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Hassan, Nazim

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Effects of grazing on C:N:P stoichiometry attenuate from soils to plants and insect herbivores in a semi-arid grassland

Nazim Hassan[1], Xiaofei Li[2], Jianyong Wang[1], Hui Zhu[1], Petri Nummi[3], Deli Wang[1*], Deborah Finke[4], Zhiwei Zhong[1*]

[1]*Institute of Grassland Science/School of Environment, Northeast Normal University, and Key Laboratory of Vegetation Ecology/Key Laboratory for Wetland Ecology and Vegetation Restoration, Changchun, Jilin 130024, China*

[2]*College of Resources and Environmental Sciences/Key Laboratory of Sustainable Utilization of Soil Resources in the Commodity Grain Bases in Jilin Province, Jilin Agricultural University, Changchun, Jilin 130118, China*

[3]*Wetland Ecology Group, Department of Forest Sciences, University of Helsinki, PO Box 27, 00014 University of Helsinki, Finland*

[4]*Division of Plant Sciences, University of Missouri, Columbia, USA*

***Correspondence:** Deli Wang, Email: wangd@nenu.edu.cn; Zhiwei Zhong, Email: zhongzw822@nenu.edu.cn

Author contribution statement NH and XFL contributed equally to this work. ZWZ, XFL, and DLW designed experiments; NH, JYW, and HZ performed the experiments and analyzed the data; ZWZ, XFL, DF, PN, DLW, and NH drafted the manuscript; all the authors contributed to the interpretation of results and the critical revision of the manuscript.

Abstract

Understanding the processing of limiting nutrients among organisms is an important goal of community ecology. Less known is how human disturbances may alter the stoichiometric patterns among organisms from different trophic levels within communities. Here, we investigated how livestock grazing affects the C:N:P ecological stoichiometry of soils, plants (*Leymus chinensis*), and grasshoppers (*Euchorthippus* spp.) in a semi-arid grassland in northeastern China. We found that grazing significantly enhanced soil available N and leaf N content of the dominant *L. chinensis* grass by 15% and 20%, respectively. Grazing also reduced (soluble) C:N of *L. chinensis* leaves by 22%. However, grazing did not affect total C, N, or P contents nor their ratios in *Euchorthippus* grasshoppers. Our results reveal that the effects of grazing disturbances on elemental composition attenuated from lower to higher trophic levels. These findings support the theory that organisms from higher trophic levels have relatively stronger stoichiometric homeostasis compared to those from the lower trophic levels. Moreover, grasshopper abundance dropped by 66% in the grazed areas, and they reduced the feeding time on their host *L. chinensis* grass by 43%, presumably to limit the intake of excess nitrogen from host plants. The energetic costs associated with the maintenance of elemental homeostasis likely reduced grasshopper individual performance and population abundance in the grazed areas. A comprehensive investigation of stoichiometric properties of organisms across trophic levels may enable a better understanding of the nature of species interactions, and facilitate predictions of the consequences of future environmental changes for community organization.

Key words: Stoichiometric homeostasis, population dynamics, C:N:P, plant-insect interactions.

Introduction

Ecological stoichiometry is a body of theory concerning the balance of energy and chemical elements in living systems (Sterner and Elser 2002). It has been widely applied as a framework for ecological questions ranging from organism nutrition, food web structure, population and community organization to eco-evolutionary dynamics (Schade et al. 2003; Sterner et al. 2008; Sperfeld et al. 2017; Branco et al. 2018; Riggi and Bommarco 2019). Until recently, most studies have focused on the elemental composition of individual abiotic or biotic components, such as soils (Cleveland and Liptzin 2007; Tian et al. 2010; Hume et al. 2016), plants (Gusewell 2004; Elser et al. 2007, 2010), and herbivorous insects (Elser et al. 2000; Fagan et al. 2002), as well as their pairwise (e.g. plant-soil, plant-herbivore, or prey-predator) stoichiometric relationships (Daufresne and Loreau 2001; Fagan and Denno 2004; Yu et al. 2011; Boersma et al. 2016). The overall stoichiometric patterns and linkages within an ecological community, such as from soils to plants and insect herbivores, which are often closely interconnected in biogeochemical cycling, remain poorly understood (but see Schade et al. 2003; Mulder and Elser 2009).

Coincidental with the appreciation of the importance of ecological stoichiometry, there has also been a wide realization that many ecosystems experience dramatic shifts in structure and functioning driven primarily by human disturbances. For example, human disturbances such as livestock grazing, mowing, and fire are known to exert profound impacts on pools and cycling rates of soil C, N and P by altering the abundance and activities of soil microorganisms like bacteria and fungi (Patra et al. 2005). Such alterations in soil nutrient availability may scale up across trophic levels from soils to plants and animals, altering the stoichiometric properties of whole food webs (Schade et al. 2003; Delgado-Baquerizo et al. 2018). Given that most of our knowledge about ecological stoichiometry is gained from the study of unmanaged ecosystems

(Schade et al. 2003; Vanni and McIntyre 2016), it is critical to explore how human activities can influence stoichiometric properties of key components of an ecosystem and thereby provide guidelines for improving management practices.

Livestock grazing is one of the most pervasive management strategies in grassland ecosystems across the globe. Grazing has the potential to exert impacts on elemental composition of co-occurring organisms (Bai et al. 2012; Cease et al. 2015; Zhou et al. 2017). Grazers can directly regulate soil nutrient pools via the deposition of feces and urine (Stout et al. 1997; Wang et al. 2018). Furthermore, they can indirectly change soil nutrients via foraging activities that alter the quantity and quality of plant litter that returns to the soil and their trampling effects (Olff and Ritchie 1998; Schrama et al. 2013; Liu et al. 2015a-c; Wang et al. 2019). Compared to plant litter, nitrogen and phosphorous in feces and urine are more soluble and can be transferred into plants more rapidly. Thus, in grazing grasslands the cycling of soil nutrients from animal excretion is referred to as “the rapid cycle pathway”, whereas plant litter is considered to be “the slow cycle pathway” (McNaughton et al. 1988; Hobbs 1996; Frank and Evans 1997). In the past decades, many studies have examined the effects of large herbivore grazing on the elemental content of soils (Shan et al. 2011; McSherry and Ritchie 2013; Risch et al. 2015). It has been found that the impact of grazing varies and is often related to a number of biotic and abiotic factors, such as grazing intensity, grazing history and precipitation (Zhou et al. 2017). There are also a small number of studies that have investigated the bottom-up regulation of C:N:P stoichiometric linkages between soils and plants in response to livestock grazing (Bai et al. 2012; Mipam et al. 2019).

Natural grassland ecosystems harbor not only soils and plants, but also numerous heterotrophic animals from multiple trophic levels that interact to shape the food webs (Elser et al. 2007). Heterotrophic animals, especially insect herbivores, feed directly on their host plants and thus their body elemental composition is highly dependent on the plants they consume. Indeed, specialist insect herbivores are reported to track the composition of soils and their host food plants in nutrient-limited habitats, such as desert ecosystems (Schade et al. 2003). Given that many arid or semi-arid grassland ecosystems are poor in nutrients such as nitrogen and phosphorus (Elser et al. 2007), a similar stoichiometric linkage among soils, plants, and insect herbivores may exist in these ecosystems as well. Moreover, organisms from higher trophic levels (e.g. insect herbivores) often regulate nutrient intake and utilization to meet nutritional demands (Behmer 2009) and demonstrate a stronger stoichiometric homeostasis compared to organisms from lower trophic levels (e.g. plants) in the face of disturbances (Sterner and Elser 2002). Insect herbivores could regulate their nutrient intake and body element balance by several mechanisms, including preingestive regulation, postingestive regulation, and learning (Behmer and Joern 2008; Behmer 2009). Nevertheless, the regulation of such nutrient balance and stoichiometric homeostasis is energetically expensive and often at the cost of individual performance (Behmer and Joern 2008; Behmer 2009; Zhu et al. 2019), with potential consequences for the population dynamics of organisms. Until recently, very few studies have simultaneously investigated how body element balance, feeding behaviors, and population dynamics of insect herbivores respond to human disturbances, limiting our understanding of the mechanisms driving insect abundance and distribution in ecosystems.

