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# Linking nutrient strategies with plant size along a grazing gradient: Evidence from *Leymus chinensis* in a natural pasture

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## Abstract

Studying the changes in nutrient use strategies induced by grazing can provide insight into the process of grassland degradation and is important for improving grassland quality and enhancing ecosystem function.  Dominant species in meadow steppe can optimize their use of limiting resources; however, the regulation of nutrient use strategies across grazing gradients is not fully understood.  Therefore, in this study, we report an *in situ* study in which the impact of grazing rates on nutrient use strategies of *Leymus chinensis*, the dominant plant species in eastern Eurasian temperate steppes, was investigated.  We conducted a large randomized controlled experiment (conducted continuously for five years in grassland plots in a natural pasture in Hailar, eastern Mongolia Plateau, China) to assess the effects of grazing rate treatments (0.00, 0.23, 0.34, 0.46, 0.69, and 0.92 adult cattle unit (AU) ha$^{-1}$) on *L. chinensis* along a grazing gradient and employed a random sampling approach to compare the accumulation, allocation, and stoichiometry of C, N, and P in leaves and stems.  Our findings demonstrated the follows: (i) The height of *L. chinensis* decreased with an increase in the grazing gradient, and the concentrations of C, N, and P significantly increased; (ii) the accumulation of C, N, and P per individual was negatively correlated with the concentration of aboveground tissues, suggesting that there was a tradeoff in *L. chinensis* between nutrient accumulation and concentration at the individual scale; (iii) the leaf-to-stem ratio of C, N, and P accumulation increased with grazing intensity, indicating a tradeoff in nutrient allocation and plant size at the individual plant level; and (iv) grazing rates were negatively correlated with the ratios of C:N and C:P in the stem; however, these ratios in leaves significantly increased with grazing intensity.  Our findings suggest that *L. chinensis* in meadow steppe adapts to grazing disturbance through tradeoffs between plant size and nutrient use strategies.  Moreover, our results imply that grazing produces a compensatory effect on nutrient use efficiency between the stems and leaves of *L. chinensis*.

**Keywords:** meadow steppe, nutrient-use strategy, overgrazing, stoichiometry, Inner Mongolia, *Leymus chinensis*

## 1. Introduction

Natural grasslands that occupy approximately half of the world's land area and support the livelihoods of around 1 billion people (Wang *et al.* 2011; Kemp *et al.* 2013), have historically been subjected to continuous grazing by large herbivores.  However, long-term overgrazing has driven dramatic shifts in the structure and function of grasslands

worldwide (McSherry and Ritchie 2013). Since the 1980s, the grassland regions of northern China have undergone degradation of more than 90% of grassland areas due to the dramatic imbalance between vegetation and herbivores (Gan *et al.* 2012). The influence of grazing on grassland vegetation production, biodiversity, soil properties, and their interactions in grassland ecosystems is an issue of great interest among researchers (Bai *et al.* 2004; Zhang *et al.* 2014).

Increased grazing intensity and grazing-induced grassland degradation can impact plant functional traits including morphological, phenological, and physiological traits. In general, as the main functional organ of a plant, leaves have stability strategies for self-protection against grazing (McIntire and Hik 2002; Cruz *et al.* 2010). For example, the specific leaf area (SLA) of plants subjected to long-term grazing increases in comparison to plants in native (ungrazed) grassland ecosystems (Zheng *et al.* 2009), which is a strategy related to improving photosynthesis capabilities. Moreover, the aboveground portion of plants are generally more sensitive to grazing than the below-ground portion (Cruz *et al.* 2010; Guo *et al.* 2012); root traits have a lagging or a stable effect compared with the soil microenvironment (Milchunas and Vandever 2013). Additionally, different plant species respond differently to grazing (Díaz *et al.* 2001). For instance, taller plants produce leaves with a lower SLA and greater toughness, whereas shorter species produce leaves with intermediate toughness (that are selected more often by sheep) and have a SLA that increases with grazing (Cingolani *et al.* 2005). Previous studies have indicated that grazing causes a decline in aboveground biomass in individual plants primarily through a 'bottom-up' effect on plant miniaturization that is mediated by stem functional traits (Li *et al.* 2015).

Moreover, understanding the mechanisms of grazing-induced phenotypic plasticity is a challenge for grassland ecologists. Ecological theories provide different explanations for this problem (McKinney and Fowler 1991; Fu *et al.* 2005; Verón *et al.* 2011). For example, two major controversial and opposing hypotheses have been used to explain the mechanism of plant phenotypic plasticity in response to grazing. The first, the grazing avoidance hypothesis, suggested that the plants involved in plant-herbivore interactions get smaller in order to avoid the selective intake of animals, and this was achieved *via* genetic modification pathways (especially at the epigenetic inheritance level) (McKinney and Fowler 1991; Damhoureyeh and Hartnett 2002; Fu *et al.* 2005). The second hypothesis, the plant growth limitation hypothesis, suggested that these genetic modification pathways do not exist. Instead, plant phenotypic plasticity and tradeoffs between different traits were primarily caused by human

disturbance, which altered aspects of the soil microenvironment such as fertility, hydrology, and soil structure (Bardgett *et al.* 2002; McIntire and Hik 2002; Verón *et al.* 2011). The controversy surrounding the contrasting is mainly due to the lack of specific experimental evidence.

