

# Successive sheep grazing reduces population density of Brandt's voles in steppe grassland by altering food resources: a large manipulative experiment

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**Abstract** Livestock grazing has shaped grassland ecosystems around the world. Previous studies indicated grazing showed various impacts on small rodents; however, most studies were conducted over 1–2 years without controlling for confounding factors such as immigration/emigration and predation in rodents. Brandt's voles (*Lasiopodomys brandtii*) are generally recognized as pests because of food overlap with domestic herbivores, but are also important for biodiversity conservation because they provide nests or food to many birds. Fully understanding the ecological relationship between domestic herbivores and small mammals is essential to making ecosystem management decisions. To address these needs, we carried out a field experiment during the period 2010–2013 to assess the effects of sheep grazing on vegetation and the population density of

Brandt's voles along a gradient of three grazing intensities by using 12 large-scale enclosures. Responses of Brandt's voles to livestock grazing varied with grazing intensity and year. As compared to the control group, sheep grazing had no effect on vole abundance in the first year but an overall negative effect on vole abundance in the following 3 years. Successive grazing caused decreases in survival and male body mass of voles, but had no significant effect on fecundity. Negative effects of grazing were associated with a grazing-induced deterioration in both food quantity (reflected by biomass and cover of less-preferred plants), and food quality (measured by tannin and total phenol content). Our findings highlight the urgent need for more flexible management of yearly rotational grazing to optimize livestock production while maintaining species diversity and ecosystem health.

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## Introduction

As human populations grow, anthropogenic disturbances are imposing increasing threats to biodiversity and ecosystem functioning (Dirzo et al. 2014; Zhang et al. 2014). Livestock grazing has been recognized as one of the oldest and most geographically expansive forms of terrestrial anthropogenic activities (Diaz et al. 2007; Pielke et al. 2011), and has significantly shaped grassland ecosystems through both direct and indirect effects (Golluscio et al. 2009). Understanding of the effects of grazing on key species is urgently needed to address and thereby provide guidelines for managing grassland ecosystems under accelerated human disturbances (Lindsey et al. 2013).

Small rodents play important roles in helping to maintain biodiversity and functioning of grassland ecosystems (Davidson et al. 2012). They also play a significant role in promoting nutrient cycling of grassland ecosystems as consumers. However, they occasionally cause damage to grasslands as pests when their populations become dense (Zhong et al. 1999). There is a need to find a trade-off between conservation and control in managing small rodents in grassland ecosystems.

Grazing has been recognized to influence populations of small rodents in grasslands, but responses of small rodents to grazing may vary significantly due to differences in species or systems. Some studies have demonstrated that grazing has negative effects on small rodents due to the deterioration of food quantity [i.e., a shortage of food resources caused by livestock as competitors (e.g., Milchunas et al. 1998; Steen et al. 2005)] or food quality [a grazing-induced increase of secondary chemicals (e.g., Bryant et al. 1985; Lindroth and Batzli 1984)]. Some studies indicated that grazing benefited small rodents from a reduction of vegetation and the creation of preferred open habitats (Davidson et al. 2010) or stimulation of grass regrowth during the growing season (Arsenault and Owen-Smith 2002). Several other studies did not reveal any significant grazing effects (Saetnan et al. 2012).

Previous studies on this subject have three limitations. First, most of the previous studies were conducted in open grasslands, which allowed the confounding influence of immigration/emigration and predation in rodents. Second, the grazing experiments lasted only for 1–2 years (Foster et al. 2014); in such studies it is impossible to detect the differences between short-duration and extended-duration effects of grazing. Finally, the underlying mechanisms which cause change are not fully understood due to a lack of manipulative experiments with information on both the demography of the rodents and on plant-animal interactions.

Brandt's vole is a small herbivore and the predominant rodent species in the steppe grassland of Inner Mongolia. They are social animals, and their breeding season mainly lasts from May to August. Females give birth from one to three times each year, with each litter consisting of usually two to 13 pups. Its population oscillates greatly among years (Jiang et al. 2011; Zhang et al. 2003b; Zhong et al. 1999), but the underlying mechanisms have not been tested with manipulative experiments. In this study, by the use of large replicated enclosures which excluded the confounding influences of immigration/emigration and predation, we investigated the effects of sheep grazing on the population dynamics and the underlying response mechanisms of Brandt's vole populations in Inner Mongolia grasslands during the period 2010–2013. This is the first study which addresses the underlying demographic mechanisms for the

impacts of grazing on small mammal populations in the context of the plant-vole-sheep system.

## Materials and methods

### Study area

The study was conducted in the Maodeng pasture (44°11'N, 116°27'E; 1100 m in elevation; Fig. 1), 38 km northeast of Xilinhot, Inner Mongolia, China. Meteorological data gathered from 1999 to 2009 showed that the study site has a very cold winter (average daily maximum temperature approximately  $-10^{\circ}\text{C}$ ) and a warm summer (average daily maximum temperature approximately  $26^{\circ}\text{C}$ ). Mean annual precipitation at the study site was 241 mm, with 75 % occurring during the growing season (May–August). The study site is located in a semiarid typical steppe, dominated by Krylov's needle grass (*Stipa krylovii*), Chinese lyme grass (*Leymus chinensis*), and scabrous *Cleistogenes* (*Cleistogenes squarrosa*). Brandt's voles are the most abundant small herbivores in the grassland. The experimental area had been grazed mainly by sheep until the start of 2008, and subsequently rested for 2 years prior to this study. The local stock rate at the study site was about six sheep  $\text{ha}^{-1}$  during the growing season.

