ECOSYSTEM ECOLOGY - ORIGINAL PAPER

Rapid top–down regulation of plant C:N:P stoichiometry by grasshoppers in an Inner Mongolia grassland ecosystem

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Abstract Understanding how food web interactions alter the processing of limiting nutrient elements is an important goal of ecosystem ecology. An experiment manipulating densities of the grasshopper *Oedaleus asiaticus* was performed to assess top–down effects of grasshoppers on C:N:P stoichiometry of plants and soil in a grassland ecosystem in Inner Mongolia (China). With increased grasshopper feeding, plant biomass declined fourfold, litter abundance increased 30%, and the plant community became dominated by non-host plant taxa. Plant stoichiometric response depended on whether or not the plant was a grasshopper host food species: C:N and C:P ratios increased with increasing grasshopper density (GD) for host plants but decreased in non-host plants. These data suggest either a direct transfer of grasshopper-recycled nutrients from host to non-host plants or a release of non-host plants from nutrient competition with heavily grazed host plants. Litterfall C:N and C:P decreased across moderate levels of grasshopper density but no effects on C:N:P stoichiometry in the surface soil were observed, possibly due to the short experimental period. Our observations of divergent C:N:P stoichiometric response among plant species highlight the important role of grasshopper herbivory in regulating plant community structure and nutrient cycling in grassland ecosystems.

Keywords Stoichiometry · Herbivory · Nutrient cycling · Steppe · Grasshopper outbreak

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Introduction

Ecological stoichiometry studies the balance of energy and multiple chemical elements in ecological interactions and processes and has increasingly been applied to a variety of ecological questions, ranging from lake food web dynamics to evolutionary ecology of terrestrial insects (Sterner and Elser 2002). Much of this work deals with the three most important structural and functional elements in organisms, carbon (C), nitrogen (N), and phosphorus (P) (Elser et al. 2000b; He et al. 2006; Sterner et al. 2008). Stoichiometric analysis has proven useful in studying species interactions and trophic relationships among different components in both aquatic (Elser and Hassett 1994; Small and Pringle 2010) and terrestrial ecosystems (Zhang et al. 2004), although the latter has received considerably less attention up to now (Elser et al. 2000a). A consistent pattern emerging from this work is that plant biomass has considerably higher C:nutrient ratios than animal biomass (Sterner and Elser 2002). For example, in terrestrial ecosystems, the C:nutrient ratio of foliage exceeds that of insect herbivores by more than five- to tenfold, on average (Elser et al. 2000a). This considerable stoichiometric

mismatch between autotrophs and herbivores has strong impacts on primary production (Cebrian 1999), herbivore success (Mattson 1980; Sterner and Schulz 1998; Behmer 2009), and consumer-driven nutrient recycling in ecosystems (Elser and Urabe 1999; Frost et al. 2005). Thus, stoichiometric analysis takes a two-pronged approach to study of trophic interactions by considering effects both of stoichiometric food quality and of indirect effects via consumer-driven nutrient recycling (Sterner 1986; Grover 2004; Elser et al. 2010).

Grasshoppers are the dominant invertebrate herbivore in grassland ecosystems and are important in maintaining normal ecosystem function in grasslands (Uvarov 1977; Chapman and Joern 1990; Belovsky 2000). However, outbreaks of grasshoppers are frequently reported worldwide and can have tremendous influence on the ecosystem services provided by grasslands (Guo et al. 1991; Lomer et al. 2001). Due to the considerable economic impact of grasshopper outbreaks, grasshopper control has long been a central issue in study of plant–herbivore interactions (Dyer and Bokhari 1976; Bernays and Chapman 1978; Joern and Behmer 1997; Guo et al. 2006). It is well known that top-down impacts from herbivores can have major influences on plant communities and the functioning of ecosystems (Olff and Ritchie 1998; Frost and Hunter 2007), and the response of plants to herbivory is largely density-dependent (Kemp and Dennis 1993; Zhang 2007). On the one hand, foliage consumption by herbivores may reduce plant abundance and production (Pastor and Naiman 1992). On the other hand, herbivory may also accelerate nutrient cycling and increase plant production depending on temporal scale (McNaughton et al. 1997; Belovsky 2000; Belovsky and Slade 2000, 2002). When the enhancing effect of nutrient cycling exceeds the negative effect of direct consumption, faster nutrient cycling by grasshoppers may actually lead to increases in total plant abundance and production (Belovsky and Slade 2000). However, the contrary responses of plant production to light and heavy degree of grasshopper herbivory remain largely unknown (Lu et al. 2008). Furthermore, effects of herbivory on N cycling in grassland ecosystems are relatively well studied (Belovsky and Slade 2000; Schmitz 2009), but our understanding of the influence of grasshoppers on availability and cycling of P, which is also a widespread limiting element in terrestrial ecosystems (Elser et al. 2007), is extremely limited. In addition, we especially lack knowledge concerning the different responses of different components of plant communities to grasshopper herbivory.

Food plant preferences are well known in grasshoppers (Joern 1983; Joern and Alward 1988; Behmer and Joern 1993; Bernays and Chapman 1994; Raubenheimer and Simpson 2003). In our study site (the Inner Mongolia grassland, hereafter: IM), many of the principal plant species making up "typical steppe" vegetation belong to the family Poaceae (Gramineae) (Li et al. 1988). While the different species of IM grasshoppers have different daily food consumption rates on various plant species, grasses are preferred by the dominant species of IM grasshoppers including our focal study species, *Oedaleus asiaticus* (Li and Chen 1985; Li et al. 1987). Furthermore, IM grasshoppers show species-specific characteristics in food plant height selection (Yan and Chen 1997). For instance, *O. asiaticus* can feed on plants across a wide range of heights, while *Dasyhippus barbipes* and *Pararcyptera microptera meridionalis* mainly prefer plants that are shorter than 20 cm. Species-specific feeding characteristics in grasshoppers may also reflect species-specific nutrient niches (Kang and Chen 1994; Behmer and Joern 2008). While these studies have improved our understanding of plant–grasshopper relationships in this region, little is known about the response of plant community characteristics to grasshopper feeding beyond effects on net primary production. We need information in particular about the immediate influence of rapid increases in herbivory intensity, such as those that occur in grasshopper outbreaks, on grassland dynamics in term of nutrient cycling and C:N:P stoichiometry of primary producers, as these can have very important effects on various aspects of ecosystem functioning (Sterner and Elser 2002).