Here, we examined the effects of short-term (3 years) livestock grazing on a grassland food chain using the framework of ecological stoichiometry. The system studied was comprised of a widespread livestock species, domestic cattle (*Bos taurus*), the grass-specialist grasshoppers (*Euchorthippus* spp.), and their grass host plants (*Leymus chinensis*). The goal of this study was to examine how livestock grazing affects the stoichiometric properties of the soil, *L. chinensis* grass, and *Euchorthippus* grasshoppers by evaluating the total and relative contents of C, N, and P. We also investigated how grazing affected the quantity of host *L. chinensis* grass and the feeding behaviors and population abundance of *Euchorthippuss* spp. grasshoppers. We test the general hypothesis that the effects of livestock grazing will increase the available and total N and P contents and decrease C:N and C:P ratios of the soil, plants, and insect herbivores. We predict that such effects on the elemental contents and composition of organisms will attenuate from soils to plants to insect herbivores, because organisms at higher trophic levels have relatively stronger stoichiometric homeostasis (Sterner and Elser 2002). Finally, we explored the potential mechanisms by which grasshoppers maintain stoichiometric homeostasis, and how such homeostasis may affect the population abundance of insect herbivores in grassland ecosystems.

Materials and methods

Study area

The study was conducted in a semi-arid meadow steppe at Grassland Ecological Research Station of Northeast Normal University, Jilin Province, China (44°45′ N, 123°47′ E). The climate is semi-arid, continental with annual mean temperature ranging from 4.6°C to 6.5°C and annual precipitation ranges from 280 to 400 mm. The soil is a mixed salt-alkali meadow steppe (Salid Aridisol, US Soil Taxonomy) of 29% sand, 40% silt and 31% clay (top 10 cm) and is nutrient poor with total N content ranging from 2.2 to 2.5 mg g^{-1}, and total P content ranging from 0.23 to 0.27 mg g^{-1} (Li et al. 2015). The perennial grass *L. chinensis* is the dominant plant species, accounting for $\geq$ 60% of the annual total aboveground biomass in the area. Other plant species include grasses such as *Phragmites australis* and *Calamagrostis epigejos*, and forbs such as

Artemisia scoparia and *Lespedeza davurica*. The dominant invertebrate herbivores are grasshoppers (*Euchorthippus* spp.). These grasshoppers are grass specialists and feed mainly on the *L. chinensis* grass (Zhong et al. 2014, 2017). *Euchorthippus* grasshoppers are weak fliers, spending most of their time resting and feeding on leaves of *L. chinensis* and only walking or jumping short distances (0.3-0.5 m) when disturbances (e.g. livestock trampling) occur. Hence, these insects have a relatively small spatial range (≤10 m radius per month, Nazim Hassan et al., unpublished data) and tend to persist within a vegetation patch. Therefore, while grasshoppers are mobile and move freely from plant to plant, they are unlikely to move from one patch to another, which makes them susceptible to the influence of the stoichiometric properties of their host *L. chinensis* grass at the patch scale. *Euchorthippus* grasshopper density ranges from 10 to 35 individuals per m^2 during the peak of the growing season (mid-August). The study area has a long history (over 30 years) of mowing, but it was fenced in 2005 to protect against uncontrolled human disturbances.

Experimental design

In June 2009, we randomly established six 100 × 200 m blocks, each block contained a pair of 50 × 50 m enclosure plots. Within each block, one plot was assigned to the "grazing" treatment, while the other served as the "ungrazed" control treatment. Distance between experimental blocks was 150–300m, and the distance between plots in a block was approximately 30 m (see Li et al. 2018). From 2010 to 2013, the plots were grazed by cattle (mean weight 300 ±8 kg, mean ± S.E.) at an equal light to moderate intensity (0.1-0.3 animal units per ha), a recommended grazing intensity by local governments. Grazing occurred each year from June to September during the first two weeks of each month, creating grazing activities similar with local grazing habit.

Soil, plant, and grasshopper sampling

From late-July to early-August (peak of the growing season) 2012, we collected soil, plant, and grasshopper samples in the grasslands. We kept a 10 m buffer zone along the perimeter of each control and grazed plot and randomly sampled five 1 × 1 m quadrats within the inner 30 × 30 m areas of the plots. For *L. chinensis* grass, we clipped all the plants to ground surface within the five quadrats in the plots. For soils, we used a 4 cm diameter soil auger to collect two 0–30 cm soil samples within the same five quadrats, the soils from the five quadrats were pooled to homogenize the samples. For *Euchorthippus* grasshoppers, we used the standard sweep net survey method (40 cm diameter) to collect samples (Haddad et al. 2001). We conducted 20 sweeps per sample and one sample in each plot. We identified *Euchorthippus* grasshoppers (Ren 2004) and recorded their abundance by counting the total number of individuals in 20 sweeps for use in the statistical analyses.

C, N, and P content analyses for soils, plants, and grasshoppers

We measured total C, available N, and available P contents in the soils. Total organic C in soil samples was analyzed by the Walkley-Black modified acid-dichromate $FeSO_4$ titration method (Sparks et al. 1996). For total available N, a 10 g subsample soil was extracted with 70 mL 2 mol L^{-1} KCl. Extracts were frozen at 20 °C for analysis of NH_4^+ and NO_3^- content by continuous flow analyzer (Futura; AMS-Alliance Instruments, Frépillon, France). Total available N was the sum of NH_4^+ and NO_3^- concentrations. For total available P, another 10 g subsample soil was extracted using acidified NH_4OAc-EDTA and analyzed by the inductively coupled plasma-atomic emission spectrometer (ICP-AES; Spectroflame, Spectro Analytical Instruments, Kleve, Germany).

We measured soluble C (sugars), total N, and total P contents in the leaves of *L. chinensis* grasses. Soluble C were extracted from leaf samples by hot ethanol extraction. Approximately 50 mg ground leaf material from each sample was boiled in 70% ethanol (v/v) for 1 h at 80 ºC. The samples were then centrifuged at 12 000 × *g* for 10 min and the supernatant collected. This procedure was repeated three times for each sample and the supernatants combined. Samples were then treated with activated charcoal, dried in a vacuum evaporator, and redissolved in distilled water prior to soluble sugar analysis using the anthrone-sulfuric acid method (Ebell 1965; Chen and Wang 2009). Total N content of the leaf samples was determined with an automatic Kjeldahl nitrogen analyzer (Kjeltecw 2300 Analyzer Unit, Foss Analytical AB, Hoganas, Sweden), and total P content was measured through persulphate and sulphuric acid digestion followed by colorimetric analysis.

We measured total C, N, and P contents in the bodies of *Euchorthippus* grasshoppers. Grasshopper samples were dried in an oven for 72 h at 65 ºC and then analyzed for total C, N, and P contents as described above.

