decline in grasshopper population density and individual performance (survival, size, and weight of both female and male *E. unicolor*), despite a 38% increase in grass nitrogen (N) content in grazed plots. Experiments manipulating N levels of *L. chinensis* in the field and the laboratory confirmed that enriching plant N had a negative effect on grasshopper individual performance and population size. Therefore, enhanced quality (N content) of plant resources appears to be an important driver in mediating the negative effect of vertebrate grazing on grasshoppers.
4. *Synthesis.* We document that phylogenetic relatedness and trait similarity can be poor predictors of interaction strength in some cases, since distantly related herbivores of disparate size can interact indirectly via changes in plant quality. Counter-intuitively, the observed negative effect of cattle on grasshoppers was mediated, at least in part, by an increase in plant quality in cattle grazed areas. The implication is that light to moderate grazing, a common management strategy, may contribute to suppression of grasshoppers in the Eurasian steppe grassland system by altering plant nutrient supplies.

## KEYWORDS

body size, density-mediated indirect effect, indirect interactions, large herbivores, nutrient requirement, plant quality, species coexistence, trait-mediated indirect effect

## 1 | INTRODUCTION

Competition theory predicts that ecological similarity among species enhances the intensity of their interactions (Connell, 1983; Schoener, 1974). Thus, in the case of interactions among species within the herbivorous guild, the vast majority of work has traditionally focused on closely related species of similar size (e.g., Denno et al., 2000; Karban, Grof-Tisza, & Holyoak, 2012; Odadi, Karachi, Abdulrazak, & Young, 2011). However, competition among herbivores does not always conform to traditional paradigms, and phylogenetic relatedness and trait similarity can be poor predictors of interaction strength in some cases (Eubanks & Finke, 2014; Gurevitch, Morrow, Wallace, & Walsh, 1992; Hochberg & Lawton, 1990; Kaplan & Denno, 2007). Increasingly, ecologists are recognising the fact that interactions among distantly related herbivores are prevalent and relevant, with far-reaching effects on community assembly, organisation, and dynamics (Bakker, Dobrescu, Straile, & Holmgren, 2013; Belovsky, 1986; Pringle, Young, Rubenstein, & McCauley, 2007).

Competition for shared host plants between large grazing mammalian herbivores and smaller herbivores, such as arthropods, is a classic example of interaction among phylogenetically distinct groups. Recent meta-analytical reviews of the literature find that, on average, the effects of large herbivores on smaller animal taxa are negative, both in terms of abundance and diversity (Foster, Barton, & Lindenmayer, 2014; Takagi & Miyashita, 2014; van Klink, van der Plas, van Noordwijk, WallisDeVries, & Olff, 2015); however, there is considerable variation in the outcome of individual studies including positive (Cao et al., 2015), negative (Pringle et al., 2007), and neutral effects (Riipi, Lempa, Haukioja, Ossipov, & Pihlaja, 2005) of large herbivores on insect populations. While some of this context-dependency is attributed to differences in the spatial and temporal scale of the study, differences across systems in the processes linking large and small herbivores also play a significant role (Takagi & Miyashita, 2014).

The mechanisms underlying the effects of large herbivores on smaller herbivores include both direct and indirect pathways (as reviewed by van Klink et al., 2015). Direct interactions result from incidental ingestion or trampling of smaller herbivores by large herbivores (Gómez & González-Megías, 2002). Indirect effects may result from changes in plant quantity (i.e., density-mediated indirect effect), such as when large herbivores reduce plant biomass (Huntzinger, Karban, & Cushman, 2008; Vandegehuchte, Schütz, de Schaetzen, & Risch, 2017), thus limiting the availability of resources for other herbivores. The outcome of competition between differently sized herbivores tends to be highly asymmetrical; thus, these indirect effects are typically negative for the abundance of

the smaller herbivore and neutral for the larger (Gómez & González-Megías, 2002). However, in situations where the smaller herbivore prefers a different host plant than that consumed by the large herbivore, facilitation (i.e., commensalism/mutualism) may occur. As an example, Zhong et al. (2014) found that domestic sheep benefit grasshoppers by selectively consuming *Artemisia* forbs. The reduction in forb biomass enhances the ability of grasshoppers to locate their preferred *Leymus* grass host plant. Indirect effects may also result from herbivore-induced changes in host plant quality or architecture (i.e., trait-mediated indirect effect) (e.g., Ohgushi, 2005). For example, in the African savanna large grazers are reported to benefit other grazers (McNaughton, 1976), either by stimulating regrowth of plants, which is usually of better quality than mature leaves, or by returning nutrients in the form of faeces and urine (McNaughton, 1979). Feeding and trampling may also decrease the height and structural complexity of vegetation, thus altering the physical environment encountered by smaller herbivores (Huntzinger et al., 2008; Pagès et al., 2012; Pringle, 2008). Simplification of plant structure may expose herbivores to harsh abiotic conditions or render them more vulnerable to predators (Suominen, Danell, & Bergström, 1999; van Klink et al., 2015), a type of indirect effect known as environment-mediated interaction modification (Wootton, 1992).

As highlighted above, large and small herbivores might interact through a diversity of pathways, which depend on the traits of the entire suite of herbivores, the traits of the plant community and environmental factors, making the prediction of the direction and strength of interactions a complex matter. For arthropods, in particular, responses are most often attributed to altered plant biomass and structure rather than changes in plant quality (Krueß & Tscharnke, 2002; Lind et al., 2017; Takagi & Miyashita, 2014), but the impacts of grazer-induced changes in plant quality are often not investigated directly (Lind et al., 2017; Moran, 2014). Indeed, alteration of plant quality is likely to have profound impacts on small herbivores, especially for some specialist insects (Behmer & Joern, 2008; Belovsky, 1997). Current paradigms usually assume that insect herbivores in terrestrial systems are N limited due to the relatively lower N content of plant tissues (Mattson, 1980). Most N in plants is in the form of protein; therefore, an increase in plant N content can result in higher herbivore performance by relieving constraints on growth and reproduction (White, 1993). However, studies with grasshoppers (Cease et al., 2012; Clissold, Sanson, & Read, 2006) and butterflies (Fischer & Fiedler, 2000; Tao, Berns, & Hunter, 2014) have demonstrated that ingesting excessive N can lead to negative effects on insect performance by disrupting their nutritive equilibrium (Raubenheimer & Simpson, 1993). Also, the enhancement of plant N could drive detrimental effects on herbivorous insect performance by increasing the concentration of alkaloids or other N-based

allelochemicals (Behmer, 2009). In the latter case, high N content should not be equated to higher plant quality. Given the uncertainty in the response of insect herbivores to N, a direct test of the effects of large mammalian herbivory on plant quality and its consequences for phytophagous insects is needed.