In this study, we address the following questions. (1) How do the C:N:P stoichiometric patterns of plants, litterfall, and soil change in response to differences in grasshopper density? (2) Do C:N:P stoichiometric responses to grasshopper grazing differ for plant taxa preferred by grasshoppers relative to other plant taxa? (3) What effects does grasshopper herbivory have on the plant community with regard to its C:N:P stoichiometric characteristics and community structure? Answers to these questions may aid in understanding the complex top–down effects of Acridid herbivore outbreaks on primary producer communities, on soil properties, and ultimately on ecosystem functioning in the world's largest contiguous grassland, and indeed in other grasslands worldwide.

Materials and methods

Study site

Our research site in Inner Mongolia lies in the eastern extent of the Eurasian Steppe, the largest continuous biome on Earth. This study was conducted in "typical steppe" vegetation that is part of a permanent experimental plot at the Inner Mongolia Grassland Ecosystem Research Station located in the Xilin River Basin, Inner Mongolia, China (43°26′–44°08′N, 116°04′–117°05′E, average elevation

1,200 m). This area has a continental semiarid climate, characterized by a cold and dry winter and a warm and moist summer. Based on the meteorological records from 1982–2001, the average annual precipitation is 345 mm and the mean monthly temperature ranges from $-21.5°C$ (January) to 18.9°C (July). More than 80% of annual precipitation occurs in the plant growing season (from May to September, about 150 days). The soil type is dark chestnut (namely Calcic Luvisols in the FAO classification system), with an average depth of ~ 100 cm. Soil pH ranges between 7.22 and 8.71 (Zhang et al. 2004). Vegetation at the study site is *Leymus chinensis* steppe, one of the dominant steppe types in Inner Mongolia and widely distributed in the east part of Eurasian steppe (Li et al. 1988). The height of plant community is about 0.60–0.90 m and the dominant species are *L. chinensis* and *Stipa grandis*. Other common species include *Achnatherum sibiricum*, *Agropyron cristatum*, *Cleistogenes squarrosa*, *Carex korshinskyi*, and *Koeleria cristata*. Aboveground biomass of these seven species accounts for more than 80% of the total community primary production (Zhang 2007). The grasshopper *O. asiaticus* (formerly *O. decorus asiaticus*), a member of the subfamily Oedipodinae (Orthoptera:Acrididaes:Oedipodidae), is a dominant species in the study area, with a population density ranging from <10 individuals m^{-2} before 1990 (Kang and Chen 1992) to >50 individuals m^{-2} after 1990 (Xu et al. 2006). It is a relatively large-sized Acrid species (2.64 ± 0.10 cm in length and 0.24 ± 0.03 g in weight for males, 3.56 ± 0.24 cm and 0.78 ± 0.16 g for females). Male and female adults of *O. asiaticus* can be easily identified by visual inspection (Zhang 2007).

Experimental design and field investigation

Based on results from a pilot experiment in summer 2004, during July and August 2005 we established 72 enclosures ($1 \times 1 \times 1$ m) within a permanent grazer-exclusion experimental plot (fenced in 1980 in order to exclude sheep grazing). The minimum distance between any two units was 1 m. Each enclosure consisted of a steel-framed and nylon-netted cage with the lower edge sealed against the soil to prevent escape of experimental animals and intrusion of other species. A 0.5×0.5 m window on the upper side of each cage allowed experimental operations such as introduction of grasshoppers, checking of grasshopper densities, and removal of predators (mostly spiders).

We designed a density gradient experiment to imitate the grasshopper outbreaks of different intensities. On 24 July 2005, adult *O. asiaticus* were introduced to cages at 8 treatment levels: 0 (control), 2, 5, 10, 20, 30, 40, 50 grasshoppers m^{-2}. The sex ratio of each treatment was 1:1 (male to female) but 3:2 or 2:3 (at random) for the

5-grasshopper treatment. There were nine replicates for each treatment, randomly allocated among cages. After the experimental grasshoppers were introduced, we checked each cage every 3 days to confirm the number of living grasshoppers (dead grasshoppers were removed and replaced with a similarly-sized animal of the same sex), repair damaged cages, and eliminate spiders and other species of grasshoppers that occurred naturally within the cages.

On 24 August 2005 (1 month after the start), cages were removed and litterfall was collected before sampling of living plants. The aboveground standing biomass was harvested by clipping off all plants at the soil surface. We sorted plant biomass at the species-level and weighed all samples to obtain fresh and dry weight. Soil samples consisted of duplicate 5-cm soil cores that were collected from each cage and then mixed prior to further analyses.

Chemical content analyses for organic C, total N and total P

Plant samples were dried at 65°C in an oven for 48 h, soil samples were air-dried for 48 h, and then both plant and soil samples were milled and ground to fine powder to pass through mesh screens (80 μm for plant samples, 100 μm for soil samples) before chemical analysis. Organic C contents of plant and soil samples were analyzed by using external heating method (Bao 2000), total N (TKN) of plant and soil samples was determined by applying a micro-Kjeldahl procedure with sulfuric acid and digestion catalyst. Total phosphorus was analyzed colorimetrically by employing the ammonium molybdate method (Bao 2000). All our results for element concentration were expressed as g kg^{-1} on a dry weight basis, and elemental ratios were converted to atomic ratios by correcting for each element's atomic mass. C, N, and P concentrations at the community level were weighted by the relative biomass abundance of the different species.