All results are expressed on a dry weight basis as g kg^{-1} or mg kg^{-1} (e.g. soil available N and P), and elemental ratios were converted to atomic ratios by correcting for the atomic mass of each element (Schade et al. 2003).

Grasshopper feeding behaviors

In late-July 2012, when the grazing was temporarily halted, we conducted field observation experiments to investigate how livestock grazing affects the feeding behaviors of grasshoppers in the grasslands. We measured the frequency and duration of time feeding on different vegetation groups (*L. chinensis*, other grasses, and forbs) by *Euchorthippus* grasshoppers. On a sunny day, one cylindrical cage (diameter of 2.0 m and 1.5 m in height) wrapped with aluminum mesh was randomly installed in each of the control and grazed plots. We then stocked *Euchorthippus* grasshoppers into each cage based on the density of grasshoppers in the plot in which the cage was installed. We randomly chose one grasshopper and put an identifying red paint mark on its thorax and abdomen to help observers see and relocate it. Twelve observers simultaneously monitored the feeding activities of the marked grasshoppers in the six control and the six grazed plots. We recorded the frequency (number of times) and duration (in seconds) of feeding by grasshoppers on different plant groups using a voice recorder (Lenovo B316þ; Lenovo Group, Beijing, China) and an electronic timer (PS-306, Pursun, Shenzhen, China). All individual grasshoppers were observed for six hours continuously from 10:00 to 16:00 h, during the peak of their activities. The grasshopper feeding behavior data from the cage in each plot were used directly in the statistical analyses.

Statistical analyses

All statistical analyses were performed in the open source software R 3.1.0 (R Development Core Team. 2014). To assess the effects of cattle grazing on the elemental composition of soil, plants, and insects, we used linear mixed effect models with “treatment” included as a fixed effect (two levels: “grazed” and “control”) and “replicate block” included as a random effect. The specific response variables assessed were: total/soluble C, total/available N, and total/available P contents, as well as C:N, C:P, and N:P of soils, *L. chinensis* grass, and *Euchorthippus* grasshoppers. We also used the same method to assess the effects of grazing on *L. chinensis* biomass, grasshopper feeding frequency, feeding time, and population abundance. Models were fitted using the function lmer from the package lme4 and the package lmer-Test was used to calculate *P*-values. Treatment effect sizes for each variable were calculated as

Cohen's *d* and the magnitude of the effects were compared across trophic levels. All response variables were tested for homogeneity of residual variances using the Levene's test. If needed, data were normalized by log, square root, or arcsine square root transformations.

Results

Effects of grazing on C, N, P contents and C:N:P stoichiometry of soils, plants, and grasshoppers

Soil available N was 15% higher in the grazed plots than the control plots ($F_{1,5}$ = 9.65, *P* = 0.027; *d* = 1.32; Fig. 1c). However, we did not detect a significant effect of grazing on soil total C and available P contents (*d* = 0.39 and 0.40, respectively; Fig. 1a, e). There were also no detectable differences in soil C:(available)N, C:(available)P, and N:P in the control and grazed plots (*d* = -0.67, 0.03, and 0.95, respectively; Fig. 1b, d, f).

For *L. chinensis* grass, grazing significantly increased total N concentration by 20% ($F_{1,5}$= 8.96, *P* = 0.030; *d* = 1.84; Fig. 2c), but had no detectable effect on soluble C and P contents (*d* = -0.63 and 0.45, respectively; Fig. 2a, e). Grazing significantly decreased (soluble)C:N by 22% ($F_{1,5}$ = 8.16, *P* = 0.036; Fig. 2b), but did not have a detectable effect on (soluble)C:P and N:P of *L. chinensis* grass (*d* = -1.59, -0.86, and 0.79, respectively; Fig. 2d, f).

Grazing had no detectable effects on total C, N, and P of male or female *Euchorthippus* grasshoppers (male *d* = -1.00, 0.40, and 0.09, respectively; female *d* = -0.58, 0.23, and 0.29, respectively; Fig. 3a, c, e). Grazing also did not have a significant effects on C:N, C:P, and N:P of both male and female of *Euchorthippus* grasshoppers (male *d* = -0.61, -0.37, and 0.16, respectively; female *d* = -0.35, -0.41, and 0.10, respectively; Fig. 3b, d, f).

Effects of grazing on feeding behaviors of grasshoppers

Grazing did not significantly affect the diet selections of *Euchorthippus* grasshoppers: for both control and grazed plots, the average feeding frequency of grasshoppers on *L. chinensis*, other grasses, and forbs was 10%, 1.5%, and 0%, respectively (Fig. 4a). However, grazing significantly reduced the time that grasshoppers spent feeding on *L. chinensis* grass by 43% ($F_{1,5} = 8.43$, $P = 0.034$, Fig. 4b). The time that grasshoppers spent feeding on other grasses was low overall and unaffected by grazing (Fig. 4b).

Effects of grazing on plant biomass and grasshopper abundance

L. chinensis biomass was not different between the control and grazed treatments ($d = -0.63$; Fig. 5a). However, grazing reduced the abundance of *Euchorthippus* grasshoppers by 66% ($d = -3.20$; $F_{1,5} = 23.82$, $P = 0.005$; Fig. 5b).

Discussion

Very few studies have investigated the C:N:P stoichiometric patterns and linkages of organisms across trophic levels, especially under human disturbances. This study simultaneously explored how grazing, a pervasive and important grassland management strategy, affects the stoichiometry of organisms in a grassland food chain, including soil, plants, and insect herbivores. We found that grazing significantly enhanced soil available N and the total N content of leaves of *L. chinensis* grasses, which in turn reduced C:N in leaves of these plants. However, we did not detect an effect of grazing on total C, N, and P contents nor the elemental ratios of female or male *Euchorthippus* grasshoppers. Furthermore, the effect size of cattle grazing on grasshopper stoichiometry was on average 2-3 times less than that documented for soils or plants. Our results reveal that the effects of grazing disturbance on the elemental composition of organisms attenuated from lower to higher trophic levels (e.g. from soil to plants to insect herbivores) in a grassland ecosystem (Fig. 6). These results are in line with our hypotheses and with previous studies, which found that animals often have a stronger homeostasis in the elemental composition of their body compared to plants (Sterner and Elser 2002; Sitters et al. 2017; Metcalfe et al. 2019). However, while previous studies are mostly conducted in the laboratory with artificial diets, we document that such stoichiometric patterns across trophic levels also exist in natural communities.

Livestock grazing is generally reported to increase N and P nutrient availability but have limited effects on C concentrations of soils in a variety of ecosystems (Chaneton et al. 1996; Liu et al. 2015b,c; Sato et al. 2019), and our results add to these findings. Soil C responses to short-term grazing disturbance are difficult to detect because soil C pools are relatively large (Jobbágy and Jackson 2000). However, grazing can rapidly alter soil N and P cycling via multiple mechanisms. First, selective grazing can change the abundance and species composition of the plant community, which in turn alters the quantity and quality of litter that enters soil systems (Augustine and McNaughton 1998; Young et al. 2013; Liu et al. 2015a-c). Second, trampling activities of large herbivores can accelerate the physical decomposition processes of plant litter (Schrama et al. 2013; Liu et al. 2015c). Third, feces and urine deposition of livestock can be important sources of soil available nutrients (Wang et al. 2018). In our ecosystem, cattle grazing tends to increase the relative abundance of N-rich forb species (Liu et al. 2015a, b), and dung and urine deposition from cattle serve as an important source of soil nutrient availability (Wang et al. 2018). The increase in soil available N in the grazed areas (Fig. 1c) appears mainly driven by the activities of cattle, including changes in litter quantity and quality, trampling, and feces and urine deposition, the relative contributions of which are difficult to distinguish in the field. The higher soil nutrients in turn increase total N content and reduce (soluble)C:N in leaves of *L. chinensis* grass (Fig. 2b,c) in the grazed areas. In addition to cattle, grasshoppers may also contribute to soil nutrient availability by their effects on the quantity and quality (e.g. C:N) of plant litter and the inputs of their frass and carcasses, especially at high densities (Belovsky and Slade 2000; Schmitz 2008; Hawlena and Schmitz 2010). Nevertheless, given that cattle grazing tended to suppress grasshopper density (about 5 individual/m^2 in the grazed areas, see Zhu et al. 2019) and the relative short duration of our study, it is unlikely that these insect herbivores played a large role in influencing soil nutrient availability in our system.