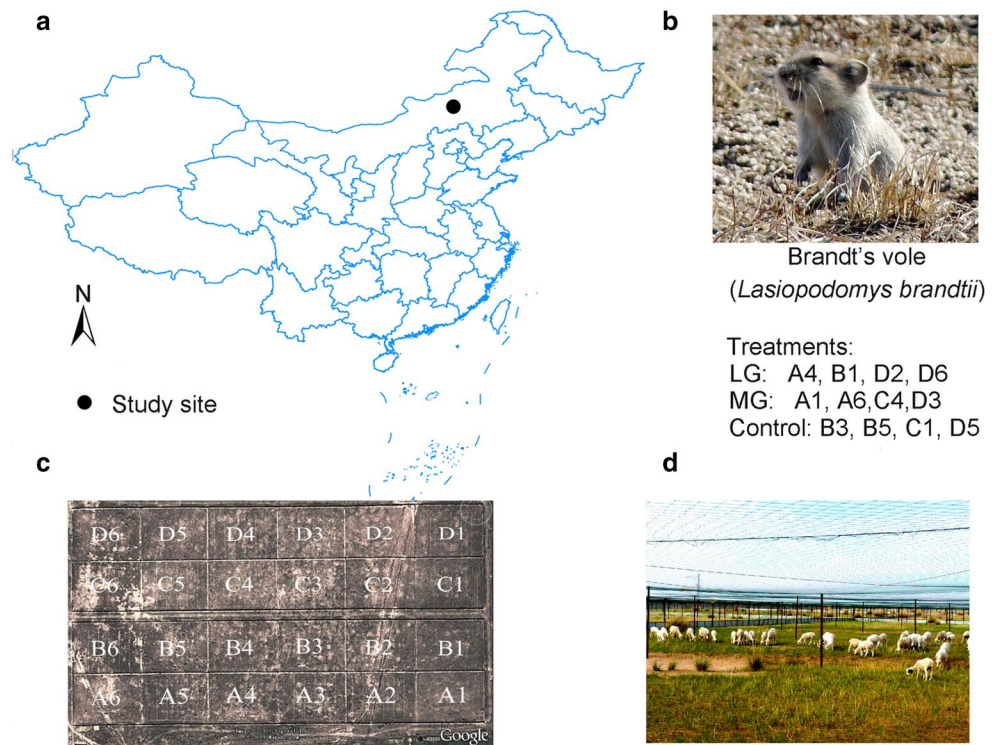
### Experimental design

We constructed a set of twenty-four 0.48-ha enclosures of  $80 \times 60$  m with galvanized iron sheets extending 1 m underground and 1.4 m above ground to prevent movements of burrowing rodents, sheep, and carnivores into or out of enclosure (see Li et al. 2015). Each enclosure was covered completely by 50-cm-high wire netting (1-cm mesh size) on all four sides and by nylon netting (10-cm mesh size) on the top to exclude avian predators. These enclosures eliminated all emigration and immigration of rodents and excluded all their predators.

Twelve enclosures were randomly assigned into three treatments: control, light grazing (LG), moderate grazing (MG), with four replicates for each treatment (the other 12 enclosures were used for other study purposes; Fig. 1c). According to the local standard of sheep grazing intensity (Schonbach et al. 2011), the grazing intensity for the three treatments C, LG and MG was set at 0, 2, and 4 sheep  $\text{ha}^{-1}$ , respectively. We did not set up a heavy grazing treatment to avoid serious vegetation deterioration. We grazed 40 adult sheep for a half day or a whole day biweekly during the growing season, which was equivalent to the grazing intensity of the LG group or the MG group, respectively.

Experimental voles were trapped in Abaga Qi (43°31'30"N, 116°04'50"E) or Dongwu Qi (45°33'39"N,

**Fig. 1** Location of the study site in China (a), Brandt's vole *Lasiopodomys brandtii* (b), enclosure layout from Google Earth in 2011 (c), a grazing enclosure (d)



116°37'48"E) of Xilingol League in the early spring (April) of the year of experiment and in autumn (September and October) of the previous year using live traps made of wire mesh [trap size, height  $\times$  width  $\times$  length = 10  $\times$  10  $\times$  24 cm; mesh size, 1 cm; Table S1, Electronic supplementary material (ESM)]. Voles captured in autumn were raised in an open enclosure (480  $\times$  300 m; near to experimental enclosures) as supplemental source populations. Founder individuals of experimental populations were mostly from spring-captured individuals; if the number of voles was not sufficient, individuals from the supplemental pool population were also used. In 2010, due to a shortage of field voles, we introduced five or six non-sibling laboratory-bred voles to each enclosure. During the period 2010–2013, thirteen to 15 pairs of adult voles were randomly assigned to each of the 12 enclosures in early spring, and allowed to adapt to the enclosure environments for 10–15 days before the grazing was initiated. We added new voles into enclosures to compensate for losses of voles due to mortalities during the adaptation period and to have an average founder population size of 13–15 pairs at the beginning for each year (see Table S1 of the ESM). The grazing began when the 10- to 15-day adaptation period finished. Voles in enclosures were allowed to survive to the following spring to determine impacts of grazing during the non-breeding season. All voles were removed from enclosures at the beginning of the ensuing spring so as to repeat the experiments the following year to evaluate the impacts of

successive grazing. This resetting of the experimental system was designed to minimize the differences of founder populations, and to test the successive effects of grazing on voles in multiple years. Studies on the carry-over effects of grazing on voles in 2 successive years started in 2014.