Here, we explore the interaction between large domestic herbivores and small herbivorous insects in a semi-arid grassland ecosystem. The dominant plant community in this system is the grass *Leymus chinensis*, with a variety of less abundant forb species (Wang, Du, Zhang, Ba, & Hodgkinson, 2017; Zhu et al., 2012). The herbivore community is dominated by the large vertebrate *Bos taurus* (i.e., domestic cattle) and the small insect grasshopper *Euchorthippus unicolor*. Both cattle and *E. unicolor* grasshoppers prefer *L. chinensis* grass, and rarely feed on forbs (Liu et al., 2015; Zhong et al., 2014). Previous studies have found negative effects of ungulate grazing on the populations of grass-feeding grasshoppers due to reductions in plant biomass (Capinera & Sechrist, 2012) and positive effects of ungulate grazing due to increased structural heterogeneity (Joern, 2004), but did not directly measure plant quality. One previous study specifically investigated the response of grasshoppers to plant quality and found higher abundance of grasshoppers in heavily grazed areas where grass N content was reduced, but did not measure the possibly confounding effects of grazing on plant abundance and structure (Cease et al., 2012). No studies have directly manipulated the presence of grazing herbivores and systematically examined multiple mechanistic pathways of impact on grasshoppers, including altered plant quantity, microclimate conditions, and plant quality.

Our work is novel in that we used a set of field and laboratory manipulative experiments to directly investigate the potential pathways whereby large domestic herbivores affect *E. unicolor* grasshoppers, including plant quantity, microclimate conditions, and plant quality. We addressed the questions: (i) does large herbivore grazing affect the individual performance and population abundance of co-occurring *E. unicolor* grasshoppers, and if so, what is the underlying mechanism? Specifically, we ask (ii) does large herbivore grazing modify plant resource availability (i.e., density-mediated indirect effect)?, (iii) does large herbivore grazing modify the microclimatic conditions experienced by small herbivores (i.e., environmentally mediated indirect interaction)?, and (iv) does large herbivore grazing modify plant resource quality (i.e., trait-mediated indirect effect)?

## 2 | MATERIALS AND METHODS

### 2.1 | Study system and background

Our study was conducted at the Grassland Ecological Research Station, Northeast Normal University, Jilin Province, China (44°45'N, 123°45'E). The study site is located in the eastern region of the Eurasian steppe, where the climate is semi-arid, with cold, arid winters and hot, rainy monsoonal summers. Annual mean temperature ranges from 4.6°C to 6.4°C, and annual precipitation varies between 280 and 400 mm, with 70% falling during the growing season, especially between June and August.

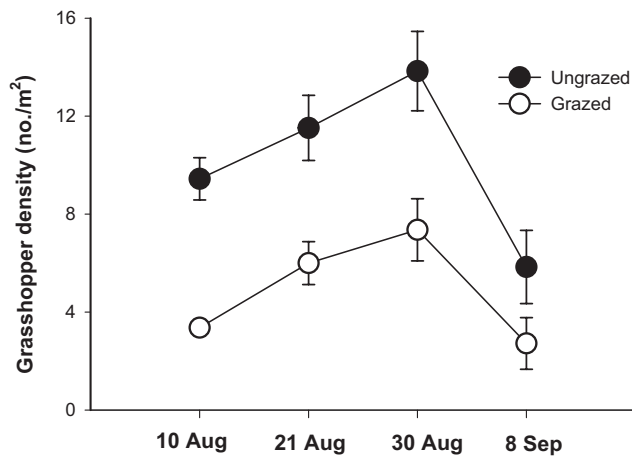
The meadow steppe in the area is dominated by the perennial rhizomatous grass *L. chinensis*, which has strong compensatory regrowth after grazing or clipping disturbance (Gao, Wang, Ba, Bai, & Liu, 2008; Wang et al., 2017). *Leymus chinensis* provides food and substrate to a great number and variety of native and domestic herbivores. Other common species at this site include grasses such as *Phragmites australis*, *Calamagrostis epigejos*, and *Setaria viridis*; and forbs such as *Artemisia scoparia* and *Messerschmidia sibirica* (Liu et al., 2015). From the 1970s to 2011, the study area was fenced to exclude livestock and human residents for grassland management and conservation purposes.

The main large vertebrate herbivores in the area are domestic cattle (*B. taurus*), which prefer the dominant grass *L. chinensis* and rarely feed on forb species (Liu et al., 2015, also see Supporting Information Appendix S1). The major invertebrate herbivores at the site are grasshoppers (Oedipodinae, Acrididae) and leafhoppers (Hemiptera, Cicadellidae) (Zhong et al., 2017; Zhu et al., 2012). The *Euchorthippus* grasshopper dominates the herbivorous insect community, accounting for >65% of all insect abundance annually (Zhong et al., 2017). Grasshopper *E. unicolor* usually displays one generation a year and displays five nymphal stages: eggs typically hatch in late July to early August, becoming adults by mid-August, and reaching peak densities by the end of August (Zhong et al., 2014). *Euchorthippus unicolor* shares similar dietary preferences with domestic cattle: it feeds predominately on *L. chinensis* grass, and only seldom on forbs (Supporting Information Appendix S1). Thus, the diets of both dominant vertebrate and invertebrate herbivorous species in this semi-arid grassland ecosystem are completely overlapping.