Euchorthippus grasshoppers in our semi-arid grassland system are grass-specialists, and they appear to have evolved to feed on these low-N host plants (Zhu et al. 2019). Several grasshopper species such as *Phoetaliotes nebrascensis* in North America (Joern and Behmer 1998) and *Chortoicetes terminifera* in Australia (Clissold et al. 2006) also prefer low-N grasses instead of other more nutrient-rich forbs and legumes, which might be a consequence of their adaptation to N-limited environments. The presence of grazing, however, increased the N concentration of grasshoppers' host *L. chinensis* by 22% (Fig. 2), causing a nutritional imbalance in the food of grasshoppers, with an excess of N relative to C. Surprisingly, despite such alterations in the nutrient concentrations of their food plants, the elemental composition of grasshoppers showed no response to such changes (Fig. 3). These results support the view that terrestrial heterotrophs, including insect herbivores, commonly show an ability to maintain elemental homeostasis despite changes in the chemical composition of their resources (Sterner and Elser 2002).

In the face of environmental changes, there are at least three non-mutually exclusive mechanisms by which insect herbivores could regulate their nutrient intake and body element balance (Behmer 2009). First, preingestive regulation. Insect herbivores can use chemoreceptors on their mouthparts to detect the nutrient concentrations on the leaf surface and mix their low C:N diet with a high C:N diet to reach specific dietary intake targets (Joern and Behmer 1998; Boersma and Elser 2006). However, we found no evidence that grasshoppers changed their diet preference for *L. chinensis* grass in the grazed areas (Fig. 4a). In fact, a shift in diet selection was unlikely to be a feasible option for grasshoppers to deal with excess N in our study system, as the alternative forb plants are even higher in N content and lower C:N than the grasshoppers' preferred grasses (Hassan et al., unpublished data). Instead, it appears that grasshoppers deal with excess N by reducing the amount of food they intake: they reduced their feeding time on *L. chinensis* by 43% in the grazed areas (Fig. 4b). Our field behavioral observations were performed during the period when cattle grazing was temporarily halted (late-July) and there were no direct influences of cattle on grasshoppers. One reasonable inference is that the differences in grasshopper feeding time may be driven by the differences in grasshopper densities in the grazed and ungrazed sites (see *Grasshopper feeding behaviors* in the *Materials and methods* section). Another potential but more cryptic mechanism is that the reduction in grasshopper feeding time may be a response to the nutritional imbalance of the grass and that the change in food intake is an adaptive behavior for homeostasis maintenance by these insects. Further study is needed to distinguish the relative contribution of grasshopper density and food plant quality in affecting grasshopper feeding behaviors in our ecosystem. Second, postingestive regulation. When insect herbivores consume nutrients in amounts that exceed demand, they may eliminate excess nutrients as waste. For example, excess N may be eliminated as uric acid or in volatile form (e.g. ammonium) (Simpson and Raubenheimer 2001). We did not measure the elemental composition of grasshopper frass or their body volatile compounds. Nevertheless, studies with *Oedaleus asiaticus* grasshoppers, which also mainly feed on *L. chinensis* grass in Inner Mongolia grasslands, have shown postingestive regulation in the form of P in frass (Zhang et al. 2014), indicating this mechanism may potentially operate in our system as well. Finally, insect herbivores can regulate nutrient intake by learning (Behmer 2009), and further studies are needed to explore the potential of such mechanism in regulating grasshopper elemental homeostasis in our system.

The maintenance of stoichiometric homeostasis is commonly energetically expensive (Boersma and Elser 2006) and thus may affect individual performance and population size (Joern and Behmer 1998). We found that grasshopper abundance dropped by more than 60% in the grazed areas (Fig. 5b). It is unlikely that the quantity of food available to grasshoppers played a role in this response, because grazing by cattle did not significantly alter the biomass of the grasshopper's host plant *L. chinensis* (Fig. 5a). Rather, our results suggest that a mismatch between the intake target of the grasshoppers and the nutrient composition of their host negatively affected their performance, contributing to the population reduction in the grazed areas. Indeed, Zhu et al. (2019) found that higher N content in *L. chinensis* leaves decreased the growth and survival of *Euchorthippus* grasshoppers. Similar effects of changing food quality on *O. asiaticus* grasshoppers were found in temperate grasslands (Cease et al. 2012). Diet soluble C level is also critical in affecting grasshopper performance (Joern and Behmer 1997), yet we did not detect an effect of cattle grazing on this variable in our system (Fig. 2a). The overall impacts of large herbivore grazing on insect abundance are complex and can occur through several mechanisms, including changing insect thermoregulation activities, microclimates, and the abundance and diversity of predators (e.g. spiders and birds) for insect herbivores (Belovsky et al. 1990; van Klink et al. 2015). Nevertheless, our results along with a previous study (Zhu et al.

2019), suggest that grazing-induced changes in N content and C:N ratio of *L. chinensis* grass may potentially play a role in grasshopper population dynamics in this system (see Fig. 6). In addition, other limiting nutrients in food plants (Liebeg's law of the minimum) (Odum 1959) or changes in anti-herbivore defenses may also explain the observed responses of grasshoppers in the grazed areas. A comprehensive investigation of the mechanisms why grasshopper abundance declines in the presence of cattle is beyond the scope of our study, but further exploration of the underlying mechanisms behind these responses will give us a clearer understanding of the assembly of herbivore communities.

Taken together, our study finds that livestock grazing can cause significant changes in the chemical elemental composition of soil and plants, but had limited effects on insect herbivores. These results indicate that body stoichiometry of organisms from higher trophic levels may be more resistant to disturbances compared to those of organisms from lower trophic levels in a community (Sterner and Elser 2002). However, the present study investigated the effects of cattle grazing on nutrient dynamics over a relatively short 3-year timescale. Whether soil and plant nutrients gradually build up to result in a significant effect on the nutrient dynamics of insect herbivores over a longer time period remains to be seen. Many human-mediated environmental changes, including fire, N deposition, and altered precipitation, influence the availability of soil nutrients, and thus the results of our study likely apply to a broad range of ecosystems experiencing human disturbances. Though having a stronger stoichiometric homeostasis, organisms from higher levels (e.g. predators) are commonly more vulnerable to environmental changes as compared to organisms from lower trophic levels (e.g. plants) (Pereira et al. 2010). Whether and how the physiological and performance costs to maintain elemental homeostasis can partially explain the asymmetric biodiversity decline patterns across trophic levels deserves further attention. A comprehensive investigation of the stoichiometric properties of organisms in food webs will enable a better understanding of the nature of species interactions and may lead to better predictions of the consequences of environmental change for community organization and assembly in the future.