### Animal trapping

Vole populations were monitored biweekly from May to October using mark-capture-recapture methods and each trapping session lasted for 2 successive days. We checked the traps three times during each trapping session. About 180 traps baited with peanuts were set near burrows in each enclosure. The voles were marked with a numbered metal ear tag at their first capture. The tag number, body mass, sex and reproductive condition of voles were recorded. After measurements, voles were immediately released at the capture site. In order to minimize the effects of the bait on the chemical composition of fecal samples, fresh samples of feces were collected within 1 h after the traps were set. Diet compositions of voles and relative frequency (RF) indices of each plant species were estimated by identifying food items based on counting of 100 microscope fields of feces sampled in each enclosure. The RF index for each plant species was calculated following Gill et al. (1983):

$$RF_i = \frac{\text{Number of fields where species } i \text{ is identified}}{\text{Number of fields with fragments of any species}} \times 100.$$

## Vegetation survey

Vegetation was sampled in the first week following a trapping session once each month. We evaluated changes in food quantity by surveying plant cover, biomass and density. The cover (%), density (the number of plants rooted within each quadrat) and biomass (grams per square meter) of each plant species were measured in five randomly selected quadrats (1 × 1 m) within each enclosure. Subsequently, each 1 × 1-m quadrat was further subdivided into 16 subquadrats (0.25 × 0.25 m), and aboveground material of each plant species in one of these subquadrats was clipped at ground level. Locations of clipped quadrats differed in each sampling session to minimize the impact of clipping. Clipped plants were separated by species, stored temporarily in paper envelopes, and then dried in an oven at 65 °C to a constant mass. Plant samples were also analyzed for food quality using the chemical and nutritional properties including crude protein, hydrolysable tannins (hereafter “tannins”) and total phenols. Crude protein content was determined using the Kjeldahl method. Total phenols and tannins were measured by using a modified Folin-Ciocalteu method (Makkar et al. 1993). Plant samples were not taken within about 8 m of the enclosure wall to avoid the extra heat effect of galvanized iron sheet flashing on the vegetation.

The preference for different plant species by voles was evaluated by the relative preference index (RPI) (Vandyne and Heady 1965). RPI was calculated as the ratio of the percent of a plant species in the diet of voles from fecal analysis to the percent of the plant species present in the enclosure. If the mean RPI was greater than 1.0 and the 95 % confidence interval (CI) did not include 1.0, the particular plant species was defined as a “preferred plant” for voles; otherwise, it was defined as a “less-preferred plant.”

## Data analysis

The population density was first estimated by the minimum number of animals known alive (MNA) method (Krebs 1966). It is a reasonable index for estimating population size when the recapture rate is high (Hilborn et al. 1976; Ozgul et al. 2004). In our study, the average probability of recapture during these 4 years was 86.8 % (95 % CI 86–87.6 %). Thus, MNA was used as an index of population size of Brandt’s voles in the enclosures. The population density for each enclosure was also estimated by the jackknife estimator for model  $M_h$  (where  $h$  = heterogeneity in capture probabilities among animals) with the program CAPTURE (Otis et al. 1978; available at <http://www.mbr-pwrc.usgs.gov/software.html>). The population density of voles was defined as the number of voles per hectare. As suggested by Redfield et al. (1978), recruits were defined

simply as the number of any previously unmarked voles, regardless of age or body size of animals.

Significant differences in population parameters of voles (i.e., density, recruits, body mass) and parameters of plants (i.e., cover, biomass, density, crude protein content, tannin content and total phenol content) between control and grazing groups were tested by means of linear mixed models (LMM) and package nlme (Pinheiro et al. 2014) in R (R Core Team 2013). In the LMM analysis, a repeated covariance structure was used to take account of variables measured over time. Enclosure and year were both specified as random effects, which allowed for correlated responses within years and enclosures. Factor enclosure was nested within factor year. Trapping session and treatment as well as their interaction were used as fixed effects in all analyses. Firstly, we used the data of the first year to detect the short-duration grazing effects; secondly, we used the pooled data from the 4 years to detect the extended-duration grazing effects. We applied the function `glht` of the `multcomp` package to perform the multiple comparisons. Three enclosures [one for the control (B3), one for LG (B1) and one for MG (A1); Fig. 1] in 2010 were not included in the analysis due to their invasion by domestic cats. Data of male body mass were analyzed to avoid the effects of pregnancy in females. In order to identify differences of total tannin or phenol content of the three preferred plant species in the diets of voles between the control and grazing groups, we weighted tannin or phenol content by the RF for each plant species in the fecal analysis of voles.

We estimated apparent survival (hereafter “survival”) and recapture probabilities with the classic Cormack-Jolly-Seber (CJS) models implemented in program MARK 7.1 (Lebreton et al. 1992; White and Burnham 1999). Instead of the “all possible models” approach (Anderson et al. 2001), we constructed a set of candidate models a priori, of which each represented survival as a function of the combination of covariates, including treatment group, time and sex. The CJS models assume statistical homogeneity both on survival rates from time  $i$  to time  $i + 1$  and on recapture rates at time  $i$  among different individuals. We estimated overdispersion in our trapping data using the median  $\hat{c}$  (an estimate of the variance inflation factor) to scale the variance estimate. If the assumptions of the CJS model were met, then  $\hat{c} = 1$ ; otherwise  $\hat{c} > 1$ . We used quasi Akaike’s information criterion corrected for small sample size (QAICc) and adjusted for overdispersion when  $\hat{c} > 1$  for model selection and statistical inference (Burnham and Anderson 2002).