Domestic cattle were introduced in our study site in 2012, seasonally grazed from June to September and their grazing is maintained at a light to moderate intensity (0.1–0.3 animal units/ha). Before the onset of cattle grazing in May 2012, five 50 × 50 m fenced (1.5 m high) cattle enclosures (ungrazed plots) were randomly established across the study site at 50 m intervals. Additional unfenced 50 × 50 m plots served as controls (grazed plots) in areas adjacent to each of the five ungrazed plots, about 2 m apart. Control grazed plots were used to monitor the responses of plant communities to cattle grazing.

### 2.2 | Effects of cattle grazing on *E. unicolor* population density and performance

To investigate the effects of cattle grazing on *E. unicolor* density in grazed and ungrazed plots after 3 years of cattle grazing (question (i)), two parallel transects with a series of 10 0.25 m<sup>2</sup> rings were placed at about 6.5 m intervals and 10 m away from the plot boundary to minimise edge effects. Rings were left undisturbed for at least 1 day before grasshopper surveys. Densities were estimated by counting the number of *E. unicolor* in each ring (Joern, 2004). Surveys were conducted on 10, 21, 30 August, and 8 September 2014. All surveys were conducted on sunny days with minimal cloud cover and no wind. We averaged *E. unicolor* density



**FIGURE 1** Population density of grasshopper *Euchorthippus unicolor* in the grazed and ungrazed plots over four sampling dates in August and September 2014. Values are  $M \pm SE$

for the two transects to yield a single value for each plot ( $n = 5$ ) on each of the four sampling days.

To assess the effects of cattle grazing on *E. unicolor* performance, we determined survival rates, body size, and mass gain in the grazed and ungrazed plots. In August 2014, we installed two circular cages ( $0.75 \text{ m}^2$  basal area  $\times$  1.1 m high) in each plot (20 cages in total). Each circular cage was constructed of iron rod frames, covered with 2-mm plastic mesh screen, and sunk 10 cm into the ground. Just before the deployment of the circular cages, the area was cleared of all above-ground invertebrates and predators using a vacuum cleaner. We then collected fourth-instar *E. unicolor* using sweep-nets on the adjacent grassland area. Before the nymphs were introduced into the cages, *E. unicolor* were separated according to sex, and their initial body mass was determined by weighing seven individuals at a time in one vessel using an analytical scale (Top Instrument, Zhejiang, China). *Euchorthippus unicolor* were introduced into each circular cage in groups of 14 individuals (seven females and seven males), which matched observed densities in the study site. Survival was estimated by counting the number of *E. unicolor* per cage every 3 days, starting on 8 August and ending on 28 August. Adult body mass and size measurements were determined again for all the remaining females and males from each circular cage. Mass gain was calculated by subtracting the initial mass from the adult mass (Specht, Scherber, Unsicker, Köhler, & Weisser, 2008). We measured *E. unicolor* body length and femur length using an Electronic Digital Caliper (Guanglu Inc., Guilin, China). The average for the two circular cages within each plot was used in statistical analyses.

### 2.3 | Effects of cattle grazing on vegetation abundance and microclimatic conditions

In order to test question (ii), i.e., whether cattle grazing influenced plant resource availability, we examined the differences in plant communities in grazed and ungrazed plots. In August 2014, we established two 50-m parallel transects within each plot and measured

plant community characteristics in ten  $0.5 \times 0.5 \text{ m}$  quadrats along each transect, at about 4-m intervals. Within each quadrat, we identified plant species, counted the number of shoots for each species (i.e., if the species were clonal, this means we counted the number of ramets), visually estimated the percentage cover of all plant groups, and measured plant height on five individual shoots for each plant group (to the nearest centimetre) using a ruler. We estimated the volume ( $\text{m}^3$ ) of three different plant groups (*L. chinensis*, other grasses and forbs) by multiplying the mean area covered with the mean height of each of the plant groups in 20 quadrats in each plot (Huntzinger et al., 2008). We measured aboveground biomass by clipping standing plant material to 1 cm above ground level from eight randomly located  $0.5 \times 0.5 \text{ m}$  quadrats within each plot. For each plant variable, the values were averaged to yield a single value for each plot (the level of replication for this field experiment, i.e.,  $n = 5$ ).