Ecological stoichiometry provides a powerful framework for studying the effects of grazing on plant morphological characteristics *via* changes to the stoichiometry of carbon (C), nitrogen (N), phosphorus (P) and plant-soil interactions (Zheng *et al.* 2012). In general, stoichiometry, and C, N, and P concentrations in plant tissues are associated with plant growth strategies, which strongly influence the capacity for adaptation to various environmental stresses such as grazing and clipping (Hillebrand and Kahlert 2001). Several previous studies have indicated that grazing-induced shifts in nutrition allocation patterns among different tissues (e.g., leaves, stems, roots, and fruit) are influenced by the availability of soil resources (e.g., soil available N and P) (Bai *et al.* 2012; Zheng *et al.* 2012). At the community scale, stoichiometric homoeostasis appears to be an important mechanism that is responsible for the structure, function, and stability of grassland ecosystems (Yu *et al.* 2010).

*Leymus chinensis,* a native, perennial rhizomatous grass with high palatability and forage value, is the dominant species of steppe vegetation distributed across the eastern Eurasian temperate grassland (Zhang *et al.* 2013). Previous studies showed that *L. chinensis* displayed increasing leaf nutrient concentration and decreasing nutrient resorption with time since grazing exclusion for both N and P (Lü *et al.* 2014). At the organ level, however, there is a dearth of information on the effects of grazing intensity on nutrient use strategies that are mediated by plant size in natural pastures. By investigating this question further, we will gain a better understanding of the mechanisms that underlie nutrient use strategies in plants subjected to variations in grazing pressure.

Therefore, we examined the effects of grazing on stoichiometry and the concentrations of C, N, and P in *L. chinensis* along a consistent grazing gradient in a meadow steppe located in the Hulun Buir Plateau of the Inner Mongolian Autonomous Region, China. Our overall objective was to establish a grassland management regime that would maximize hay production. We investigated the relationship of these nutrients in correlation with plant size and plant tissue. Specifically, we used a large randomized controlled experiment to investigate the effects of different grazing intensities and we randomly sampled individual plants to compare the tradeoffs of nutrient use strategies in *L. chinensis* along the grazing gradient. We addressed the following three questions: (i) How does grazing affect the relationship between the size of *L. chinensis* and the

concentrations of C, N, and P in leaves and stems?  (ii) How does grazing affect C, N, and P allocation strategies in *L. chinensis* with varying plant size?  (iii) How does grazing intensity affect the relationship between leaf and stem C:N:P stoichiometry in *L. chinensis*?

## 2. Results

### 2.1. Grazing increase C, N, and P concentrations

The plant height of *L. chinensis* significantly decreased with increasing grazing intensity ($P<0.01$; Appendix A), whereas the concentrations of C, N, and P in leaf, stem and the whole aboveground portion significantly increased along the grazing intensities gradient ($P<0.05$), with the exception of leaf C and stem N (Fig. 1).  Moreover, the concentrations of C, N, and P were ranked as follows: stem>aboveground portion>leaf ($P<0.05$; Fig. 2).  In general, C, N, and P concentrations in the stem and leaves of *L. chinensis*, as well as the whole plant, were significantly negatively correlated with plant height according to the six grazing intensities ($P<0.01$; Fig. 2).  Additionally, the grazing-induced concentrations of C, N, and P in the stem, leaves, and the whole *L. chinensis* plant were significantly positively correlated to some extent (Table 1).

### 2.2. Tradeoffs between C, N, and P accumulations and concentrations

At the individual level, the amount of C, N, and P accumulations in stem, leaf and the whole aboveground portion of *L. chinensis* individuals significantly decreased with increasing grazing intensity ($P<0.05$; Fig. 3).  At the organ level, the accumulation of N and P were ranked as follows: aboveground portion>leaf>stem ($P<0.05$; Fig. 3).  However, there had no significant difference in the C accumulation between leaf and stem ($P>0.05$; Fig. 4).  Additionally, according to a model of exponential decay, C, N, and P accumulations per *L. chinensis* individual were significantly correlated with the C, N, and P concentrations in the whole plant ($P<0.01$; Fig. 4).  More specifically, the tradeoffs between C, N, and P accumulations and concentrations were similar in stems and leaves ($P<0.05$), except for leaf P and stem C (Table 2).