Overwinter survival and density changes of voles from autumn (October) to the spring (April) were also estimated. Statistical differences in overwinter survivals and density changes between control and grazing groups were analyzed by one-way ANOVA followed by the post hoc multiple



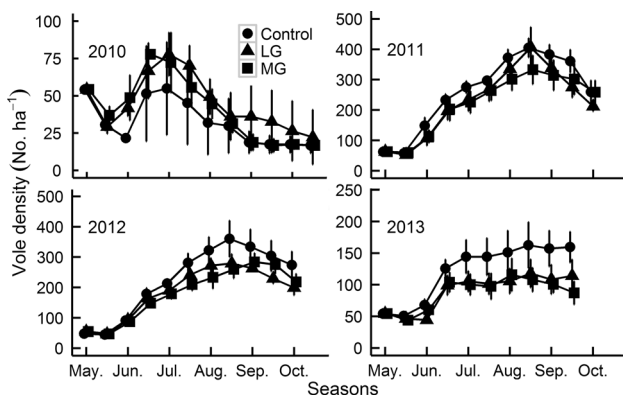
comparisons by Tukey’s honest significant difference with a significance level of  $P < 0.05$ . The CJS model could not estimate the overwinter survival since it is the last capture period, so overwinter survival per year was calculated as the proportion of animals captured during the last trapping session of the previous October that were recaptured in April of the current year, as suggested by Karels et al. (2000). October vole densities were also used as covariates (density dependence) when overwinter survival was analyzed. Due to an insufficient sample size of overwinter populations in 2011, the overwinter survival and density changes from autumn 2010 to spring 2011 could not be estimated.

The current study emphasizes comparisons between grazing and control groups in 4 years. Because of a limited sampling number of years, the yearly variations will be not analyzed and discussed.

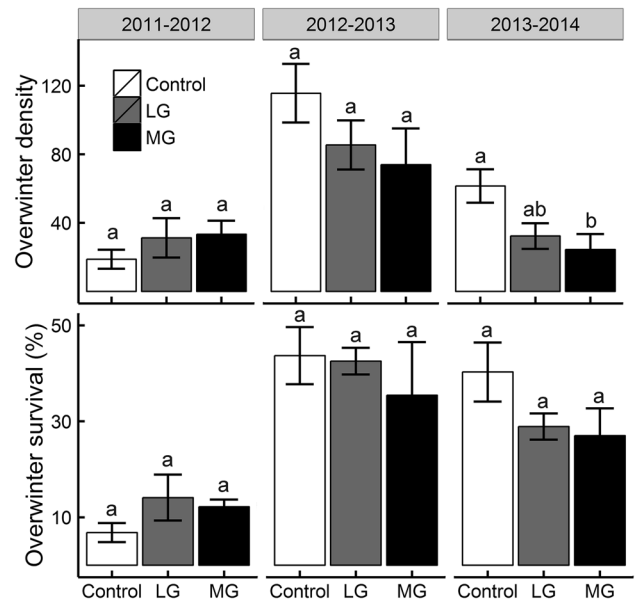
## Results

### Population density

In the breeding season, data pooled from the 4 years showed that vole density was significantly affected by livestock grazing ( $F_{2, 39} = 4.2, P = 0.023$ ) and trapping sessions ( $F_{10, 420} = 51.3, P < 0.001$ ), with no interaction between trapping sessions  $\times$  grazing ( $F_{20, 420} = 0.74, P = 0.79$ ). Tukey’s tests revealed that MG lowered vole density by 23 % on average compared to control vole density ( $t = -2.67, P = 0.02$ ; Fig. 2); LG also marginally lowered vole density by 21 % relative to the control group ( $t = -2.28, P = 0.058$ ; Fig. 2). A separate analysis of the first-year data showed that grazing has no effect on vole density in 2010 ( $F_{2, 6} = 0.31, P = 0.75$ ). Results using population density



**Fig. 2** Changes in population densities (mean  $\pm$  SE,  $n = 4$ ) of Brandt’s vole of control, light grazing (LG) and moderate grazing (MG) treatments. Note that the density scale varies in the different years



**Fig. 3** Changes in overwinter densities in April and survival rates ( $n_{\text{April}}/n_{\text{October}}$ ) of control, LG and MG treatments. Data represent mean  $\pm$  SE. Different letters indicate significant treatment effects. For abbreviations, see Fig. 2

estimated by using the jackknife estimator were similar to those determined by MNA (see Table S3 of the ESM). In non-breeding seasons, ANOVA results showed significant differences in overwinter density among control and grazing groups in 2013–2014 ( $F_{2,9} = 4.9, P = 0.036$ ); the MG group had a 60 % lower overwinter density of voles than the control group ( $t = -2.97, P = 0.038$ ; Fig. 3).

### Survival

The median  $\hat{c}$ -test significantly indicated a lack of fit or overdispersion in the general model (in 2010,  $\hat{c} = 1.4$ ; in 2011,  $\hat{c} = 1.6$ ; in 2012,  $\hat{c} = 1.47$ ; in 2013,  $\hat{c} = 1.5$ ); therefore, the variance inflation factor was applied for all subsequent model fitting.