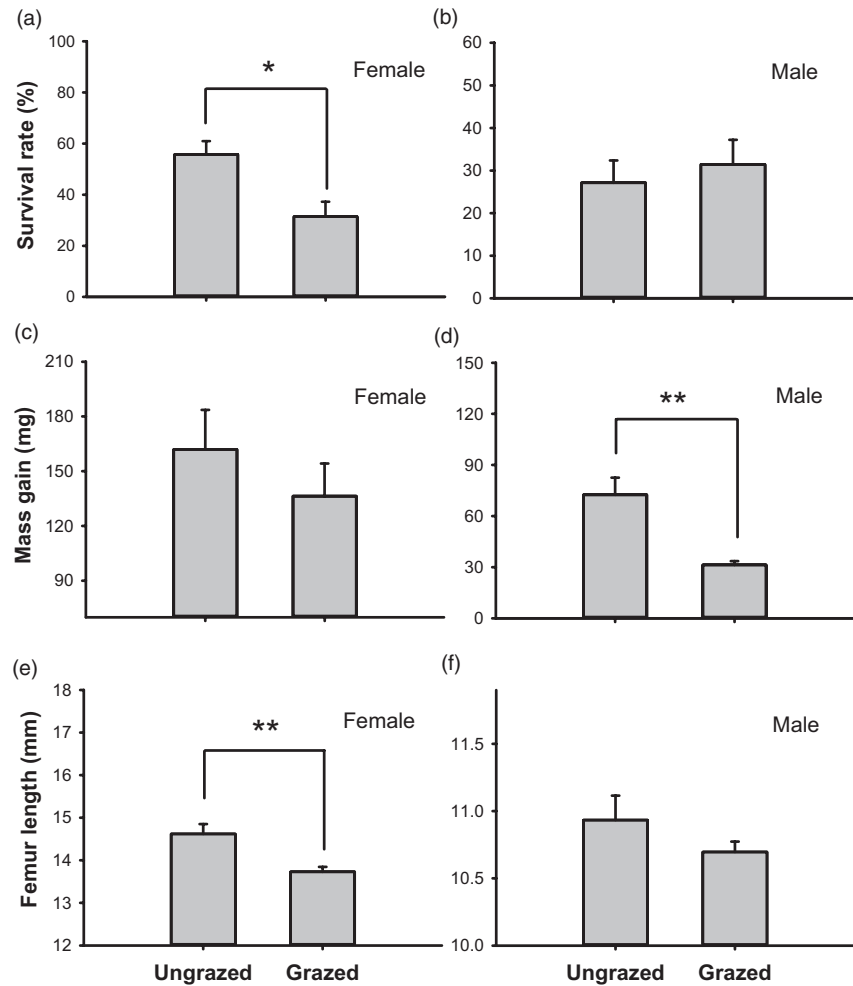
In order to test question (iii), i.e., whether cattle grazing influenced within-canopy microclimatic conditions, we evaluated light, temperature, and humidity at 5 and 35 cm above ground (grasshopper active zones) in August 2014, within the same quadrats used for vegetation variable sampling. Photosynthetically active radiation (PAR) was measured on a clear day with a GLZ-C-G PAR point sensor (Top Instrument, Zhejiang, China). Ambient air temperature and relative humidity were measured using an AR-847 digital thermo-hygrometer (Jinzhao Inc., Shenzhen, China).

### 2.4 | Effects of cattle grazing on plant quality

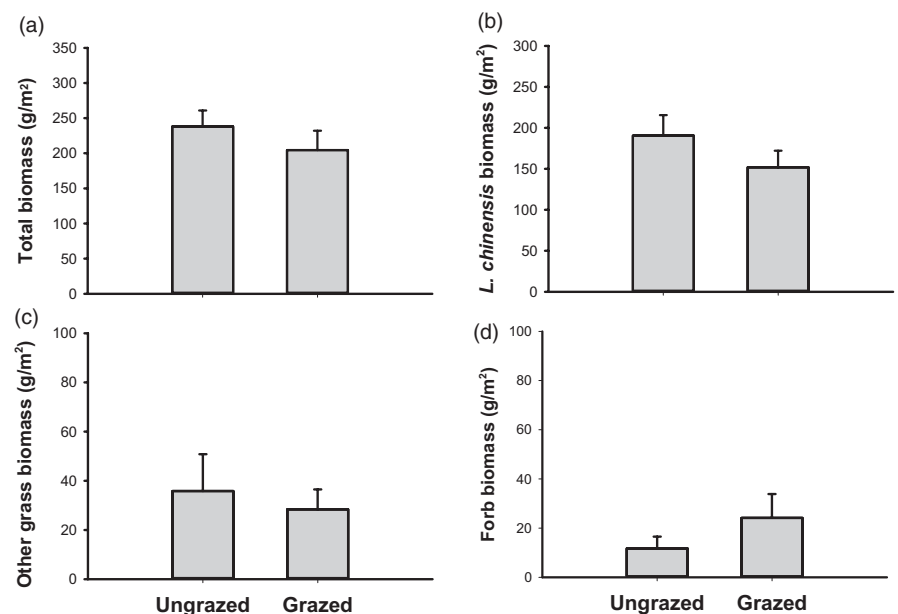
To test question (iv), i.e., whether large grazers influenced plant quality, the chemical and physical traits of the dominant grass *L. chinensis* were measured in grazed and ungrazed plots. We collected *L. chinensis* leaves from eight randomly located  $0.5 \times 0.5 \text{ m}$  quadrats within each plot, in mid-August in 2014. Two of the uppermost (second or third leaves from the top) fully expanded leaves that had not been damaged by cattle or grasshoppers were measured. These tend to be the leaves that are most commonly used by *E. unicolor* (Y. Zhu, field observations). Leaves were fresh- and dry-weighed to determine water content. Then, dried samples were ground using a ball-mill and analysed for nitrogen (N) and carbon (C) contents, and C:N ratio using a Vario MICRO cube Elemental Analyzer (Elementar GmbH, Hanau, Germany). Leaf toughness of *L. chinensis* was measured on 10 individual leaves in each plot using an improved SY-S03 penetrometer (Shiya Inc., Shijiazhuang, China). All values were averaged to yield a single value for each plot (resulting in five replicates,  $n = 5$ ).

### 2.5 | Effects of nitrogen addition on *E. unicolor* grasshopper and *L. chinensis* grass

Our results in 2014 suggested that the negative influences of cattle on *E. unicolor* grasshoppers were not a result of reduced availability of shared resources, nor a consequence of a change in microclimatic conditions, but a result of alterations of plant quality (N content) (see Figures 1–4 and tables in the Section 3 below). Therefore, in 2015,



**FIGURE 2** Performances of female and male grasshopper *Euchorthippus unicolor* in the grazed and ungrazed plots in August 2014: (a, b) survival rate, (c, d) mass gain, (e, f) body size. Seven female and seven male fourth-stage nymphs were monitored in field circular cages installed on each plot for 3 weeks. Values are  $M \pm SE$ . The data were analysed separately by sex, because female grasshoppers are significantly larger than males. Asterisks indicate a significant difference (\* $p < 0.05$ , \*\* $p \leq 0.01$ )



**FIGURE 3** Biomass of (a) all plants combined, (b) *Leymus chinensis*, (c) other grasses, and (d) forbs in the grazed and ungrazed plots in August 2014. The difference between treatments in none of the four panels was significant. Values are  $M \pm SE$

we conducted a set of field and laboratory fertilisation experiments to investigate how increased N content in plants affected *E. unicolor* grasshoppers.