Host and non-host plant identification

Based on previous research (Li et al. 1987) of food plant selection by three grasshopper species (including our study species), we compared responses of C:N:P stoichiometry to grasshopper herbivory for two groups of plants, those preferred for feeding by *O. asiaticus* ("host" plants, several species of monocots, including *L. chinensis*, *S. grandis*, *A. cristatum*, *C. squarrosa*, *C. korshinskyi*, and *K. cristata*), and those that are not preferred by this species ("non-host", including monocot *A. sibiricum* and plants belonging to genus *Allium* of family Liliaceae, as well as all other dicots). More than 20 species of plants in our experimental plots were identified as non-hosts, although they accounted

for 36.4% in total aboveground biomass of plant community based on our control plot investigation. We summed biomass and nutrient contents across taxa classified into these two groups and then analyzed the data with respect to grasshopper density.

Statistical analyses

Statistical analyses were performed by using SPSS version 13.0 (SPSS). The linear relationships of grasshopper density with various indices of C:N:P stoichiometry were examined by calculating Spearman coefficients. To establish the final function to describe our results fitting the non-linear but not linear model, we employed Akaike information criterion (Burnham and Anderson 2002) to compare the fit of different functions to the data, and regression curve fits were analyzed using ANOVA. All data in our results met the requirements of ANOVA; no data transformations were needed.

Results

Grasshopper effects on aboveground biomass and litterfall

As expected, aboveground biomass of plants was strongly influenced by grasshopper feeding and had a significant negative linear relationship with grasshopper density (GD, hereafter) ($r^2 = 0.97$, $F = 183.4$, $P < 0.0001$; Fig. 1a). As the relationship between litterfall dry weight and GD was not linear, a quadratic relationship (concave downward) was fit to the data and found to be highly significant ($r^2 = 0.96$, $F = 53.2$, $P = 0.0004$) (Fig. 1b) with maximum litterfall at ~ 37 grasshoppers m^{-2}.

Grasshopper effects on C:N:P stoichiometry of plants, litterfall, and soil

Plant C concentration of overall biomass decreased significantly with increasing GD ($r^2 = 0.83$, $F = 30.0$, $P = 0.002$), while N ($r^2 = 0.78$, $F = 21.4$, $P = 0.004$) and P ($r^2 = 0.87$, $F = 40.2$, $P = 0.001$) concentrations increased (Fig. 2a–c). Consequently, C:N ($r^2 = 0.90$, $F = 55.4$, $P < 0.001$) and C:P ($r^2 = 0.90$, $F = 56.5$, $P < 0.001$) ratios of overall plant biomass declined considerably (Fig. 2d, e). However, the relationship between plant N:P ratio and GD was not statistically significant, although it did show a fairly visible quadratic trend (Fig. 2f).

For litterfall, C concentration had a negative relationship with GD but this trend was not statistically significant ($r^2 = 0.31$, $F = 2.72$, $P = 0.15$; Fig. 2g). However, significant or marginally significant quadratic regressions with
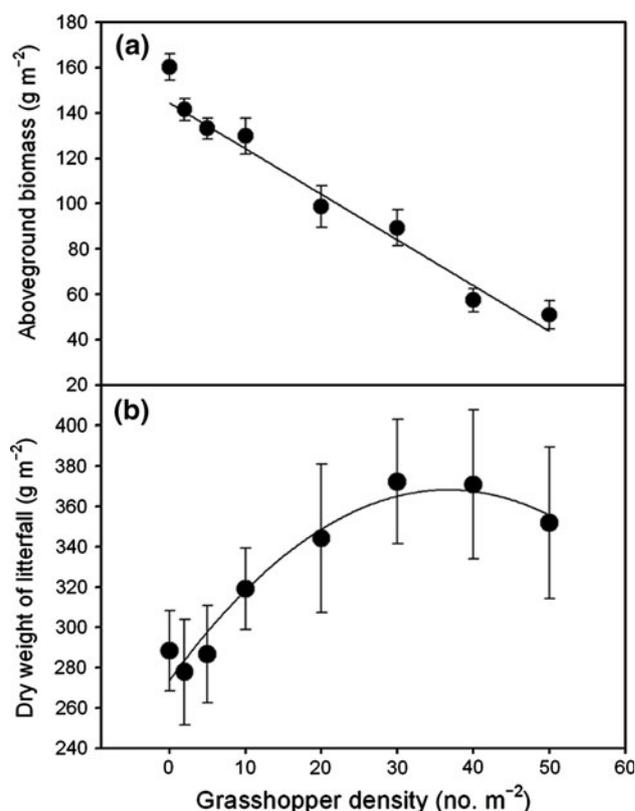
Fig. 1 Responses of **a** total aboveground plant biomass and **b** dry weight of total litterfall to grasshopper density (GD) for the grasshopper *Oedaleus asiaticus*. *Error bars* indicate ±1 SE. In (**a**), a strong negative relationship was found between aboveground biomass of plant and GD (ANOVA: $r^2 = 0.97$, $F = 183$, $P < 0.0001$) and yielded the following equation: $y = -2.11\,x + 149.0$. In (**b**), litterfall biomass included all pre-existing litter plus new litter produced during the experiment. A quadratic function fit to the data was significant (ANOVA: $R^2 = 0.96$, $F = 53.2$, $P = 0.0004$) and yielded the following equation: $y = -0.07\,x^2 + 5.15\,x + 274$. Both the linear and quadratic terms were statistically significant ($P < 0.05$)

GD (quadratic downwards for nutrient concentrations, upwards for C:nutrient ratios; Fig. 2h–k) were observed for N concentration ($r^2 = 0.71$, $F = 6.09$, $P = 0.05$), P concentration ($r^2 = 0.74$, $F = 6.95$, $P = 0.04$), C:N ($r^2 = 0.68$, $F = 5.21$, $P = 0.06$), and C:P ($r^2 = 0.72$, $F = 6.57$, $P = 0.04$). In contrast, the N:P ratio of litterfall did not change with grasshopper density (Fig. 2l).