### 2.3. Effects of grazing intensity on C, N, and P allocations

The leaf-to-stem ratio of the concentration of C increased with grazing intensity, whereas the leaf-to-stem ratios of N and P decreased with grazing intensity ($P<0.01$; Fig. 5).  The leaf-to-stem ratio of C, N, and P accumulation signifi-
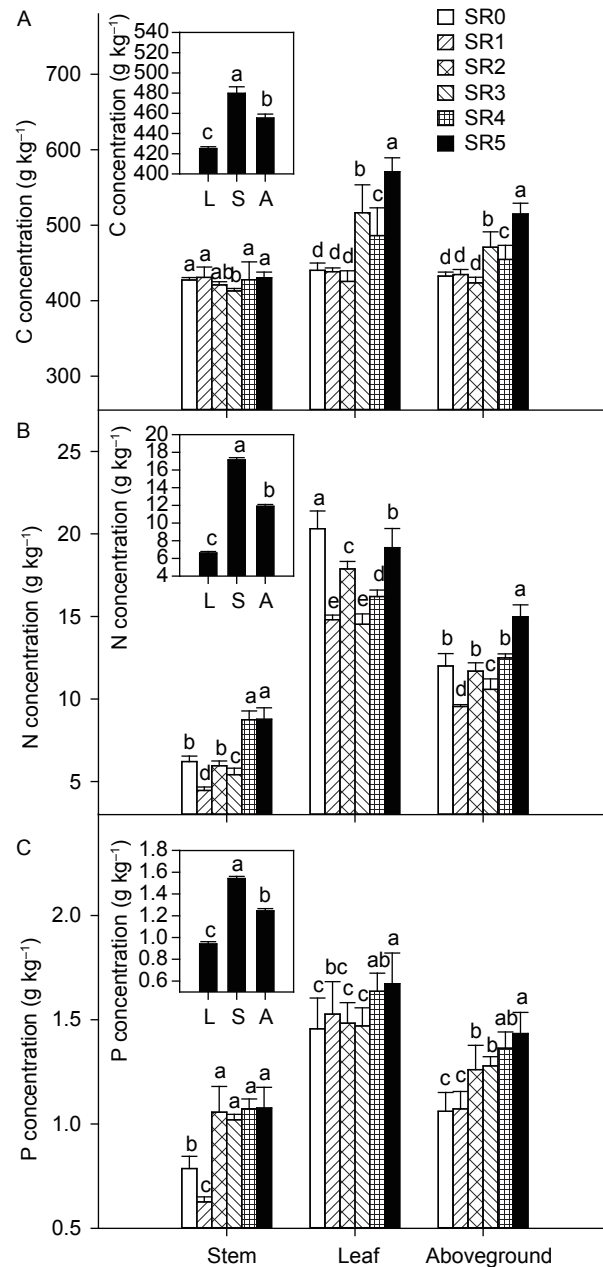