In April 2015, we set up eighteen  $4 \times 4$  m plots that were randomly allocated to three levels of N enrichment (0, 10, and  $17.5 \text{ g N/m}^2$ ,  $n = 6$ ). N levels were chosen to produce an increase in plant

N equivalent to the actual effects of grazing seen in our 2014 study ( $10 \text{ g N/m}^2$ ) and a much higher level equal to that used by Cease et al. (2012) ( $17.5 \text{ g N/m}^2$ ). Nitrogen was applied as an aqueous solution of  $\text{NH}_4\text{NO}_3$ ; with the control, unfertilised treatment receiving an equivalent volume of just water. Plots were fertilised in early May and again at the end of June.

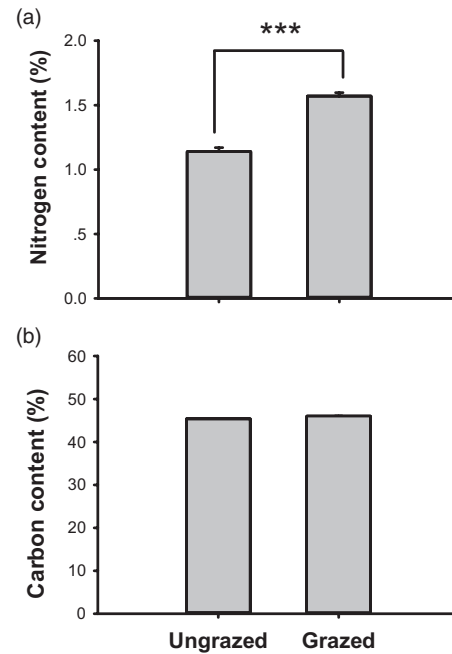
In late August 2015, we measured the density of *E. unicolor* within each treatment field plot. We randomly placed one  $1 \text{ m}^2$  quadrat in each of the 18 plots. Each quadrat was carefully enclosed using a  $1.5 \text{ m}$  high shading screen barrier. All grasshoppers in each quadrat were then removed using a sweep-net and the number of *E. unicolor* was counted (Heidorn & Joern, 1987). The survey was conducted only on sunny days with minimal cloud cover and no wind, with three replications. In early August 2015, we installed one cage into each of the treatment plots (18 cages in total). We then introduced 14 fourth-instar nymphs (seven females and seven males) to each cage, and counted the survivors every 3 days for 3 weeks. We measured *E. unicolor* survival rate, body size, and mass gain in each cage using the methods described above.

In order to control for the potential confounding factors present in the field fertilisation experiment, we simultaneously conducted a laboratory experiment to test the response of *E. unicolor* to plant N content. In early August 2015, we collected fourth-instar nymphs from the field and separated them according to sex. Then, we randomly assigned each nymph to one of three treatments containing plant material that had received 0, 10, or  $17.5 \text{ g N/m}^2$ . We stocked 10 individuals per sex per cage (in cages  $0.08 \text{ m}^2$  basal area  $\times 0.3 \text{ m}$  high, 36 cages in total,  $n = 6$ ). Fresh *L. chinensis* grass was collected from each of the treatments from the field plots, offered in small water-filled plastic vials, and replaced every other day. Grasshoppers were monitored for 3 weeks, and then we measured grasshopper survival rate, body size, and mass gain in each cage.

In mid-August 2015, we collected *L. chinensis* leaves from each of the field treatment plots, and measured their N content, C content, toughness, and water content using the same methods previously described. We also measured the volume of *L. chinensis* grass in  $0.5 \times 0.5 \text{ m}$  quadrat within each plot.

## 2.6 | Data analyses

To assess the effects of cattle grazing on the response variables studied in the large herbivore exclusion field experiment, we used linear mixed effect models, with “treatment” included as a fixed effect (two levels: “grazed” and “ungrazed”) and “replicate plots” included as a random effect. The specific response variables assessed were: performance variables (i.e., survival, body size, and mass gain) from male and female *E. unicolor* grasshoppers; plant biomass, cover, density, height, and volume for each plant functional group separately and also across all groups combined (*L. chinensis*, other grasses, and forbs); the microclimatic conditions (air temperature, air humidity, and light penetration) *E. unicolor* experienced in the plots; and the physical and chemical leaf traits of *L. chinensis* grass (N content, C content, C:N ratio, toughness, and



**FIGURE 4** Total (a) nitrogen content (percentage dry mass) and (b) carbon content (percentage dry mass) of *Leymus chinensis* leaves in the grazed and ungrazed plots in August 2014. Asterisks indicate significant difference ( $***p \leq 0.001$ ). Values are  $M \pm SE$

water content). Models were fitted using the function lmer from the package lme4 (Bates, Mächler, Bolker, & Walker, 2015) and the package lmerTest was used to calculate  $p$ -values (Kuznetsova, Brockhoff, & Christensen, 2016). To examine the effect of cattle grazing on the response variable “abundance of *E. unicolor* grasshoppers” over time, we added the fixed effect “sampling time” (four levels: the four sampling dates) the basic linear mixed effect model outlined above.

For both N addition experiments in the field and in the laboratory, we used linear models to determine the effects of the fixed factor “N addition treatment” (three levels: 0, 10, and  $17.5 \text{ g N/m}^2$ ) on the response variables *E. unicolor* density and performance (i.e., survival, body size, and mass gain). We also performed linear models with the fixed factor to assess the effects of “N addition treatment” on *L. chinensis* leaf N content, C content, toughness, water content, and volume. Tukey multiple comparison analyses were used whenever significant differences were found.

All response variables were tested for homogeneity of variances, using the Levene’s test. We tested the normality of residuals using the Shapiro–Wilks test and the visual inspection of residuals versus fitted values (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). The response variable “proportion of surviving of *E. unicolor* in cages” were arcsine square root transformed to ensure normality. We used Satterthwaite’s approximation to calculate the denominator degrees of freedom. Untransformed data are presented in the figures. All statistical analyses were performed in the open source software R 3.1.0 (R Development Core Team, 2014).