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Data availability

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.7d7wm37rq

Compliance with ethical standards: All experimental procedures were carried out in accordance with the Law of the People’s Republic of China on the Protection of Wildlife (1988).

Conflict of interest: The authors declare no conflicts of interest.

References

Augustine DJ, McNaughton SJ (1998) Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. J Wildl Manage 23:1165–1183.

Bai Y (2012) Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient. J Appl Ecol 49:1204–1215.

Behmer ST (2009) Insect herbivore nutrient regulation. Annu Rev Entomol 54:165–187.

Behmer ST, Joern A (2008) Coexisting generalist herbivores occupy unique nutritional feeding niches. Proc Natl Acad Sci USA 105:1977–1982.

Belovsky GE, Slade JB, Stockhoff BA (1990) Susceptibility to predation for different grasshoppers—an experimental study. Ecology 71:624–634.

Belovsky GE, Slade JB (2000) Insect herbivory accelerates nutrient cycling and increases plant production. Proc Natl Acad Sci USA 97:14412–14417.

Boersma M, Elser JJ (2006) Too much of a good thing: on stoichiometrically balanced diets and maximal growth. Ecology 87:1325–1330.

Boersma M, Mathew KA, Niehoff B, Schoo KL, Franco-Santos RM, Meunier CL (2016) Temperature driven changes in the diet preference of omnivorous copepods: no more meat when it's hot? Ecol Lett 19:45–53.

Branco P, Egas M, Elser JJ, Huisman J (2018) Eco-evolutionary dynamics of ecological stoichiometry in plankton communities. Am Nat 192:1–20.

Cease AJ et al (2012) Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. Science 335:467–469.

Cease AJ, Elser JJ, Fenichel EP, Hadrich JC, Harrison JF, Robinson BE (2015) Living with locusts: connecting soil nitrogen, locust outbreaks, livelihoods, and livestock markets. BioScience 65:551-558.

Chaneton E et al (1996) Nitrogen and phosphorus cycling in grazed and ungrazed plots in a temperate sub humid grassland in Argentina. J Appl Ecol 33:291–302.

Chen L, Wang R (2009) Anatomical and physiological divergences and compensatory effects in two *Leymus chinensis* (Poaceae) ecotypes in Northeast China. Agric Ecosyst Environ 134:46–52.

Cleveland CC, Liptzin D (2007) C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252.

Clissold FJ, Sanson GD, Read J (2006) The paradoxical effects of nutrient ratios and supply rates on an outbreaking insect herbivore, the Australian plague locust. J Anim Ecol 75:1000–1013.

Daufresne T, Loreau M (2001) Plant-herbivore interactions and ecological stoichiometry: When do herbivores determine plant nutrient limitation? Ecol Lett 4:196–206.

Delgado-Baquerizo M, Eldridge DJ, Maestre FT, Ochoa V, Gozalo B, Reich PB, Singh BK (2018) Aridity decouples C: N: P stoichiometry across multiple trophic levels in terrestrial ecosystems. Ecosystems 21:459–468.

Ebell LF (1965) Variation in total soluble sugars of conifer tissues with method of analysis. Phytochemistry 21:227–233.

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Siemann EH (2000) Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578-580.

Elser JJ et al (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142.

Elser JJ et al (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytol 186:593–608.

Fagan WF, Denno RF (2004) Stoichiometry of actual vs. potential predator-prey interactions: insights into nitrogen limitation for arthropod predators. Ecol Lett 7:876–883.

Fagan WF, Siemann E, Mitter C, Denno RF, Huberty AF, Woods HA, Elser JJ (2002) Nitrogen in insects: implications for trophic complexity and species diversification. Am Nat, 160:784-802.

Frank DA, Evans RD (1997) Effects of native grazers on grassland N cycling in yellowstone national park. Ecology 78:2238–2248.

Gusewell S (2004) N : P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266.

Haddad N et al (2001) Contrasting effects of plant richness and composition on insect communities: A field experiment. Am Nat 158:17–35.

Hawlena D, Schmitz OJ (2010) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. Proc Natl Acad Sci USA 107:15503–15507.

Hobbs N (1996) Modification of ecosystems by ungulates. J Wildl Manag 60:695–713.

Hume A et al (2016) Soil C:N:P dynamics during secondary succession following fire in the boreal forest of central Canada. Forest Ecol Manag 369:1–9.

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436.

Joern A, Behmer ST (1997) Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia 112:201–208.

Joern A, Behmer ST (1998) Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. Ecol Entomol 23:174–184.

Li X et al (2015) Combined effects of nitrogen addition and litter manipulation on nutrient resorption of *Leymus chinensis* in a semi-arid grassland of northern China. Plant Biol 17:9–15.

Li X et al (2018) Reciprocal facilitation between large herbivores and ants in a semi-arid grassland. P Roy Soc B-Biol Sci 285:20181665.

Liu C et al (2015a) Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore assemblage and pre-grazing plant diversity. J Appl Ecol 53:242–250.

Liu J, Feng C, WangD, Wang L, Wilsey BJ, Zhong Z (2015b) Impacts of grazing by different large herbivores in grassland depend on plant species diversity. J Appl Ecol 52:1053–1062.

Liu N, Kan HM, Yang GW, Zhang YJ (2015c) Changes in plant, soil, and microbes in a typical steppe from simulated grazing: explaining potential change in soil C. Ecol Monogr 85:269–286.

McNaughton SJ, Ruess RW, Seagle SW (1988) Large mammals and process dynamics in African ecosystems. Bioscience 38:794–800.

McSherry ME, Ritchie ME (2013) Effects of grazing on grassland soil carbon: a global review. Glob Chang Biol 19:1347–1357.

Metcalfe DB, Cherif M, Jepsen JU, Vindstad OPL, Kristensen JÅ, Belsing U (2019) Ecological stoichiometry and nutrient partitioning in two insect herbivores responsible for large-scale forest disturbance in the Fennoscandian subarctic. Ecol Entomol 44:118–128.

Mipam TD, Chen S, Liu J, Miehe G, Tian L (2019) Short-term yak-grazing alters plant-soil stoichiometric relations in an alpine meadow on the eastern Tibetan Plateau. Plant Soil

23:1–13.

Mulder C, Elser JJ (2009) Soil acidity, ecological stoichiometry and allometric scaling in grassland food webs. Global Change Biol 15:2730–2738.

Odum, EP (1959) Fundamentals of Ecology. (2nd ed.). Saunders, Philadelphia.

Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Trends Ecol Evol 13:261–265.

Patra AK et al (2005) Effects of grazing on microbial functional groups involved in soil N dynamics. Ecol Monogr 75:65–80.

Pereira HM et al (2010) Scenarios for global biodiversity in the 21st century. Science 330:1496–1501.

R Development Core Team. (2014) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ren BZ (2004) The biology of locusts in Songnen grassland. Science Press, Jilin, China. (*in chineses*)

Riggi LG, Bommarco R (2019) Subsidy type and quality determine direction and strength of trophic cascades in arthropod food webs in agroecosystems. J Appl Ecol 56:1982–1991.

Risch AC et al (2015) Aboveground vertebrate and invertebrate herbivore impact on net N mineralization in subalpine grasslands. Ecology 96:3312–3322.

Sato C et al (2019) Environmental and grazing management drivers of soil condition. Agric Ecosyst Environ 276:1–7.

Schade JD et al (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol Lett 6:96–101.

Schmitz OJ (2008) Effects of predator hunting mode on grassland ecosystem function. Science

319:952–954.