**Fig. 1** Carbon (C), nitrogen (N) and phosphorus (P) concentrations in *Leymus chinensis* tissue according to grazing intensity. A, carbon *vs.* grazing intensity (linear equation fitting): stem, $r=0.04$, $P>0.05$; leaf, $r=0.70$, $P<0.01$; whole plant, $r=0.69$, $P<0.01$. B, nitrogen *vs.* grazing intensity (linear equation fitting): stem, $r=0.71$, $P<0.01$; leaf, $r=-0.07$, $P>0.05$; whole plant, $r=0.58$, $P<0.01$.  C, phosphorus *vs.* grazing intensity (linear equation fitting): stem, $r=0.61$, $P<0.01$; leaf, $r=0.38$, $P<0.01$; whole plant, $r=0.68$, $P<0.01$.  The upper cases of L, S and A in the small figures represent sampling positions (L, leaf; S, stem; A, aboveground, i.e., leaf+stem).  All data are presented as means±SE values; the different letters above the error bars indicate significant difference ($P<0.05$).  Six grazing rates are labeled as follows: SR0–SR5, 0.00, 0.23, 0.34, 0.46, 0.69, and 0.92 AU ha$^{-1}$; where 1.00 AU=500 kg of adult cattle.  The same as below.
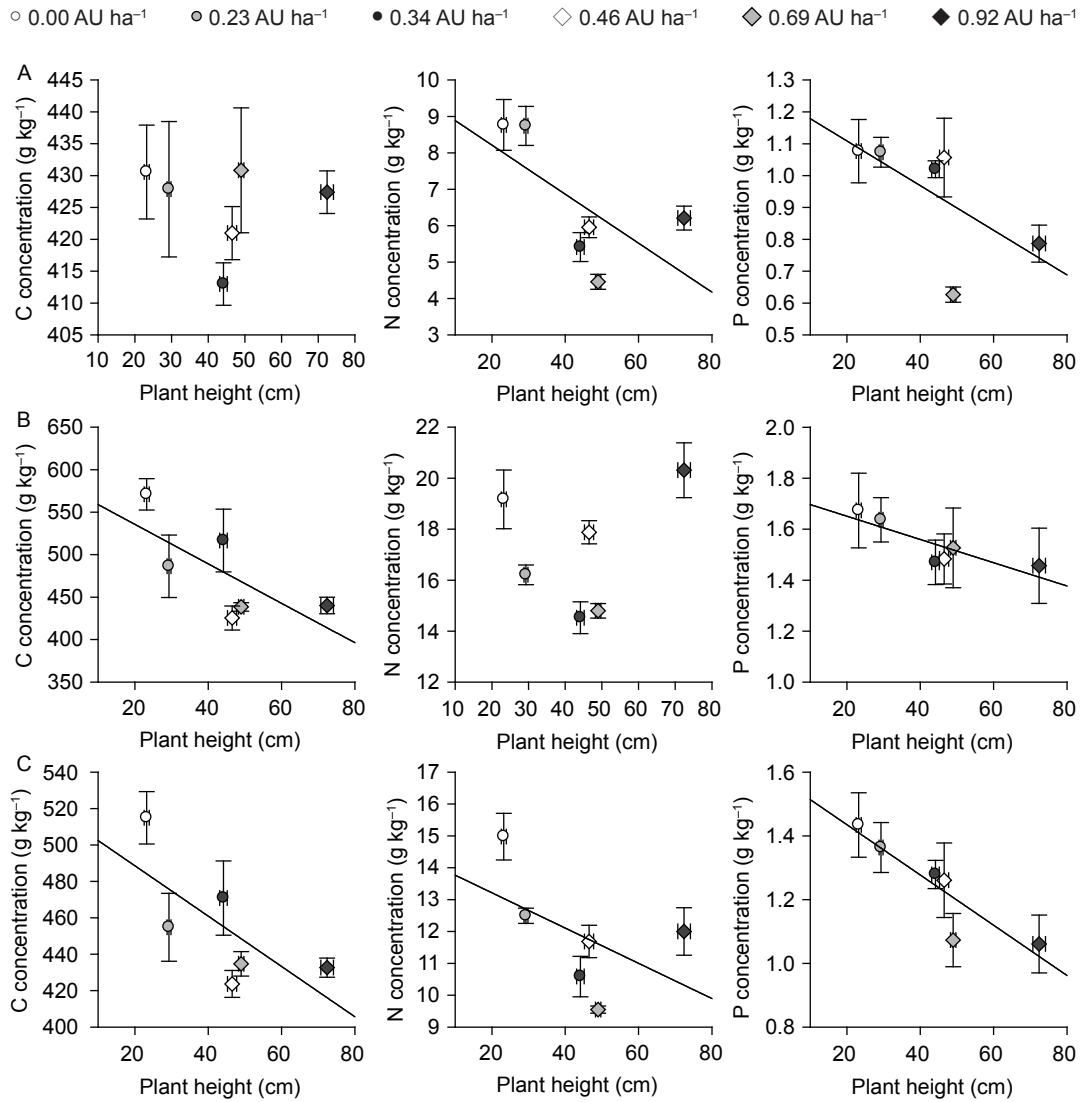
**Fig. 2** C, N, and P concentrations in *L. chinensis* tissue correlated with individual plant size along the grazing intensity gradient (*n*=90). All relationships were fitted by a linear equation.  A, stem: C concentration, *r*=–0.05, *P*>0.05; N concentration, *r*=−0.58, *P*<0.01; P concentration, *r*=–0.52, *P*<0.01.  B, leaf: C concentration, *r*=–0.59, *P*<0.01; N concentration, *r*=0.19, *P*>0.05; P concentration, *r*=−0.33, *P*<0.01.  C, whole plant: C concentration, *r*=–0.58, *P*<0.01; N concentration, *r*=–0.42, *P*<0.01; P concentration, *r*=−0.61, *P*<0.01.

**Table 1** Correlations between carbon (C), nitrogen (N) and phosphorus (P) concentrations in *Leymus chinensis* stems, leaves, and the whole plant

|  | $C_{stem}$ | $N_{stem}$ | $P_{stem}$ | $C_{leaf}$ | $N_{leaf}$ | $P_{leaf}$ | $C_{leaf}$ | $N_{leaf}$ | $P_{leaf}$ |
|---|---|---|---|---|---|---|---|---|---|
| $C_{stem}$ | 1.00 | | | | | | | | |
| $N_{stem}$ | 0.20 | 1.00 | | | | | | | |
| $P_{stem}$ | 0.08 | 0.60** | 1.00 | | | | | | |
| $C_{leaf}$ | −0.01 | 0.42** | 0.49** | 1.00 | | | | | |
| $N_{leaf}$ | 0.04 | 0.45** | 0.14 | 0.01 | 1.00 | | | | |
| $P_{leaf}$ | 0.21* | 0.54** | 0.37** | 0.15 | 0.28** | 1.00 | | | |
| $C_{leaf}$ | 0.22* | 0.46** | 0.50** | 0.95** | 0.05 | 0.23* | 1.00 | | |
| $N_{leaf}$ | 0.09 | 0.83** | 0.52** | 0.41** | 0.69** | 0.54** | 0.51** | 1.00 | |
| $P_{leaf}$ | 0.15 | 0.68** | 0.80** | 0.44** | 0.17 | 0.79** | 0.52** | 0.70** | 1.00 |