### 3 | RESULTS

#### 3.1 | Effects of cattle grazing on *E. unicolor* population density and performance

Cattle grazing had a negative effect on the abundance of *E. unicolor* grasshoppers (grazing:  $F_{1,28} = 47.54$ ,  $p < 0.001$ ; Figure 1), and the effect of grazing was consistent over time (grazing  $\times$  time:  $F_{3,28} = 0.96$ ,  $p = 0.43$ ). *Euchorthippus unicolor* abundance increased in both treatments throughout the growing season before decreasing at the end of the season (time:  $F_{3,28} = 12.85$ ,  $p < 0.001$ ).

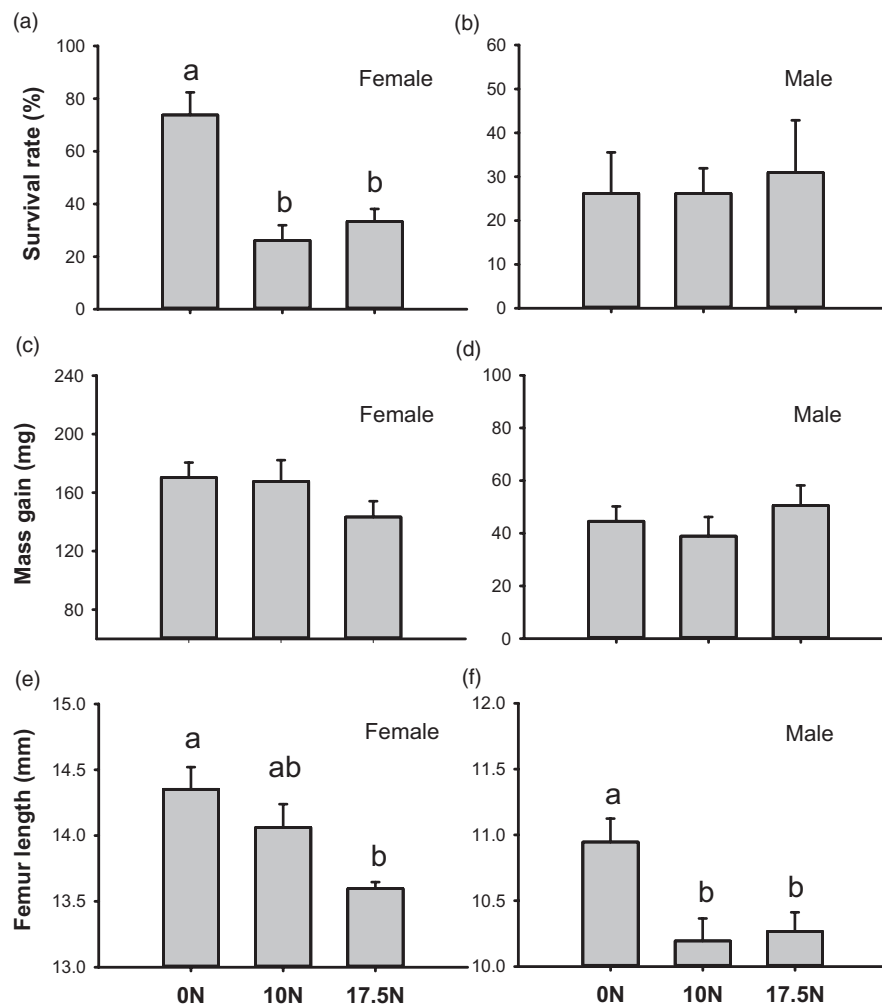
Female *E. unicolor* survival rates were 44% lower in grazed than in ungrazed plots ( $F_{1,8} = 9.525$ ,  $p = 0.015$ , Figure 2a) and size (femur length) was reduced by 6% in grazed plots ( $F_{1,4} = 23.27$ ,  $p = 0.008$ ; Figure 2e), but mass gain was unaffected (Figure 2c). Cattle grazing reduced mass gain of male *E. unicolor* ( $F_{1,8} = 15.839$ ,  $p = 0.004$ ; Figure 2d), but did not affect their survival rate or femur length (Figure 2b,f).

#### 3.2 | Effects of cattle grazing on vegetation and microclimatic conditions

Cattle grazing did not affect plant biomass or volume significantly for any of the studied plant functional groups (Figure 3, Supporting Information Appendix S2: Table S1). Cattle grazing did decrease the height of *L. chinensis* by 6% ( $F_{1,8} = 6.034$ ,  $p = 0.04$ ), but had no significant impacts on *L. chinensis* density or cover. Grazing did not affect the height, density, or cover of the other grasses, the forbs, or the total of all three plant groups (Supporting Information Appendix S2: Table S1).

Cattle grazing significantly increased air temperature by  $+0.77^{\circ}\text{C}$  on average at 35 cm above ground ( $F_{1,4} = 14.266$ ,  $p = 0.019$ ), but had no impacts on air temperature at 5 cm, air relative humidity or PAR at any of the two levels above ground (Supporting Information Appendix S2: Table S2).

Cattle grazing significantly increased N content of *L. chinensis* leaves by 38% ( $F_{1,8} = 108.14$ ,  $p < 0.001$ ; Figure 4a), but did not alter leaf carbon content (Figure 4b). This resulted in a 27% decrease in



**FIGURE 5** Effects of nitrogen application on grasshopper *Euchorthippus unicolor* performance in field circular cages. The (a, b) survival rate, (c, d) mass gain, and (e, f) body size of female and male grasshopper of different nitrogen application treatments (0, 10, and 17.5 g N/m<sup>2</sup>). In August 2015, seven female and seven male fourth-stage nymphs were monitored for 3 weeks. Different letters above the columns indicate significant differences ( $p < 0.05$ ). Values are  $M \pm SE$

C:N ratio of *L. chinensis* leaves ( $F_{1,8} = 75.992$ ,  $p < 0.001$ ). In contrast, livestock grazing did not change any of the plant physical traits assessed, with *L. chinensis* leaf toughness and water content remaining similar in grazed and ungrazed plots (Supporting Information Appendix S2: Figure S1).