Schrama M, Heijning P, Bakker JP, van Wijnen HJ, Berg MP, Olff H (2013) Herbivore trampling as an alternative pathway for explaining differences in nitrogen mineralization in moist grasslands. Oecologia 172:231–243.

Shan Y et al (2011) Seasonally dependent impacts of grazing on soil nitrogen mineralization and linkages to ecosystem functioning in Inner Mongolia grassland. Soil Biol Biochem 43:1943–1954.

Simpson SJ, Raubenheimer D (2001) The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. Ecology 82:422–439.

Sitters J, Bakker ES, Veldhuis MP, Veen GF, Olde Venterink H, Vanni MJ (2017) The stoichiometry of nutrient release by terrestrial herbivores and its ecosystem consequences. Front Earth Sci 5:1–8.

Sparks DL, Page AL, Loeppert PA, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (1996) Methods of Soil Analysis Part 3: Chemical methods. Soil Science Society of America and American Society of Agronomy, Madison, WI.

Sperfeld E, Wagner ND, Halvorson HM, Malishev M, Raubenheimer D (2017). Bridging ecological stoichiometry and nutritional geometry with homeostasis concepts and integrative models of organism nutrition. Funct Ecol 31:286–296.

Sterner R, Elser J (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton, NJ: Princeton University Press in press.

Stout WL et al (1997) Nitrate leaching from cattle urine and feces in Northeast USA. Soil Sci Soc Am J 61:1787–1794.

Tian H, Chen G, Zhang C, Melillo JM, Hall CA (2010) Pattern and variation of C: N: P ratios in

China’s soils: a synthesis of observational data. Biogeochemistry 98:139–151.

van Klink R et al (2015) Effects of large herbivores on grassland arthropod diversity. Biol Rev 90:347–366.

Vanni MJ, McIntyre PB (2016) Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: a global synthesis. Ecology 97:3460–3471.

Wang J et al (2018). Feces nitrogen release induced by different large herbivores in a dry grassland. Ecol Appl 28:201–211.

Wang L et al (2019) Diversifying livestock promotes multidiversity and multifunctionality in managed grasslands. Proc Natl Acad Sci USA 116:6187–6192.

Young HS et al (2013) Effects of mammalian herbivore declines on plant communities: observations and experiments in an African savanna. J Ecol 101:1030–1041.

Yu Q et al (2010) Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability. Ecol Lett 13:1390–1399.

Zhang Z et al (2014) Grasshoppers regulate N:P stoichiometric homeostasis by changing phosphorus contents in their frass. PLoS ONE 9:e103697.

Zhong Z, Li X, Pearson D, Wang D, Sanders D, Zhu Y, Wang L (2017) Ecosystem engineering strengthens bottom-up and weakens top-down effects via trait-mediated indirect interactions. P Roy Soc B-Biol Sci 284:20170475.

Zhong Z, Wang D, Zhu H, Wang L, Feng C, Wang Z (2014) Positive interactions between large herbivores and grasshoppers, and their consequences for grassland plant diversity. Ecology 95:1055–1064.

Zhou G, et al (2017) Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. Global Change Biol 23:1167–1179.

Zhu Y et al (2019) Negative effects of vertebrate on invertebrate herbivores mediated by enhanced plant nitrogen content. J Ecol 107:901–912.

FIGURE LEGENDS

Figure 1. Effects of grazing on soil total C, available N, and available P contents (a, c, e), and soil C:(available)N, C:(available)P, and available N:P (b, d, f) in the control and grazed plots. Asterisks indicate a significant difference between treatments ($P < 0.05$). Error bars represent ±1 SE.

Figure 2. Effects of grazing on contents of soluble C, total N, and total P (a, c, e), and (soluble)C:N, (soluble)C:P, and N:P (b, d, f) in *L. chinensis* leaves in the control and grazed plots. Asterisks indicate a significant difference between treatments ($P < 0.05$). Error bars represent ±1 SE.

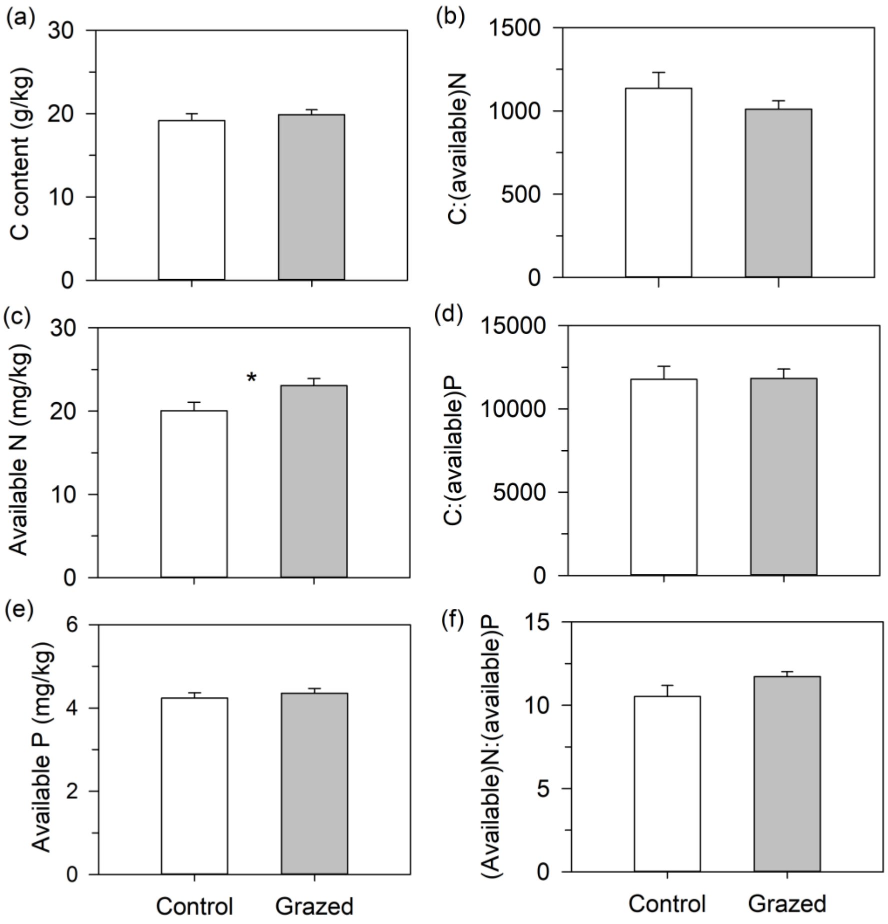
Figure 3. Effects of grazing on the body contents of total C, total N, and total P (a, c, e), and C:N, C:P, and N:P (b, d, f) of male and female *Euchorthippus* grasshoppers in the control and grazed plots. There were no significant differences ($P < 0.05$) between treatments for all the variables. Error bars represent ±1 SE.

Figure 4. Effects of grazing on the (a) feeding frequency and (b) feeding time of *Euchorthippus* grasshoppers on *L. chinenis*, other grasses, and forbs in the control and grazed plots. Asterisks indicate a significant difference between treatments ($P < 0.05$). Error bars represent ±1 SE.

Figure 5. Effects of grazing on the (a) biomass of *L. chinensis*, and (b) abundance of *Euchorthippus* grasshoppers in the control and grazed plots. Asterisks indicate a significant difference between treatments ($P < 0.05$). Error bars represent ±1 SE.