In breeding seasons, model selection (Table 1) showed that the effect of sex on estimates of survival differed among years. Males had greater survival rates than females in 2010 ( $\beta = 0.21, 95\% \text{ CI } 0.03\text{--}0.45$ ) and in 2011 ( $\beta = 0.12, 95\% \text{ CI } 0.01\text{--}0.26$ ). In 2012, the best-approximating model indicated there was no difference in survival between males and females (Table 1). In 2013, males had lower survival than females ( $\beta = -0.22, 95\% \text{ CI } -0.41\text{ to } -0.03$ ). Both light and MG significantly reduced the survival of voles in the breeding season in both 2012 (LG,  $\beta = -1.78, 95\% \text{ CI } -2.8\text{ to } -0.76$ ; MG,  $\beta = -2.93, 95\% \text{ CI } -3.89\text{ to } -1.97$ ) and 2013 (LG:  $\beta = -8.22, 95\% \text{ CI } -12.62\text{ to } -3.82$ ; MG,  $\beta = -9.84, 95\% \text{ CI } -14.92\text{ to } -4.76$ ), but no significant effects of grazing on survival

**Table 1** Modeling survival probabilities ( $\varphi$ ) of *Lasiopodomys brandtii* as functions of food, sex and time

Models	$n$	QAICc	$\Delta$ QAICc <sup>a</sup>	$\omega_i^b$	$Q_{\text{deviance}}^c$
2010					
$\varphi(\sim \text{sex} + \text{time})$	22	2148.38	0	0.83	609.51
$\varphi(\sim \text{sex} + \text{grazing} + \text{time})$	24	2151.80	3.42	0.15	608.82
$\varphi(\sim \text{sex} + \text{time} + \text{sex} \times \text{time})$	32	2156.35	7.97	0.02	596.86
2011					
$\varphi(\sim \text{sex} + \text{time} + \text{grazing} + \text{time} \times \text{grazing})$	70	10,900.83	0.00	0.63	1874.67
$\varphi(\sim \text{time} + \text{grazing} + \text{time} \times \text{grazing})$	40	10,901.87	1.04	0.37	1877.73
$\varphi(\sim \text{sex} + \text{time} + \text{grazing} + \text{sex} \times \text{time} + \text{grazing} \times \text{time})$	50	10,913.60	12.77	0	1869.31
2012					
$\varphi(\sim \text{grazing} + \text{time} + \text{grazing} \times \text{time})$	40	10,133.63	0	0.62	1786.21
$\varphi(\sim \text{grazing} + \text{time})$	21	10,136.08	2.45	0.18	1824.88
$\varphi(\sim \text{sex} + \text{time} + \text{grazing} + \text{grazing} \times \text{time})$	41	10,136.64	3.01	0.14	1785.19
2013					
$\varphi(\sim \text{sex} + \text{grazing} + \text{time} + \text{grazing} \times \text{time})$	36	4812.94	0	0.83	1148.59
$\varphi(\sim \text{grazing} + \text{time} + \text{time} \times \text{grazing})$	35	4816.19	3.24	0.16	1153.87
$\varphi(\sim \text{sex} + \text{time} + \text{grazing})$	21	4823.43	10.49	0	1189.47

The top three models are shown. The model structure for population ( $p$ ) is not shown because it was the same across all five models [ $p(\text{time})$ ]

$n$  Number of parameters, QAICc Akaike information criterion corrected for overdispersion and small sample size,  $\omega_i$  model weight, + additive effect,  $\times$  two-effect interaction

<sup>a</sup> Difference of a model from the minimum QAICc

<sup>b</sup> Level of support for a model on a scale of 0 (no support) to 1.0 (full support)

<sup>c</sup>  $-2\log \text{likelihood}/\hat{c}$ , where  $\hat{c}$  is an estimate of the variance inflation factor

rates were detected in 2010 and 2011. These results indicated that overall successive grazing decreased the survival rate of voles.

In the non-breeding season, ANOVA results showed no significant differences in overwinter survival among the control vs. grazing groups for any year (Fig. 3).

## Recruitment

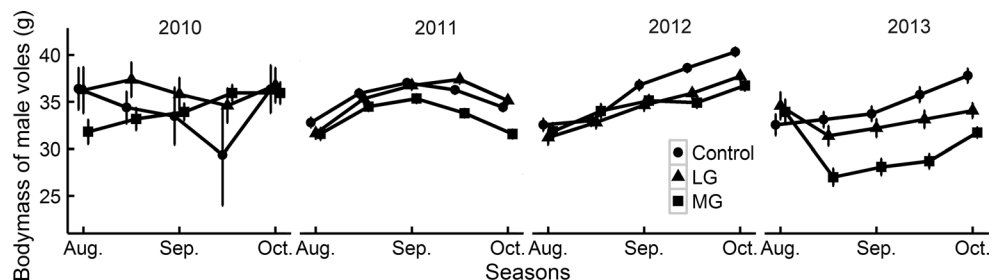
Pooled data showed that there were no significant effects of grazing ( $F_{2, 39} = 0.31$ ,  $P = 0.74$ ) and interaction between grazing and trapping session ( $F_{20, 420} = 0.59$ ,  $P = 0.92$ ) on recruits. Trapping session had significant effects on recruits

( $F_{10, 420} = 50.2$ ,  $P < 0.001$ ). Separate analysis of the first year also showed that grazing had no effect on the number of recruits ( $F_{2, 6} = 0.26$ ,  $P = 0.78$ ).

## Body mass

Pooled data showed livestock grazing significantly decreased the body mass of male voles ( $F_{2, 42} = 4.2$ ,  $P = 0.021$ ; Fig. 4). Trapping session had a significant effect on the body mass of male voles ( $F_{10, 15550} = 268$ ,  $P < 0.001$ ). However, separate analysis of the first year showed grazing had no significant effect on the mass of male voles ( $F_{2, 324} = 0.01$ ,  $P = 0.99$ ).