### 3.3 | Effects of nitrogen addition on *L. chinensis* grass and *E. unicolor* grasshopper

Fertilisation treatments in the field (10 and 17.5 g N/m<sup>2</sup>) significantly enhanced the N content of *L. chinensis* leaves by 34% and 122% compared to controls, respectively ( $F_{2,15} = 2.852$ ,  $p < 0.001$ ; Supporting Information Appendix S2: Figure S4a), but did not affect leaf carbon content (Supporting Information Appendix S2: Figure S4b). These changes led to 24% and 54% decreases in C:N ratios of *L. chinensis* leaves in 10 g N/m<sup>2</sup> and 17.5 g N/m<sup>2</sup> treatments compared to the control treatment ( $F_{2,15} = 709.4$ ,  $p < 0.001$ ; Supporting Information Appendix S2: Figure S4c). Fertilisation did not affect *L. chinensis* volume significantly, although a positive trend was observed (Supporting Information Appendix S2: Figure S4d). In contrast, fertilisation significantly decreased *L. chinensis* leaf toughness ( $F_{2,15} = 17.24$ ,  $p < 0.001$ ). There was some evidence for an effect of N addition on water content ( $F_{2,15} = 3.962$ ,  $p = 0.04$ ), but Tukey multiple comparison analysis was not significant (see Supporting Information Appendix S2: Figure S4e,f).

Nitrogen addition of 10 and 17.5 g N/m<sup>2</sup> markedly decreased *E. unicolor* density within each field plot by 44% and 51% respectively ( $F_{2,15} = 4.18$ ,  $p = 0.036$ ; Supporting Information Appendix S2: Figure S2). In addition, fertilisation inputs significantly reduced *E. unicolor* performance. Specifically, survival rate and femur length of female *E. unicolor* were reduced in N-enriched plots (survival rate,  $F_{2,15} = 11.61$ ,  $p < 0.001$ ; femur length,  $F_{2,15} = 7.019$ ,  $p = 0.007$ ; Figure 5a,e), while mass gain was unaffected (Figure 5c). Similarly, femur length of male *E. unicolor* was also reduced in fertilised plots ( $F_{2,15} = 6.331$ ,  $p = 0.01$ ; Figure 5f), while enrichment did not affect the survival rate or mass gain of male *E. unicolor* (Figure 5b,d).

Consistent with results in the field fertilisation experiment, *E. unicolor* performance was also reduced when fed N-fertilised plant material in laboratory conditions. Survival rate, mass gain, and femur length of female *E. unicolor* were significantly lower when fed N-fertilised *L. chinensis* material (survival rate,  $F_{2,15} = 13.69$ ,  $p < 0.001$ ; mass gain,  $F_{2,15} = 4.352$ ,  $p = 0.032$ ; femur length,  $F_{2,15} = 7.803$ ,  $p = 0.005$ ; see Supporting Information Appendix S2: Figure S3a,c,e). Again, the survival rate and femur length of male *E. unicolor* were not affected by N enrichment, while mass gain of male *E. unicolor* was significantly reduced when fed fertilised plant material ( $F_{2,15} = 16.08$ ,  $p < 0.001$ ; Supporting Information Appendix S2: Figure S3d).

## 4 | DISCUSSION

Interspecific interactions between closely related herbivorous species have been widely studied and typically result in negative

effects for both species as a result of the exploitation of a limiting shared plant resource (i.e., competition). As an example, competitive interactions have been found between wild ungulates and cattle (Odadi et al., 2011), and grasshoppers and caterpillars (Xi, Griffin, & Sun, 2013). In contrast, the outcomes of interactions between distantly related herbivores with disparate body sizes are complex and tend to be asymmetrical (Gómez & González-Megías, 2002). This study demonstrates that large vertebrate grazing produces a significant negative effect on a much smaller invertebrate herbivore in a grassland ecosystem. We simultaneously explored multiple potential driving factors underlying this response, including plant quality and quantity, microclimate, and vegetation structure. We found small but significant effects of vertebrate grazing on grass height and canopy air temperature, but a comparatively large 38% increase in plant N content. We were able to reproduce the reduction in grasshopper abundance and performance by using N addition to mimic the effects of cattle grazing, which enhanced plant N content and volume while reducing leaf toughness. We further isolated the contribution of plant N content from potentially confounding shifts in plant species composition, plant structure, or microclimate conditions by feeding clipped leaves to grasshoppers in the laboratory. Therefore, while we do not exclude the possibility of other contributing factors, we conclude that a change in plant quality (N content) of the shared host grass appears to be the major driver of the effect of cattle on grasshoppers.

Counter-intuitively, the negative effect of cattle on grasshoppers appeared to be the result of an increase in plant N content in cattle grazed areas. Although high leaf N content is often thought to be beneficial for herbivores (Mattson, 1980), our experiments show that N enrichment due to moderate cattle grazing resulted in lower survival rates and reduced performance for the herbivorous grasshopper. Cease et al. (2012) also found that N fertilisation and an increase in protein: carbohydrate ratio negatively affected grasshopper performance.