Nutrient contents and C:N:P stoichiometry of soil (0–5 cm) appeared to change somewhat along the grasshopper density gradient (Fig. 2m–r). However, with the exception of soil P concentration ($r^2 = 0.68$, $F = 5.59$, $P = 0.037$), these trends were not statistically significant.

Grasshopper effects on host and non-host plants

Aboveground biomass of host plants declined dramatically ($\sim$fivefold) when GD increased from 0 to 50

Fig. 2 Density-dependent effects of grasshoppers on C:N:P stoichiometry of overall plant biomass (*left column*, **a–f**), on litterfall (*middle column*, **g–l**), and on soil (0–5 cm) (*right column*, **m–r**). *Error bars* indicate ±1 SE; *conc.* concentration. The parameters and statistical significance for functions fit to each relationship are given in Table 1. *Lines* are indicated only for relationships that were statistically significant (*P* < 0.05)
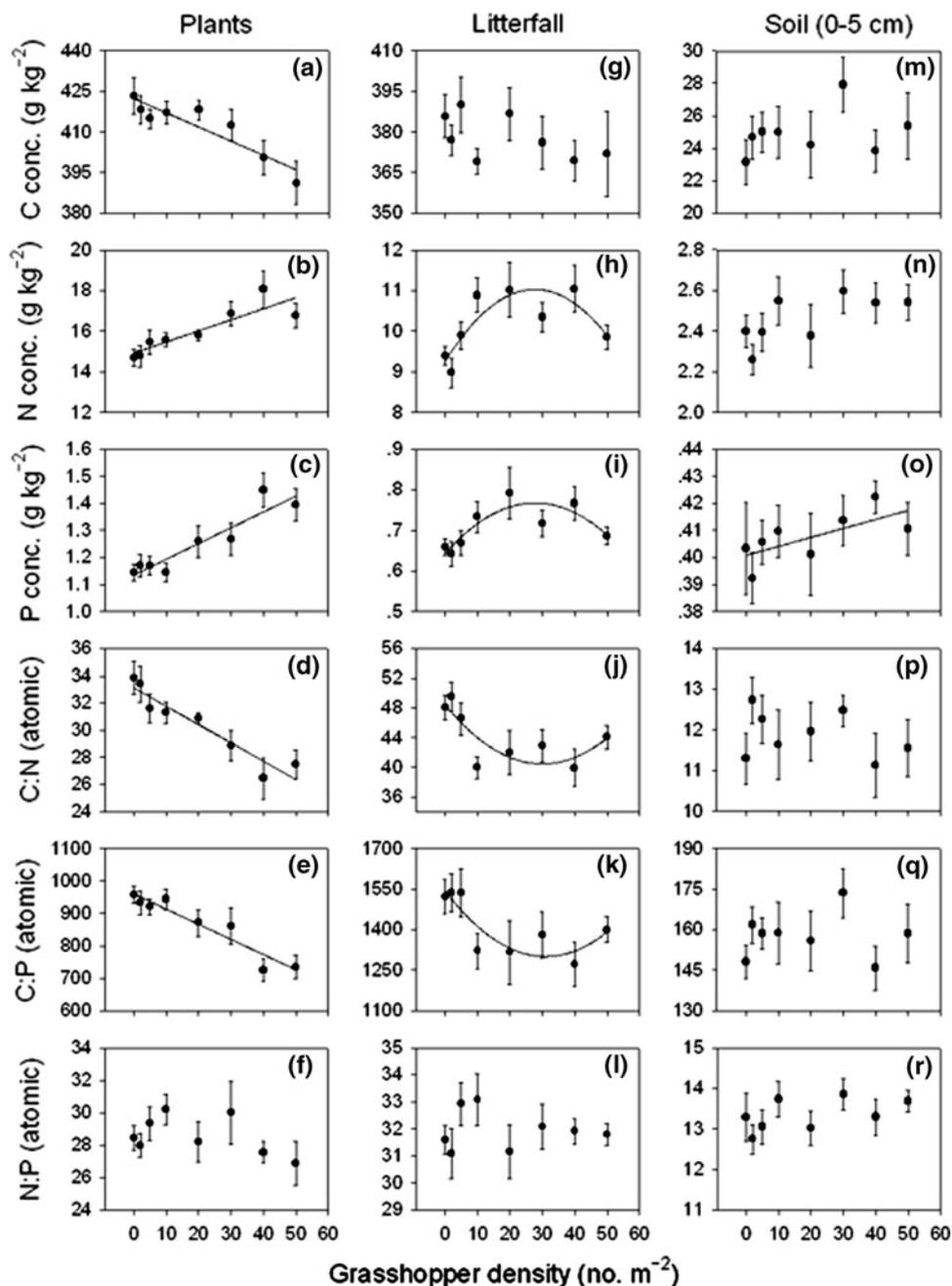
grasshoppers m^{-2}, while non-host plant biomass *increased* as GC increased from 0 to 15 individuals m^{-2} and then declined gradually. Thus, our experiment shows that intense herbivory by *O. asiaticus* can alter plant community structure from grass-dominant to dicot-dominant (Fig. 3). These contrasting changes in aboveground biomass for host and non-host plants were accompanied by notably different trends in C:N:P stoichiometry of the two groups of plants (Fig. 4). As grasshopper density increased from 0 to 30 individuals m^{-2}, N and P concentrations of non-host plant biomass *increased*, in strong contrast to the trend for host plants, where nutrient concentrations *decreased* (Fig. 4b–c). This contrasting pattern also

occurred for C concentration at low GD (i.e. 0–20 individuals m^{-2}) (Fig. 4a). As result of these trends, C:N ratio *increased* in a non-linear fashion with grasshopper density for host plants (Fig. 4d) but C:N and C:P *declined* for non-host plants (Fig. 4d–e). Finally, biomass N:P ratio declined with GD in host plants but was relatively unchanged in non-host plants (Fig. 4f).