**Fig. 4** Changes in body mass (mean  $\pm$  SE) for males of *L. brandtii* of control, LG and MG treatments. For abbreviations, see Fig. 2



**Food quantity**

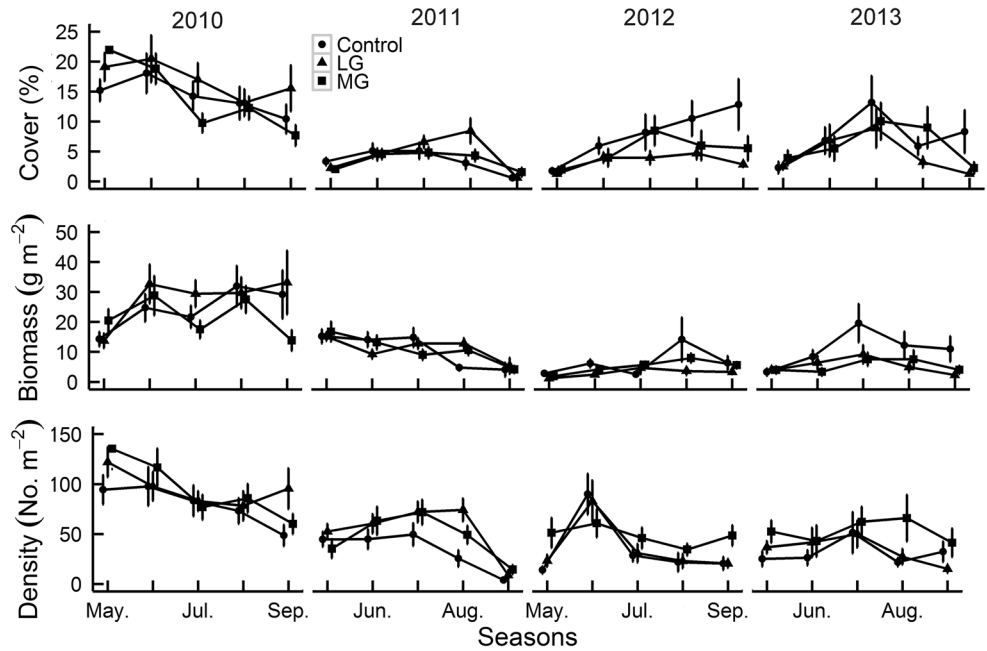
Over the 4 years of study the most preferred plant species for Brandt’s voles in our enclosures were *Leymus chinensis*, *Setaria viridis* and *Medicago sativa*, and the less-preferred plant species were most commonly *Stipa krylovii*, *Cleistogenes squarrosa*, and *Phlomis dentosa* (Table S2, ESM).

For preferred plant species, pooled data showed that there was no significant differences in plant cover ( $F_{2, 42} = 0.28, P = 0.76$ ), plant biomass ( $F_{2, 42} = 0.56,$

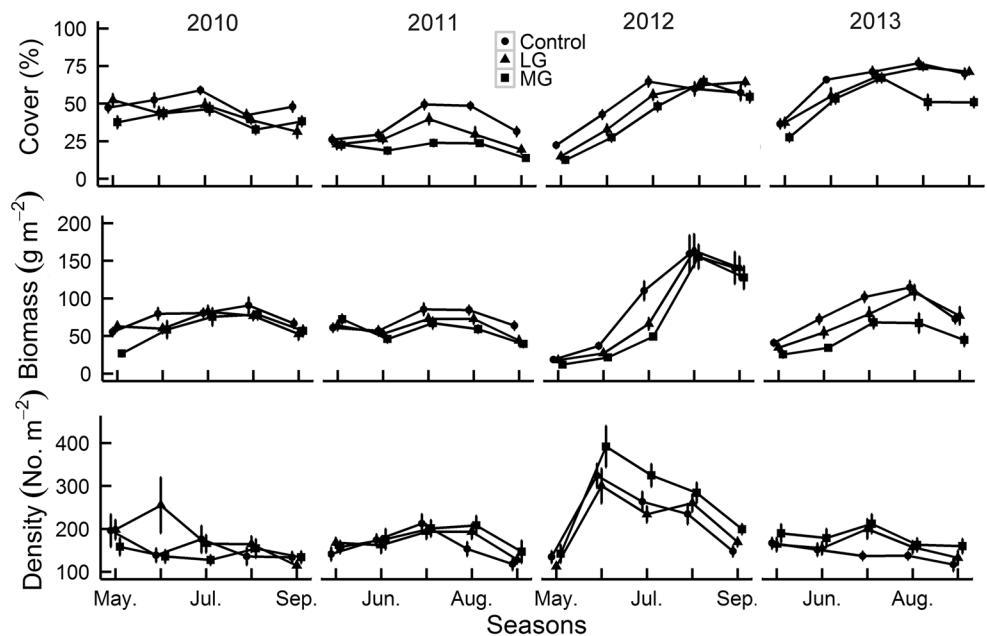
$P = 0.57$ ; Fig. 5) or plant density ( $F_{2, 42} = 1.48, P = 0.24$ ) between control and grazing groups. Separate analysis of the first year also showed that grazing exerted no significant effect on plant cover ( $F_{2, 9} = 0.3, P = 0.75$ ), plant biomass ( $F_{2, 9} = 0.29, P = 0.75$ ) or plant density ( $F_{2, 9} = 0.33, P = 0.73$ ).