Several potential mechanisms may explain the negative effect of elevated plant N content on grasshopper performance. Firstly, organisms are predicted to have specific dietary intake targets for each nutrient (i.e., the geometric framework, Raubenheimer & Simpson, 1993), and a mismatch between an organism's intake target and the nutrient content of its food may result in a surplus or a deficit of particular elements (Behmer & Joern, 2008; Ibanez, Millery, D'Ottavio, Guilhot, & Vesin, 2017). In our study, cattle grazing (as well as fertilisation) significantly decreased leaf C:N ratio, potentially producing an imbalance in nutrients available to *E. unicolor* grasshoppers. Secondly, nutrient-rich plants may reduce insect performance by affecting specific physiological processes such as increasing the metabolic costs of storing and excreting excess N (Boersma & Elser, 2006). There is indirect evidence that such a mechanism does operate in aphids (Zehnder & Hunter, 2009). Finally, some physical and chemical plant traits, such as N-based alkaloids, could change concomitantly with N content (Vannette & Hunter, 2011). In our system, the increased N content in *L. chinensis* leaves from grazed plots did



not correlate with changes in leaf toughness or water content, while leaves from N addition plots had lower leaf toughness, suggesting that at least physical leaf traits were not responsible for the negative response of *E. unicolor* grasshoppers. Grasses generally lack levels of secondary metabolites necessary for effective chemical defence (Mattson, 1980; Tschardt & Greiler, 1995), but some studies have demonstrated that grasses infected by an endophytic fungi (family Clavicipitaceae) could produce a variety of alkaloid compounds deterrent to herbivores (Clay, 2014). The study specific to endophytes in *L. chinensis* showed that the endophyte infection rate of *L. chinensis* is low, and currently there is no evidence suggesting endophytes can facilitate *Leymus* grass through production of alkaloids (Wang et al., 2016). Further detailed experiments are needed to clarify the plausibility of this mechanism.

Over an evolutionary time-scale, we suggest that the negative effects of nutrient-rich resources on *E. unicolor* population density and performance are most likely a result of their long-term adaptation to an N-limited environment. Many fertilisation experiments in the Eurasian steppe have documented that the dominant grass *L. chinensis* is limited by N (Wang et al., 2017). Hence, *E. unicolor* might have specialised to exploit this nutrient-poor resource. Given that many grassland ecosystems are similarly low in N content, we surmise that the mechanisms observed in this study might be widespread among insect herbivores feeding on low-N grasses. For example, many grasshopper species such as *Phoetaliotes nebrascensis* in North America (Joern & Behmer, 1998), *Chortocetes terminifera* in Australia (Clissold et al., 2006), and *Oedaleus asiaticus* in north Asia (Cease et al., 2012) have a preference for low-quality grasses instead of other more nutrient-rich forbs and legumes, which might be a consequence of their adaptation to N-limited environments.

Although we conclude that plant quality (N content) is a major driver of the effect of cattle on grasshoppers, we cannot rule out the possibility that changes to microclimatic conditions and plant structure also play a role (Suominen et al., 1999; van Klink et al., 2015). We documented a modest increase in air temperature of 0.77°C at 35 cm above ground in grazed plots. Although a similar magnitude increase in temperature of 1°C had no observable effect on grasshopper performance or survival in a previous study, negative effects of temperature on grasshopper survival and performance were documented in response to substantial increases of 3°C (Laws & Belovsky, 2010). Cattle also reduced grass height by 6%, suggesting that a reduction in plant structure may have contributed to the negative response of grasshoppers to grazing. However, grasshoppers also responded negatively to N addition, which tended to increase plant volume. Overall grass abundance was unaffected by cattle, despite a decrease in plant height, suggesting compensatory regrowth following moderate grazing intensity (McNaughton, 1979). Previous studies in a similar system reported that *L. chinensis* is a highly grazing-tolerant species that can compensate or overcompensate losses under moderate simulated grazing situation (Gao et al., 2008). Grazing may also improve conditions for plant growth by reducing intraspecific competition for light and increasing nutrient availability directly by adding

readily accessible N in the form of urine and faeces or indirectly by decreasing plant litter (Liu et al., 2017). Furthermore, grazed plants have been shown to increase chlorophyll and photosynthetic enzymes concentration in remaining or new leaves, which consequently results in a higher total N concentration (Briske, 1996; Price, 1991). Indeed, plant nutrient enrichment is a common result of large herbivore grazing and has been reported in many ecosystems including forests (Riipi et al., 2005) and African savannas (McNaughton, 1976) and alpine meadows (Cao et al., 2015). However, it is noted that the effect of large vertebrates on grass N content may vary based on the intensity of grazing. Interestingly, Cease et al. (2012) found a positive effect of large vertebrate herbivores on grasshoppers in the Eurasian steppe system because heavy livestock grazing resulted in lower grass N content, likely due to erosion and the loss of soil organic N. Besides grazing intensity and frequency, the effect of large vertebrate herbivory on plant quality also depends on several intrinsic and extrinsic factors, such as plant resistance strategy, plant life history, as well as environmental conditions (Briske, 1996).

Invertebrate herbivores, particularly specialist grasshoppers and caterpillars, usually reach maximal performance within a fairly limited range of plant nutrients (Behmer, 2009). The optimal range of each nutrient for a given herbivorous species can be highly variable, which is most notable between different insect species (Behmer & Joern, 2008), but it also occurs within a species as a result of developmental status, immediate physiological state and/or sex (Hawlena & Schmitz, 2010). Our results strongly suggest that large vertebrate herbivores (cattle) exert a negative indirect effect on small insect herbivores (*E. unicolor* grasshopper) whereby cattle modify plant quality, which decreases grasshopper population densities, survival, and performance. Therefore, understanding multiple pathways whereby large herbivores drive invertebrate population merits further exploration and can provide new insights into indirect interactions between distantly related and differently sized herbivores.

## 5 | CONCLUSIONS

Although large vertebrate herbivores are widely known to exert strong effects on invertebrate herbivores in terrestrial ecosystems, many questions surrounding these biotic interactions remain to be answered due to the paucity of carefully designed controlled experiments. Our study provides direct evidence for a mechanistic understanding of a competitive interaction that might be pervasive between differently sized herbivores, even in ecosystems with very light grazing regimes. Uniquely, we show that a change in plant quality appears to be an important driver of these competitive interactions. These less obvious but relevant pathways warrant more attention, given their potential generality. These findings further extend our understanding of the mechanisms for herbivore coexistence in natural ecosystems, and may have important implications for developing effective grazing management schemes.