* and ** represent *P*<0.05 and *P*<0.01, respectively.  The same as below.

cantly increased with increasing grazing intensity (*P*<0.01) (Fig. 5). In addition, at the organ level, both the leaf-to-stem

ratios of the concentration and accumulation were ranked as follows: N>P>C (*P*<0.05; Fig. 5).  More specifically, with
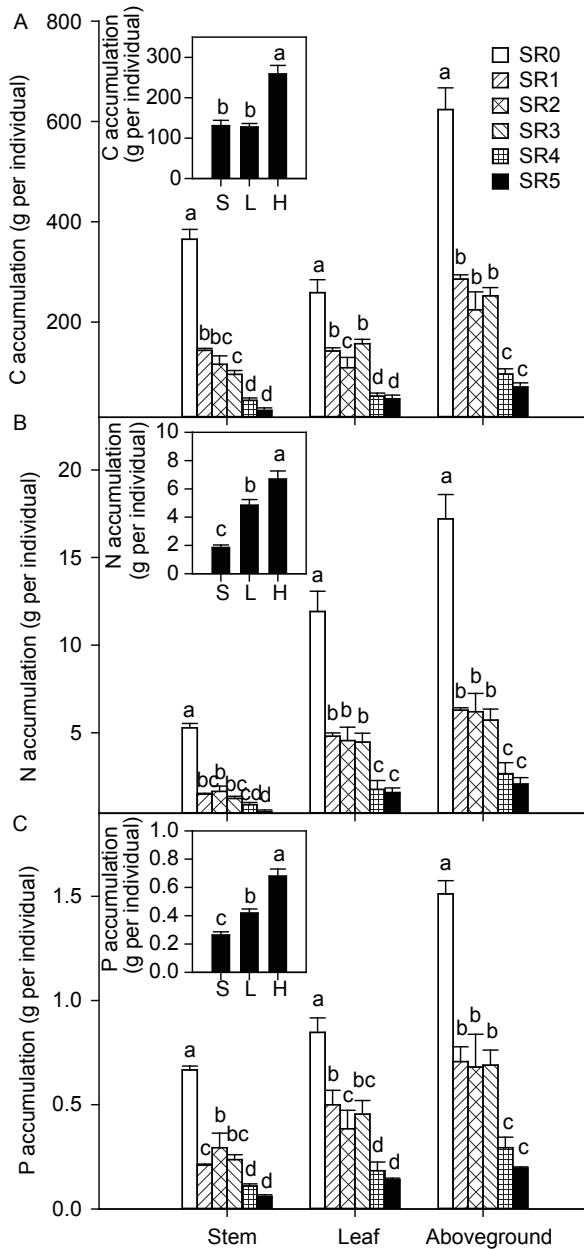
**Fig. 3** C, N, and P accumulations in *L. chinensis* tissue according to grazing intensity. A, carbon *vs.* grazing intensity (linear equation fitting): stem, $r=-0.80$, $P<0.01$; leaf, $r=-0.78$, $P<0.01$; whole plant, $r=-0.81$, $P<0.01$. B, nitrogen *vs.* grazing intensity (linear equation fitting): stem, $r=-0.73$, $P<0.01$; leaf, $r=-0.79$, $P<0.01$; whole plant, $r=-0.78$, $P<0.01$. C, phosphorus *vs.* grazing intensity (linear equation fitting): stem, $r=-0.77$, $P<0.01$; leaf, $r=-0.80$, $P<0.01$; whole plant, $r=-0.81$, $P<0.01$.