Figure 6. A conceptual framework showing how livestock (cattle) grazing affects stoichiometric properties across trophic levels from soil to plants (*L. chinensis*) to insect herbivores (*Euchorthippus* spp.) in a meadow steppe at northeastern China. Red solid and dashed lines indicate the various mechanisms by which livestock grazing has direct and indirect effects on plants and soils. Dark solid arrows indicate the propagated effects of grazing on elemental composition from soil to plants to insect herbivores. The width of the arrows indicates the strengths of the effects. Dark "+" and green "↑" indicate positive effects. The green triangle indicates that the variation in C:N:P ratios decreases from lower to higher trophic levels (e.g. from soil to insect herbivores), whereas the blue triangle indicates that homeostasis increases from lower to higher trophic levels (e.g. from soil to insect herbivores). The two boxes and arrows among them at the upper right corner indicate the potential linkages among variations in host nutrient conditions, insect stoichiometric homeostasis, insect individual performance, and insect population dynamics under environmental changes. It should be noted that, like cattle, insect herbivores may also affect soil nutrient availability by their impacts on litter quantity and quality (e.g. C:N), and the inputs of their frass and carcasses, especially at high density. In our system, cattle grazing tends to suppress grasshopper density to a relatively low level (about 5 individuals/m^2 in the grazed areas, see Zhu et al. 2019), and thus it is unlikely that these insects play a large role in influencing soil nutrient dynamics in this short-term study.

Fig. 1



(a)
C content (g/kg)
0
10
20
30
(b)
C:(available)N
0
500
1000
1500
(c)
Available N (mg/kg)
0
10
20
30
*
(d)
C:(available)P
0
5000
10000
15000
(e)
Available P (mg/kg)
0
2
4
6
(f)
(Available)N:(available)P
0
5
10
15
Control
Grazed
Control
Grazed

Fig. 2

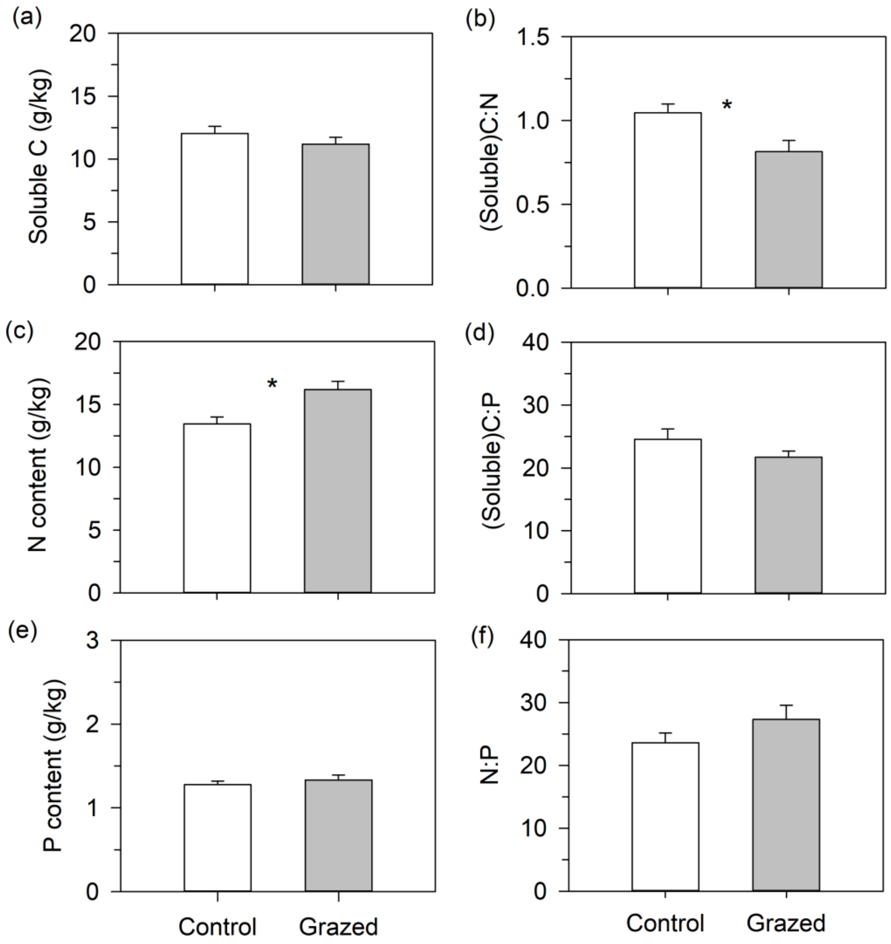

(a)
Soluble C (g/kg)
0
5
10
15
20
(b)
(Soluble)C:N
0.0
0.5
1.0
1.5
*
(c)
N content (g/kg)
0
5
10
15
20
*
(d)
(Soluble)C:P
0
10
20
30
40
(e)
P content (g/kg)
0
1
2
3
(f)
N:P
0
10
20
30
40
Control
Grazed
Control
Grazed

Fig. 3

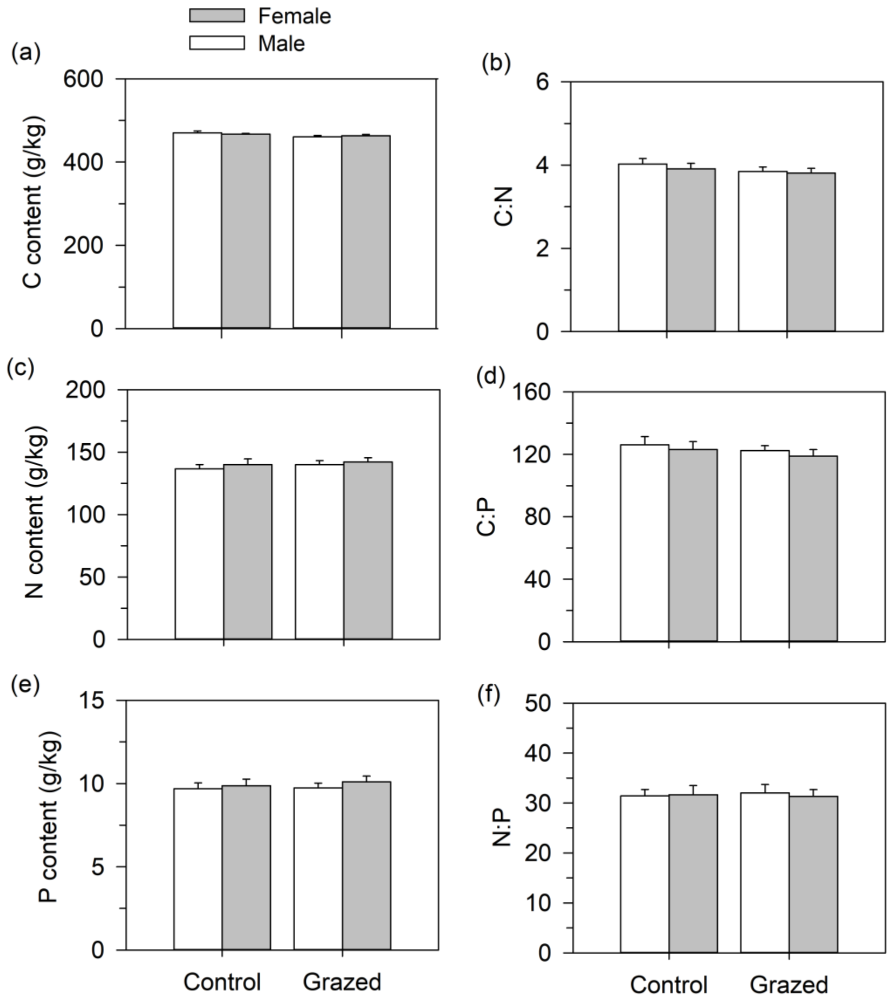

Female
Male
(a)
C content (g/kg)
0
200
400
600
(b)
C:N
0
2
4
6
(c)
N content (g/kg)
0
50
100
150
200
(d)
C:P
0
40
80
120
160
(e)
P content (g/kg)
0
5
10
15
(f)
N:P
0
10
20
30
40
50
Control
Grazed
Control
Grazed