Grasshopper effects at the species level

Our experiment showed that *O. asiaticus* did not consume *A. sibiricum* (Poaceae). This is notable because most species

Table 1 Quadratic (or linear) regression results for C:N:P stoichiometry of plants, litterfall, and soil (0–5 cm) (see note) as a function of grasshopper *Oedaleus asiaticus* density (GD), corresponding to Fig. 2

		Type of regression	Intercept	x	x^2	r^2	P
Plant	C	Linear	422	−0.53	–	0.83	0.002
	N	Linear	14.9	0.055	–	0.78	0.004
	P	Linear	1.14	0.006	–	0.87	0.001
	C:N	Linear	33.1	−0.14	–	0.90	<0.001
	C:P	Linear	960	−4.67	–	0.90	<0.001
	N:P	–	–	–	–	–	–
Litterfall	C	–	–	–	–	–	–
	N	Quadratic	9.26	0.13	-2×10^{-3}	0.71	0.046
	P	Quadratic	0.64	8.9×10^{-3}	-1.6×10^{-3}	0.74	0.036
	C:N	Quadratic	48.4	−0.53	8.9×10^{-3}	0.68	0.006
	C:P	Quadratic	1,543	−15.7	0.25	0.72	0.04
	N:P	–	–	–	–	–	–

Only parameters for statistically significant ($P < 0.05$) relationships are presented

Numbers in the columns x and x^2 represent the coefficients of x and x^2 in the quadratic (or linear) equations, respectively. P concentrations of soil had a significant linear relationship with GD, and yielded the following equation: $y = 0.00025 \, x + 0.4$ ($r^2 = 0.68$, $P = 0.037$), while all other parameters were not correlated with GD

in the Poaceae have previously been identified as host plants of grasshoppers (Li and Chen 1985; Li et al. 1987).

We analyzed the C, N and P concentrations of seven species of plants and a mixed sample of all other plant species (including dicots and some monocots such as *Allium*) in each quadrat. While each species (or group) had similar C concentrations (Figs. 4 and 5), N and P concentrations differed significantly (Figs. 4 and 5; Electronic Supplementary Material). Specifically, non-host plants generally had higher N and P concentrations than host plants (Fig. 5). These species were mostly dicotyledonous taxa (except for the monocot *A. sibiricum*). Among the grasshopper-preferred species, *L. chinensis, C. squarrosa* and *K. cristata* were the most nutrient-rich species (Fig. 5). We found that, *L. chinensis, A. cristatum* and *C. squarrosa* showed the strongest changes in C:N ratio in response to increased grasshopper density, while *A. cristatum* and *C. squarrosa* had peak C:N ratios at intermediate GD (Fig. 5, 16; Fig. 5, 28). C:N of the dominant *L. chinensis* increased up to levels of 40 grasshoppers m^{-2} (Fig. 5, 4). Contrary to other host plants, P concentration of *S. grandis* increased (and C:P and N:P declined) with GD, while *C. duriuscula* and *K. cristata* showed little change in P concentration (Fig. 5).

Discussion

Density gradient experiments as mimics of immediate grasshopper outbreak effects

Acridid outbreaks have long been a major problem that attracts considerable public attention (Uvarov 1977;
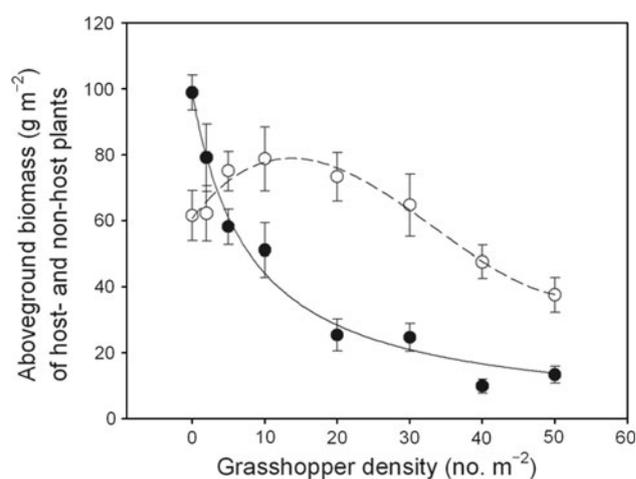
Fig. 3 Effects of grasshopper density on the aboveground biomass of host- (*filled circles*) and non-host (*open circles*) plants. *Error bars* represent ±1 SE. An exponential function fit to the data of host plants was statistically significant (ANOVA: $R^2 = 0.91$, $F = 63.0$, $P = 0.0002$) and yielded the following equation: $y = 79.4 \, e^{-0.043x}$. A logistic function fit to the data of non-host plants was statistically significant (ANOVA: $R^2 = 0.98$, $F = 55.5$, $P = 0.001$) and yielded the following equation: $y = 1.36 \times 10^{-3} \, x^3 - 0.14 \, x^2 + 2.95 \, x + 60.6$