a grazing-induced decrease in plant height, there were significant tradeoffs in C, N, and P allocation per *L. chinensis* individuals ($P<0.05$; Fig. 6). Moreover, the pathways of the grazing-induced increases in leaf C, N, and P allocation can be effectively fitted by structural equation modeling (SEM). The results demonstrated that grazing enhanced leaf C, N,
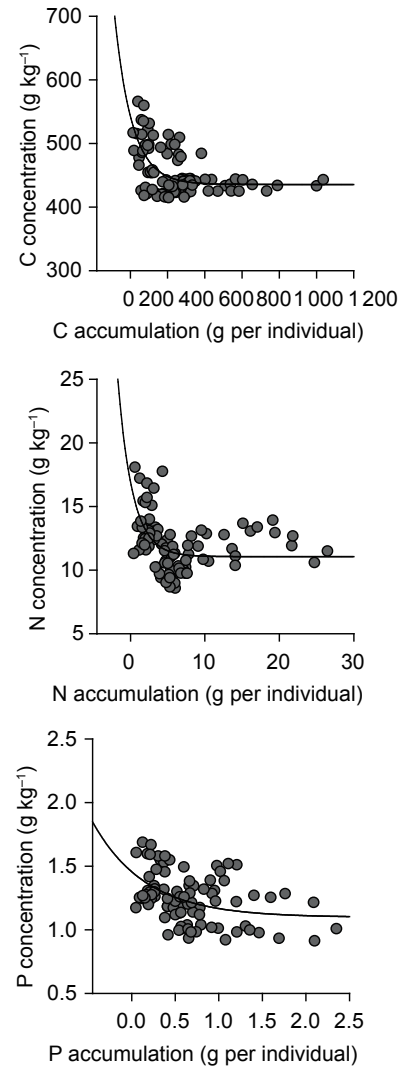


**Fig. 4** Tradeoffs between C, N, P contents per *L. chinensis* individual and C, N, P concentrations in the whole plant. All of the relationships were fitted by an equation of exponential decay ($n=90$, C: $r=-0.58$, $P<0.01$; N: $r=-0.48$, $P<0.01$; P: $r=0.42$, $P<0.01$).

and P allocation primarily *via* the pathways of the increase in leaf-to-stem ratio of biomass accumulation, followed by the ratio of nutrient concentration (Fig. 7).

## 2.4. Grazing intensity effects on C, N, and P stoichiometry

C:P and C:N ratios in the stem and the whole plant significantly decreased along the grazing intensity gradient ($P<0.05$; Fig. 8). However, C:P and C:N ratios in leaves significantly increased with increasing grazing intensity ($P<0.05$; Fig. 8). Therefore, it is likely that in response to grazing, *L. chinensis* increased usage efficiency of N and P in leaves by decreasing the usage efficiency of N and P in

**Table 2** Relationships between C, N, and P accumulations per *L. chinensis* individual and C, N, and P concentrations in stems and leaves

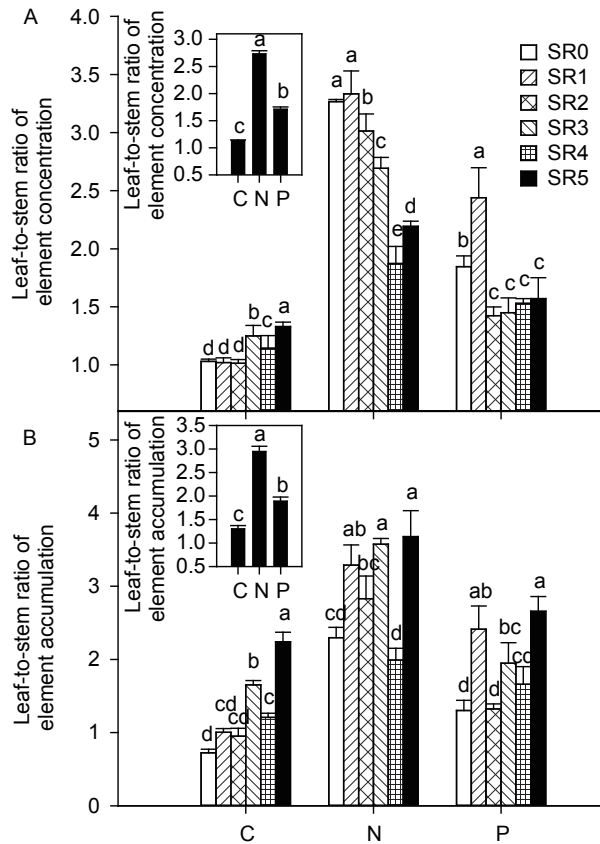| Elements | C content (g per individual) | | N content (g per individual) | | P content (g per individual) | |
|---|---|---|---|---|---|---|
| | Stem | Leaf | Stem | Leaf | Stem | Leaf |
| C | 0.05 | −0.37[**] | | | | |
| N | | | −0.23[*] | −0.39[**] | | |
| P | | | | | −0.27[*] | −0.08 |



**Fig. 5** Leaf-to-stem ratios of C, N, and P concentrations and accumulations along the grazing intensity.  A, leaf-to-stem ratio of C concentration *vs*. grazing intensity (linear equation fitting): *r*=0.66, *P*<0.01; leaf-to-stem ratio of N concentration *vs*. grazing intensity (linear equation fitting): *r*=−0.84, *P*<0.01; leaf-to-stem ratio of P concentration *vs*. grazing intensity (linear equation fitting): *r*=−0.41, *P*<0.01.  B, leaf-to-stem ratio of C accumulation *vs*. grazing intensity (linear equation fitting): *r*=0.63, *P*<0.01; leaf-to-stem ratio of N accumulation *vs*. grazing intensity (linear equation fitting): *r*=0.18, *P*>0.05; leaf-to-stem ratio of P accumulation *vs*. grazing intensity (linear equation fitting): *r*=0.33, *P*<0.01.  C: *r*=0.62, *P*<0.01; N: *r*=0.17, *P*>0.05; P: *r*=0.36, *P*<0.01.