Fig. 4

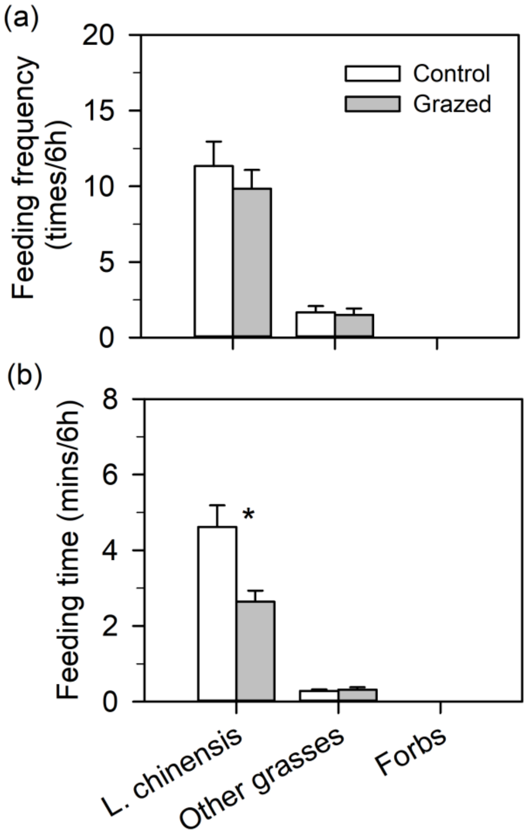
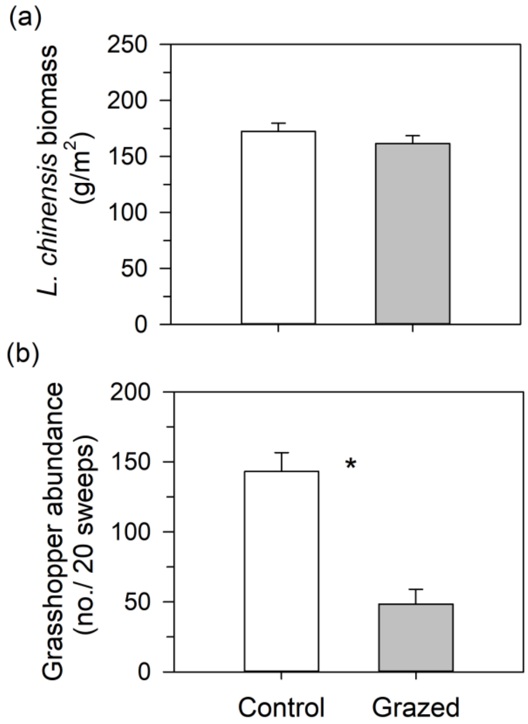
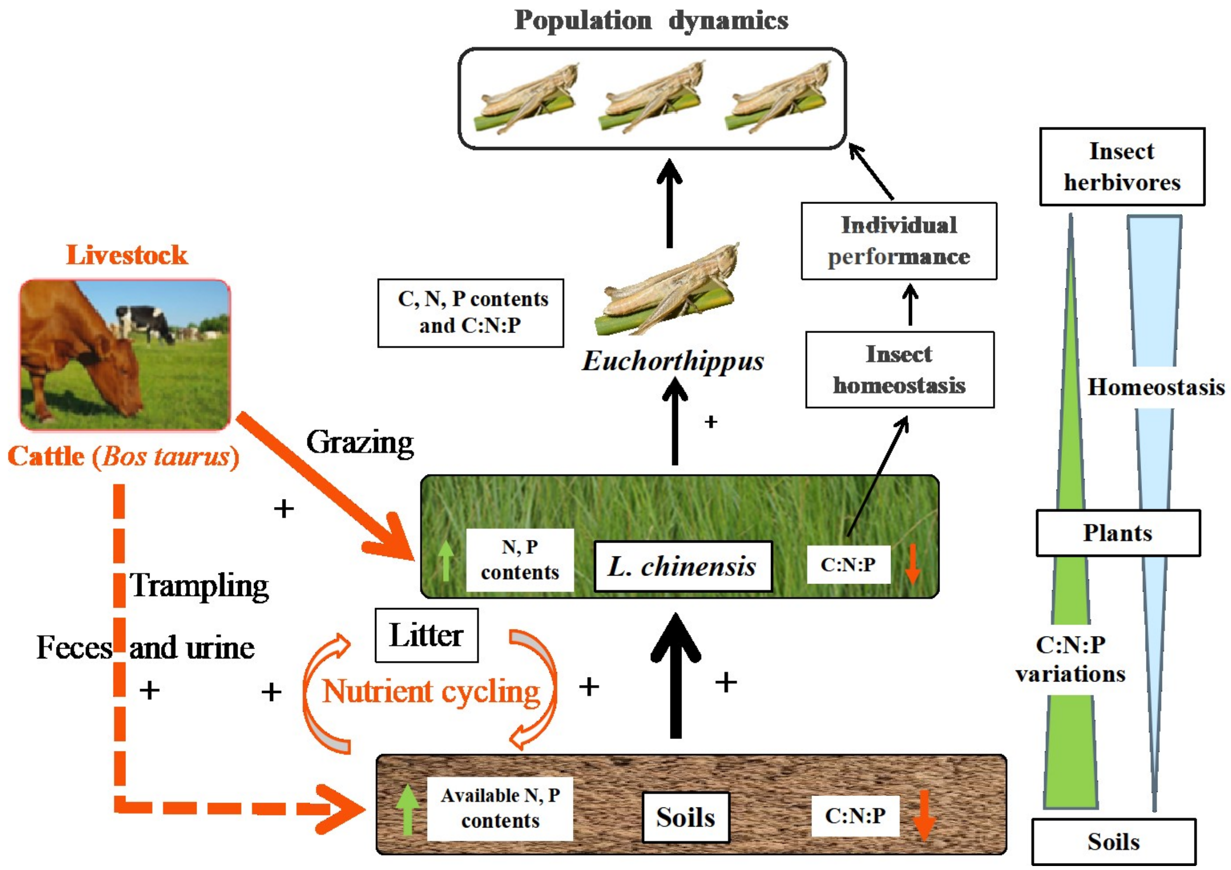

(a)
20
15
10
5
0
Feeding frequency (times/6h)
Control
Grazed
(b)
8
6
4
2
0
Feeding time (mins/6h)
*
L. chinensis
Other grasses
Forbs

Fig. 5



(a)
L. chinensis biomass (g/m^2)
250
200
150
100
50
0
(b)
Grasshopper abundance (no./ 20 sweeps)
200
150
100
50
0
*
Control
Grazed

Fig.6


Population dynamics
Insect herbivores
Individual performance
Livestock
C, N, P contents and C:N:P
Euchorthippus
Insect homeostasis
Homeostasis
+
Grazing
Cattle (Bos taurus)
+
Plants
N, P contents
L. chinensis
C:N:P
Trampling
Litter
Feces
and urine
C:N:P variations
+
+
Nutrient cycling
+
+
Available N, P contents
Soils
C:N:P
Soils