Lockwood et al. 2000; Lomer et al. 2001; Branson et al. 2006). *O. asiaticus* outbreaks in China have been reported in a wide range of grasslands in the past two decades (Xu et al. 2006). Our results clearly show that, on a seasonal time scale, total aboveground biomass of plants was significantly reduced by grasshopper feeding in a manner directly proportional to grasshopper density (GD) (Fig. 1). This supports past conclusions regarding the negative effects of intense grasshopper feeding on plant production as well as food quantity and quality for mammalian
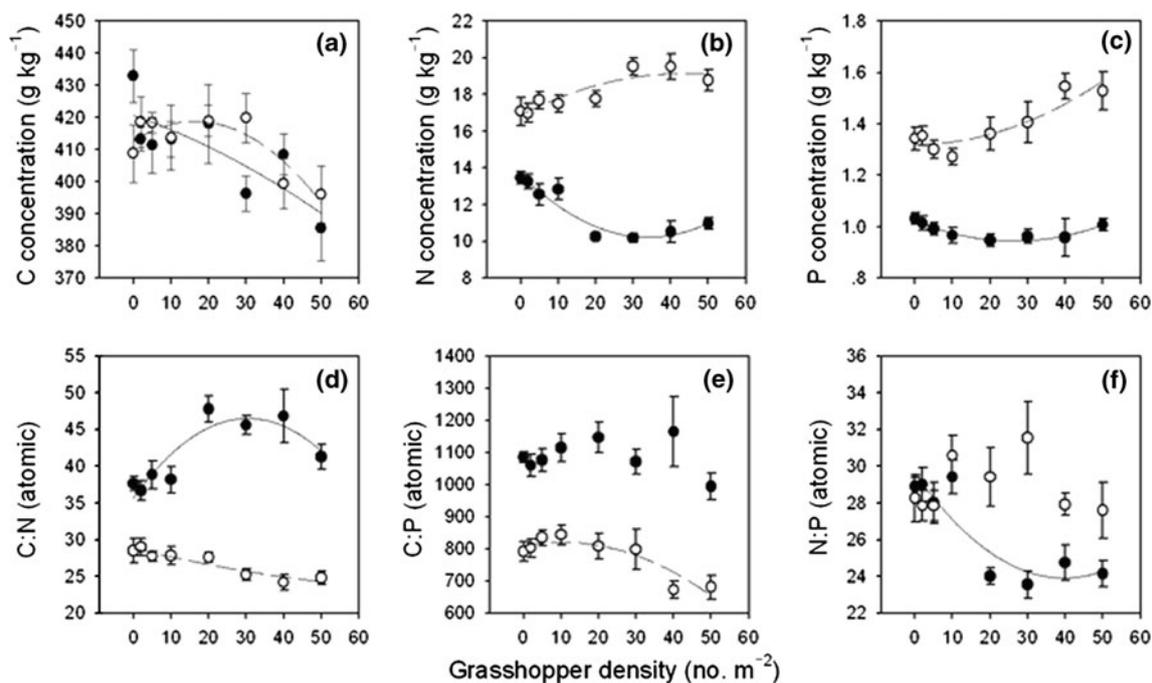
Fig. 4 C:N:P stoichiometric responses of overall host plants (*closed circles*) and non-host plants (*open circles*) to grasshopper. *Error bars* indicate ±1 SE. The parameters and statistical significance for functions fit to each relationship are given in Table 2. *Lines* are indicated only for relationships that were statistically significant ($P < 0.05$)

herbivores and other insects (Lockwood et al. 2000; but see Belovsky 2000). While moderate degrees of grasshopper consumption may increase plant production (Belovsky and Slade 2000; Lu et al. 2008), heavy herbivory in the middle of the growing season, as in our experiment, would greatly suppress plant production (Lu et al. 2008). This reflects a grasshopper outbreak scenario. Similar to an increasing intense grasshopper outbreak, increased GD in the present work resulted in increased N and P concentrations (and decreased C:N and C:P ratios) of overall plant biomass (Fig. 2), suggesting that grasshopper feeding (and perhaps associated nutrient recycling) leads to changes in stoichiometric food quality of primary producers. However, this effect is mainly due to the combined effects of shifts in the relative abundance of plant biomass between host and non-host plants and of contrasting responses of N and P concentrations between these two groups. While the actual impacts of grasshoppers in any particular setting will depend on the combined and interactive effects of overall grasshopper size, daily food consumption, abundance, living habit and feeding duration (Whitham and Mopper 1985; Bakker et al. 2004; Schmitz 2008; Hillebrand et al. 2009), rapid top–down regulation of plant C:N:P stoichiometry by grasshoppers seems to be a likely outcome, especially under conditions of large grasshopper population outbreaks.

Table 2 Quadratic (or linear) regression results for C:N:P stoichiometry of two plant groups (host plants and non-host plants) and grasshopper density, corresponding to Fig. 4

		Quadratic relationship				
		Intercept	x	x^2	r^2	P
Host plants	C[a]	–	–	–	–	–
	N	13.7	0.202	2.98×10^{-3}	0.91	0.003
	P	1.02	-6.01×10^{-3}	1.14×10^{-4}	0.93	0.001
	C:N	35.7	0.71	-0.012	0.82	0.014
	C:P	–	–	–	0.47	0.21
	N:P	29.6	-0.29	3.62×10^{-3}	0.81	0.016
Non-host plants	C	413	0.73	-0.022	0.77	0.024
	N	16.89	0.105	-1.21×10^{-3}	0.82	0.014
	P	1.32	4.99×10^{-5}	9.81×10^{-5}	0.83	0.011
	C:N	28.8	-0.12	6.44×10^{-4}	0.89	0.004
	C:P	810	2.14	-0.11	0.82	0.011
	N:P	–	–	–	0.57	0.12

Only parameters for statistically significant ($P < 0.05$) relationships are presented

Numbers in the columns x and x^2 represent the coefficients of x and x^2 in the quadratic equations, respectively