**Fig. 6** Tradeoffs between C, N, and P allocation in leaf and plant size in individual *Leymus chinensis* plants. All of the relationships were fitted with a liner equation (*n*=90) (C: *r*=−0.56, *P*<0.01; N: *r*=−0.21, *P*<0.05; P: *r*=−0.34, *P*<0.01).

the stem (Fig. 8, Table 3).  When fitted by quadratic equation, the relationships between grazing intensity and N:P ratios in the stem, leaves, and whole plant were significant (*P*<0.01).  The N:P ratios in stems, leaves, and whole plants were the lowest with intermediate grazing (Fig. 8).  Additionally, the grazing-induced variation of tissue stoichiometry in *L. chinensis* stems, leaves, and whole plants was correlated
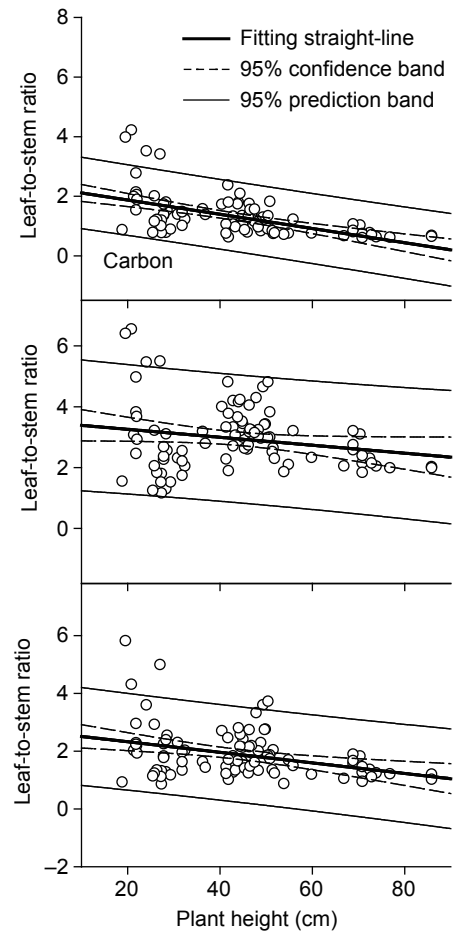
in some cases (Table 3).  The N:P ratios in the leaf and stem were positively correlated (*P*<0.05), whereas the C:N and C:P ratios in the leaf were negatively correlated with that in the stem (Table 3).

# 3. Discussion

## 3.1. Nutrient enrichment in response to grazing at the individual level

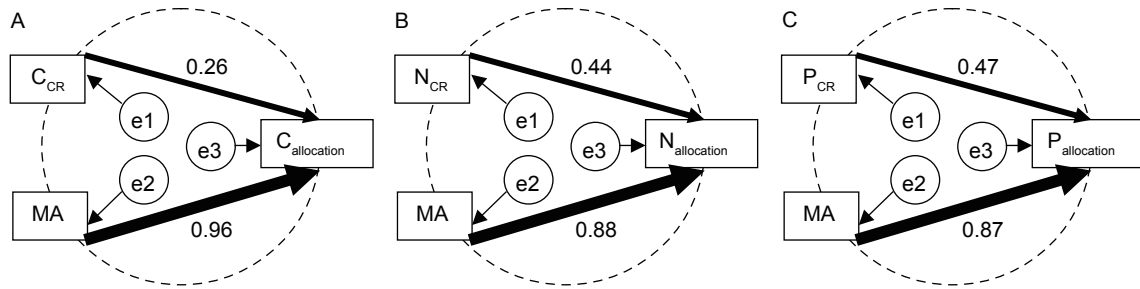Previous studies reported that plasticity in the proficiency