However, for less-preferred species, pooled data showed that grazing significantly decreased plant cover ( $F_{2, 42} = 20.6, P < 0.001$ ) and plant biomass ( $F_{2, 42} = 8.98, P < 0.001$ ; Fig. 6). Tukey’s test showed that plant cover and/

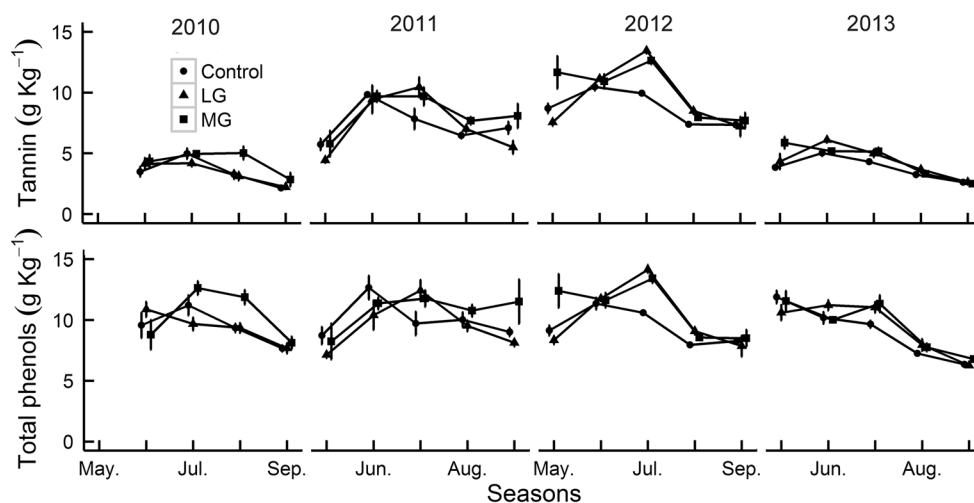
**Fig. 5** Changes in vegetation quantity (cover, biomass and density) of plants preferred by *L. brandtii* in control, LG and MG treatments. Data represent mean  $\pm$  SE. For abbreviations, see Fig. 2



**Fig. 6** Changes in vegetation quantity (cover, biomass and density) of plants less preferred by *L. brandtii* in control, LG and MG treatments. Data represent mean  $\pm$  SE. For abbreviations, see Fig. 2



**Fig. 7** Changes in food quality (weighted tannin and total phenols) in the three mostly preferred plants of *L. brandtii* in control, LG and MG treatments. Data represent mean  $\pm$  SE. Data for May 2010 are not available. For abbreviations, see Fig. 2



or plant biomass were lower in LG (13 and 12 % decreases, respectively) and MG (26 and 28 % decreases, respectively) than those in the control group. Grazing showed no significant effects on plant density ( $F_{2, 42} = 0.72$ ,  $P = 0.49$ ). Separate analysis of the first year showed grazing exerted no significant effect on plant cover ( $F_{2, 9} = 2.75$ ,  $P = 0.12$ ), plant biomass ( $F_{2, 9} = 1.7$ ,  $P = 0.24$ ) or plant density ( $F_{2, 9} = 0.77$ ,  $P = 0.5$ ) for less-preferred species.

### Food quality

The grass species *L. chinensis*, *S. krylovii* and *C. squarrosa* were common food for Brandt's vole. Based on pooled data from 4 years of study, their proportions in the dietary composition of Brandt's vole were 42.5, 36.8, and 16.6 %, respectively.

Pooled data showed that grazing had significant effects on weighted phenol contents and weighted tannin contents of the three main plant species (for phenols,  $F_{2, 42} = 4.8$ ,  $P = 0.012$ ; for tannin,  $F_{2, 42} = 9.3$ ,  $P < 0.001$ ). Contents of these two groups of secondary compounds were both higher in the MG group than in the control group (8 % increase in phenols and 13 % increase in tannin; Fig. 7), while contents in the LG and control groups showed no significant differences (phenols,  $t = 0.51$ ,  $P = 0.87$ ; tannin,  $t = 1.58$ ,  $P = 0.26$ ). Separate analysis of the first year showed that contents of weighted tannin in the MG group were marginally higher than in the control group ( $t = 0.82$ ,  $P = 0.052$ ), while grazing exerted no significant effect on weighted phenols ( $F_{2, 9} = 1.2$ ,  $P = 0.34$ ).

Pooled data showed that grazing exerted no significant effect on weighted crude protein content ( $F_{2, 42} = 1.05$ ,  $P = 0.36$ ). Trapping sessions ( $F_{4, 396} = 397.3$ ,  $P < 0.001$ ) and interaction between grazing and seasons ( $F_{8, 396} = 3.15$ ,  $P = 0.002$ ) showed significant effects on weighted crude protein content. Tukey's test showed that the MG group

had 21 % higher weighted crude protein in August than the control group ( $t = 3.8$ ,  $P = 0.012$ ). Separate analysis of the first year showed grazing had no significant effect on crude protein content ( $F_{2, 9} = 0.53$ ,  $P = 0.61$ ).

### Discussion

The impact of livestock grazing on small rodents has been recognized for a long time (Davidson et al. 2010; Milchunas et al. 1998), but the impact of grazing intensity on rodent populations and the underlying mechanisms of this have not been explored in the context of the sheep-plant-rodent system. By the use of manipulative experiments with a mechanistic approach involving both intrinsic (nutritional condition and demography of voles) and extrinsic (vegetation and grazing treatments) factors, we found that 4 years of grazing produced negative effects on vole populations, and the magnitude of the negative effect increased with grazing intensity. Decreases in survival and male body mass contributed to the decline of the vole populations.