[a] C concentrations of host plants had a significant linear relationship with grasshopper density, and yielded the following equation: $y = -0.601 x + 422.0$ ($r^2 = 0.63$, $P = 0.018$)
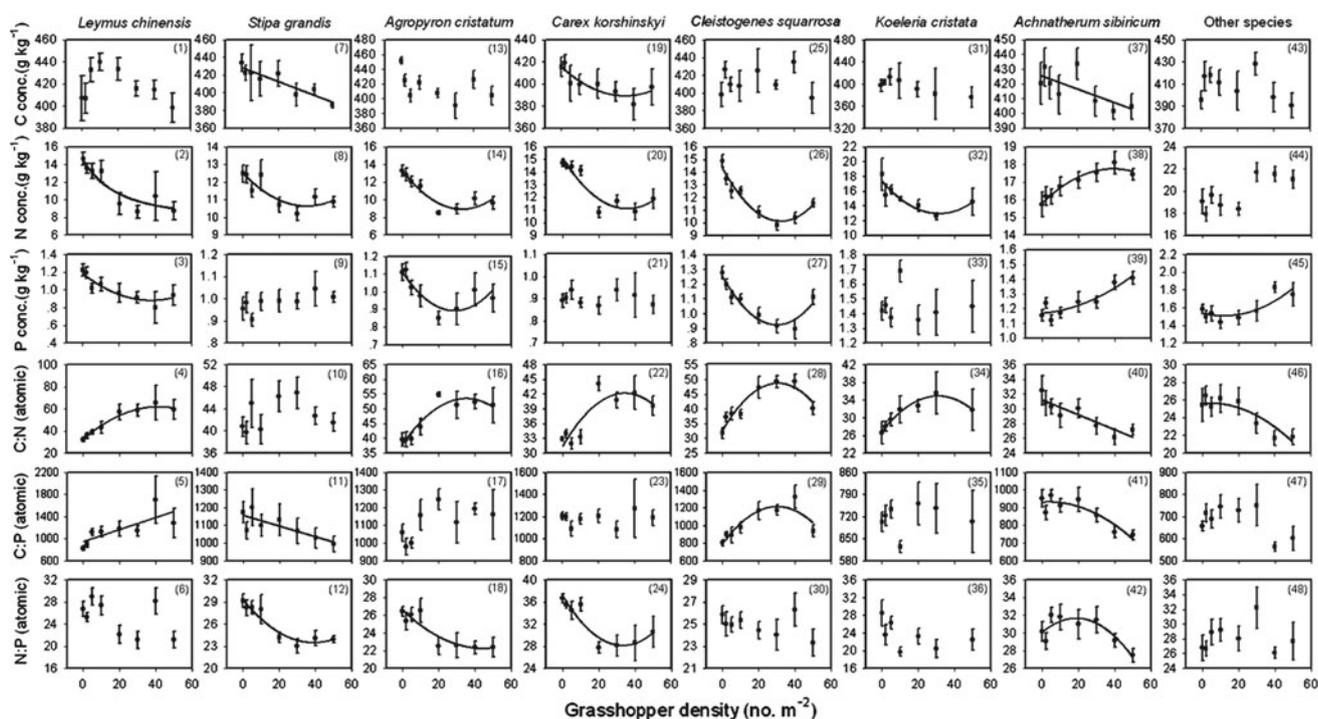
Fig. 5 Responses of C:N:P stoichiometry of seven species and one plant group to grasshopper density. *Error bars* indicate ±1 SE; *conc*. concentration. The parameters and statistical significance for functions fit to each relationship are given in the Electronic Supplementary Material. *Lines* are indicated only for relationships that were statistically significant ($P < 0.05$)

Contrasting responses in host and non-host plants

Our data demonstrate that the biomass of host plants decreased with increased GD, as expected. However, non-host plant biomass increased with GD up to about 15 individuals, above which a significant decline occurred (Fig. 3). Consumption of non-host plants by *O. asiaticus* in higher density treatments due to hunger could be responsible for this pattern, although changes in physical conditions such as light intensity may be a complementary mechanism. Regeneration rates of the two plant groups were divergent as well. For instance, at the end of our experiments, some seedlings or resprouts of *A. sibiricum* and some dicotyledonous species (non-host) were found, but there were no younger shoots observed for host plant taxa. This indicates that plant community structure could be altered from grass-dominant to dicot-dominant by intense herbivory of *O. asiaticus* (Fig. 3).

We also found contrasting responses in C:N:P stoichiometry between the two plant groups. The nutrient concentrations of host plants generally *declined* (C:nutrient ratios increased) with increased grasshopper density while non-host N and P concentrations increased (C:nutrient ratios decreased) (Fig. 4). To the extent that plant N or P contents might be a significant dietary limitation on grasshopper growth and reproduction, our data suggest strong dual density-dependent mechanisms resulting from grasshopper impacts: increased grasshopper densities not only diminish the *quantity* of available biomass of preferred food plants, they also reduce the quality of that food (i.e. low food N and P concentrations). We would expect this to affect herbivore foraging strategy and performance (Hessen et al. 2002; Raubenheimer and Simpson 2003). Our field observations of the plots suggest that tissue-specific feeding behavior by grasshoppers is likely responsible for the observed changes in host plant stoichiometry. That is, similar to other grasshoppers, *O. asiaticus* tends to feed on extended grass leaves and to cut them off in mid-leaf with the upper leaf falling to the ground. Thus, the grasshopper tends to remove, non-consumptively as well as consumptively, relatively nutrient-rich parts of the grass blade, leaving relatively low-nutrient biomass behind, and may decelerate N cycling indirectly (Ritchie et al. 1998). This morphological impact on plants due to grasshopper preferences here is similar to observations of effects of the grasshopper *Orcheliu fidicinium* on the North American wetland plant *Spartina alterniflora* (McGoff 2004). Grasshoppers have evolved powerful mechanisms (e.g., post-ingestive regulation) in dealing with food plants that differ in quality in term of their nutrient (N and P) concentrations (Simpson and Raubenheimer 1993; Yang and Joern 1994; Behmer and Joern 2008) and thus reduced quality in food plants would result in higher amounts of food consumption and impose another selection pressure on plant community dynamics.

For grasshopper effects on C:N:P ratios in non-host plants, three possibilities seem likely. First, strong negative impacts of grasshopper consumption on host plant biomass likely reduced inter-specific competition for soil nutrients, freeing up more soil resources for use by non-host plants spared from grasshopper consumption (Ritchie and Tilman 1993; Grover 1997). Second, recycling of nutrients by grasshoppers may mobilize N and P previously captured by host plants and make it available for uptake by non-host plants. Third, it may be that removing biomass of preferred host plants allowed more light to penetrate the canopy, thus leading to increased biomass accumulation of non-host taxa. Our data are unable to distinguish among these mechanisms and more work is needed.

In addition, *A. sibiricum* (a member of the family Poaceae) was identified as a non-host plant for the grasshopper *O. asiaticus* based on our investigation, whereas most species of other Poaceae plants were host plants. *A. sibiricum* had similar C:N:P stoichiometric characteristics with other Poaceae species (Fig. 5) but was not consumed by our target grasshopper in this study. The reason why *O. asiaticus* does not prefer *A. sibiricum* (with relatively higher abundance in the plant community at our study site) is not clear, although secondary metabolites and foliar physical characteristics (Zhang 2007) as well as endophytic fungi (Y.B. Gao, personal communication) may account for this result.

Implications of litterfall responses

Our data showing a strong quadratic relationship between dry weight of litterfall and grasshopper density (Fig. 1) is notable. The initial increase in litterfall with increasing GD is easy to understand, because, as discussed above, Acridid insects often feed on plants by cutting them off mid-leaf or mid-stem. This results in considerable amounts of plant material falling to the soil surface as herbivory-induced litterfall. In our data, this effect seems to dominate up to grasshopper densities of ~ 37 grasshoppers m^{-2} (Fig. 1). Above these levels, visual observation suggested that *O. asiaticus* changed its feeding behavior and began to eat fresh plant tissue on the ground, thus decreasing the accumulating mass of plant detritus that we measured in the end of experiment.

The quadratic relationship between dry weight of litterfall and grasshopper density in our study has potentially important ecological consequences for element cycling given the role of litter C:N:P stoichiometry on soil nutrient availability and eventually on the quantity and quality of food resources available to grasshoppers. The decline of C:N and C:P ratios in litterfall with increased GD (Fig. 2) implies that grasshoppers may face increasingly severe nutrient limitation due to decreased quality of their dietary

plants. This is because the more nutrient-rich is the litterfall, the poorer in nutrient should be the standing biomass of living plants, at least in the short term (Fig. 4). However, it is clear from our data that grasshopper feeding increased the total mass of litterfall and thus likely accelerated ecosystem biomass turnover. Furthermore, the lowered litterfall C:N ratio with increased GD should also increase litter decomposition rate and thus accelerate N recycling (Han et al. 1999; Wardle et al. 2002). These long-term effects might offset the short-term negative effects of grasshoppers on the quantity and quality of their food base. To more completely understand the plant–grasshopper interactions, further study is needed to trace the long-term life history strategy alterations associated with potential constraints of food C:N:P stoichiometry on the grasshoppers themselves (Joern and Alward 1988; Raubenheimer and Simpson 1993; Schmitz 1994; Simpson et al. 2004; Berner et al. 2005). The long-term effects of plant–grasshopper C:N:P stoichiometric interactions on belowground process also need to be explored (Bardgett and Wardle 2003; Wardle et al. 2002).

Short-term versus long-term effects, direct versus indirect effects

Our field experiment was conducted in a period of 1 month with an emphasis on evaluating potentially abrupt shifts in plant community structure and C:N:P stoichiometry potentially induced by grasshoppers during a population outbreak. Obviously these were short-term rather than long-term effects, and our findings necessarily resulted from the direct effects of herbivory in the current year but not the indirect effects of grasshopper consumption in ensuing years. Such indirect effects include alterations in availabilities of light and carry-over of soil nutrients as well as changed interactive effects among environmental factors caused by herbivore impacts (Sterner 1986; Lawton 1994; Joern and Mole 2005). Grasshoppers may also exert long-term effects on grassland nutrient cycling via the deposition of nutrient-rich frass, eggs, and carcasses (Zhang 2007), and this kind of consumer-driven nutrient recycling is an important mechanism through which herbivores affect ecosystem functioning (Elser and Urabe 1999). Usually, these mechanisms are not mutually exclusive (Schmitz 2008). We used this short-term experiment to mimic the effects of grasshopper outbreaks, because, in the past 20 years, our study species, *O. asiaticus*, has exhibited great potential as an outbreak species due to its relatively large body size, large daily food consumption, and higher migration capacity (Jiang et al. 2003; Xu et al. 2006). Thus, we would expect the short-term effects we have shown to accumulate and impose a long-term pressure on the plant community and thus

dramatically change the physical and chemical as well as genetic properties of the grassland ecosystem (Whitham et al. 2008). In this scenario, our results could serve as an important source of knowledge to improve our understanding of rapid shifts in plant associations in IM grasslands. For example, the overall response of plant C:N:P stoichiometry to GD indicated that grassland succession under heavy grasshopper feeding pressure would move in a direction in which the plant community has low biomass dominated by non-preferred plants with higher nutrient (N and P) content and therefore higher nutrient demand. In turn, this should alter overall rates of soil nutrient cycling (Ågren and Ernesto 1996) and alter the strength of the grassland ecosystem in C sequestration. Our data also have implications for the role of grasslands in providing ecosystem services in support of livestock production. In Inner Mongolia, domestic animals such as sheep have similar plant feeding preferences as grasshoppers (Kang and Chen 1994). Thus, heavy grasshopper grazing would alter an aspect of grassland ecosystem function that directly benefits human beings. So, our data have value in supporting biodiversity conservation and ecosystem management in typical grassland regions that are widely distributed in the Eurasian Steppe.

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