The food competition hypothesis has been proposed to explain population declines of small rodents under livestock grazing pressure in other studies (Augustine and Springer 2013). Consistent with this hypothesis, decreased forage quantity, reflected in lower plant cover and height, have been widely reported in grazed sites (Steen et al. 2005; Torre et al. 2007). Three mechanisms have been suggested for grazing-induced reductions in the population density of small rodents: direct competition as a result of a decrease in the quantity of available food (Parsons et al. 2013), an increase in predation risk resulting in higher predation losses with low vegetation height and cover (Steen et al. 2005), or a reduction in habitat quality due to the destruction of burrow systems under increased soil compaction by large mammal trampling (Torre et al. 2007).



In this study, continuous herbivory by voles sharply decreased the total biomass and cover of preferred plants in the enclosure during the 4-year study. The average ratio of non-preferred plants to preferred plants increased year by year (e.g., for biomass, 2.8 in 2010, 13.5 in 2013; for cover, 3 in 2010, 13.9 in 2013), which resulted in the less-preferred plants becoming the main supply of food for voles (Fig. S1, ESM). We found the negative effects of successive grazing were closely linked to a significant reduction in cover and biomass of less-preferred plant species in grazing enclosures, which resulted in a shortage of food for Brandt's voles. Dietary overlap was great between Brandt's vole and sheep, the grass species *L. chinensis* the main food for voles in summer was also preferred by sheep. Therefore, the disappearance of preferred plants enhanced competition between voles and sheep. This explains why the grazing effect in the first year was not significant because voles had an excess of preferred plants to eat. This observation supports the food competition hypothesis (Steen et al. 2005; Torre et al. 2007).

Grazing may change plant quality (crude protein, secondary compounds), and subsequently may affect population abundance of other herbivores in grasslands (Augustine and Springer 2013; Lindroth and Batzli 1986). Grazing-induced secondary compounds, including tannins and proteinase inhibitors, have been shown to have negative effects on small rodents in laboratory conditions (Lin et al. 2012; Lindroth and Batzli 1984), but there are few field studies of such effects (but see Saetnan et al. 2012). Repeated grazing is often necessary for maintaining high levels of secondary compounds (Gustafson and Ryan 1976), because the responses of food quality to grazing may be delayed (Saetnan et al. 2012). The response of forage nitrogen content and digestibility in plants to grazing may be positive (Augustine and Springer 2013) or neutral (Saetnan et al. 2012). In our study, MG in August showed a positive effect on crude protein. There is strong evidence in our study that grazing induced higher secondary compound contents (e.g., tannin and total phenols). Thus, grazing-induced deterioration in food quality may be treated as an additional explanation for the observation of the reduced abundance of voles.

In our study, we confirmed that grazing intensity plays a key role in explaining the mechanisms of the response of voles to livestock grazing. LG with an extended duration (4 years) would impose a negative effect only by decreasing the food quantity (e.g., cover and biomass), while MG with an extended duration would reduce the vole population both by decreasing the food quantity and by decreasing the food quality (e.g., secondary compounds). The reduction in vole abundance caused by LG was slightly lower than that of MG. Long-time high grazing intensity could lead to a decline in the population of native keystone

species by modifying plants both in their community structure and nutritional characteristics; this would cause a loss in the critical ecosystem functioning in which animals play a central role.

Overwinter survival is a critical demographic parameter for voles living in the northern hemisphere and has attracted extensive attention by field ecologists (e.g., Haapakoski et al. 2012; Karels et al. 2000). In our study, we found evidence of a lower overwinter density in years of successive grazing, which may have affected the population growth rate in the following years. In this study, we only focused on testing the effects of successive grazing on voles within four breeding seasons. Future studies should focus on testing the impact of grazing on small rodents across years.

The yearly variation in our vole populations might be caused by variations in weather in different years. As compared to 2011 and 2012, the low density of vole populations in 2010 and 2013 was linked to low values of spring precipitation and temperature, which do not favor vole breeding (Xie et al. 2012). However, due to the limited data of 4 years, we are not able to draw reliable conclusions about the effects of climate on vole populations in the long term. Long-term experiments are needed to reveal the temporal effects of climate on vole populations in our enclosures (Evans et al. 2015; Krebs 2015).

Some small rodents play a significant role in performing essential ecosystem functions and have been perceived as keystone species or ecosystem engineers in their native ranges (Davidson et al. 2012; Krebs 2014). Local extinction of these native species (e.g., pika on the Qinghai-Tibet plateau) under accelerated human disturbance or poisoning may damage ecosystem functions (Zhang et al. 2003a). Brandt's vole is an important prey for some native apex predators (e.g., mustelids, canids and raptors). Its burrows are also ideal habitat for other rodents, herpetofauna and arthropods. Thus this species is very important in maintaining biodiversity and ecosystem functioning. Voles could occasionally damage pastures during their outbreak years (Zhong et al. 1999). Our study suggests that the short duration of the LG treatment had little effect on voles, but the long duration of the MG caused population declines. Traditionally, grazing has been sustained without or with an infrequent short-duration rotation by local herders, which leads to biodiversity loss, and thereby threatens ecosystem services. To achieve an ecological balance for managing Brandt's vole, it is necessary to adjust the traditional grazing strategy. At present, moderate or heavy grazing without yearly rotation is widely adopted by local herdsman. Based on our study, we recommend that in years without obvious vole damage to grassland, low-intensity and long-duration grazing rotations should be adopted within the next 2–3 years to conserve the native species, while in years or regions with obvious vole damages, high-intensity and

short-duration grazing rotations within the next 3–4 years are recommended to reduce vole populations.

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