**Fig. 7** Path analysis of *L. chinensis* leaf-to-stem ratios of C (A), N (B), and P (C) accumulation increases induced by grazing intensity. Values associated with arrows represent standardized path coefficients. Solid arrows represent significant ($P<0.001$) paths in a fitted structural equation model depicting the impact of variables on leaf-to-stem ratios of C, N, and P accumulation. Thicker lines represented more significant. $C_{CR}$, $N_{CR}$, and $P_{CR}$ mean leaf-to-stem ratio of C, N, and P concentrations, respectively; MA, biomass allocation ratio; $C_{allocation}$, $N_{allocation}$, and $P_{allocation}$ mean leaf-to-stem ratio of C, N, and P accumulations, respectively.
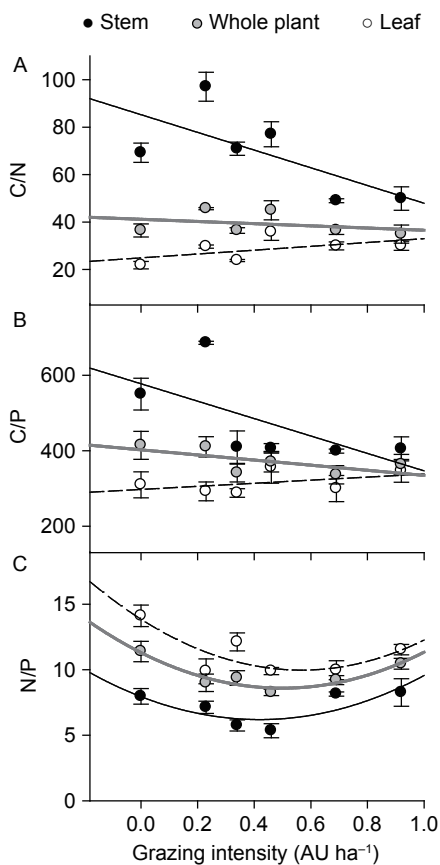


**Fig. 8** C, N, and P ecological stoichiometry in *L. chinensis* tissue along the grazing intensity gradient ($n=90$). A, C/N *vs.* grazing intensity (linear equation fitting): stem, $r=-0.64$, $P<0.01$; leaf, $r=0.47$, $P<0.01$; whole plant, $r=-0.24$, $P<0.05$. B, C/P *vs.* grazing intensity (linear equation fitting): stem, $r=-0.60$, $P<0.01$; leaf, $r=0.24$, $P<0.05$; whole plant, $r=-0.40$, $P<0.01$. C, N/P *vs.* grazing intensity (quadratic equation fitting): stem, $r=0.64$, $P<0.01$; leaf, $r=0.70$, $P<0.01$; whole plant, $r=0.76$, $P<0.01$.

of leaf and stem nutrient resorption is an important nutrient strategy for plants subjected to grazing disturbance (da

Silveira Pontes *et al.* 2010). In our study, we observed significantly higher C, N, and P concentrations in *L. chinensis* leaf and stem tissues from grazed plots compared with ungrazed plots. Additionally, nutrient concentrations were significantly higher in leaves compared to those in stems. Leaves mainly drove the increased concentrations of C in the aboveground portion of *L. chinensis* individuals, while N and P in the stem primarily drove the aboveground N and P concentrations change. In general, nutrients were adaptively accumulated in the aboveground portion of *L. chinensis* individuals in response to grazing by large herbivores in this natural semi-arid grassland in Inner Mongolia.

The nutrient usage strategies of plants in response to grazing by large herbivores, however, has long been controversial due to its high significance in the adaptation of plants to heterogeneous habitats and has clear linkages with plant growth metabolism (Zheng *et al.* 2012; Sun *et al.* 2014). Consistent with our results, many studies found that grazing by large herbivores significantly increased the concentrations of N and P in the leaves, stems, and root tissues of some plant species in natural or artificial grasslands (Bai *et al.* 2012). However, in contrast to these results, Cease *et al.* (2012) reported that N and P concentrations were significantly negatively correlated with grazing intensity. The reason for this disparity in results may be caused by complex mechanisms of nutrient usage strategies related to the interactions between plants and soil. A primary outcome of heavy livestock grazing is the loss of topsoil and organic N due to erosion and uptake by grazing animals (Stark *et al.* 2015). The N concentrations in *L. chinensis* as reported by Cease *et al.* (2012) were observed in grassland affected by heavy degradation following long-term grazing. In grazing-induced soil degradation, N content is reported to be significantly reduced in the soil (Cui *et al.* 2005). Therefore, they suggest that the N concentration in plants grown in long-term grazed grasslands decreased mainly because of the soil condition

**Table 3** Correlations between C, N, and P ecological stoichiometry in *L. chinensis* stems and leaves, and the whole plant

| | $C/N_{stem}$ | $C/P_{stem}$ | $N/P_{stem}$ | $C/N_{leaf}$ | $C/P_{leaf}$ | $N/P_{leaf}$ | $C/N_{whole}$ | $C/P_{whole}$ | $N/P_{whole}$ |
|---|---|---|---|---|---|---|---|---|---|
| $C/N_{stem}$ | 1.00 | | | | | | | | |
| $C/P_{stem}$ | 0.69** | 1.00 | | | | | | | |
| $N/P_{stem}$ | −0.51** | 0.25* | 1.00 | | | | | | |
| $C/N_{leaf}$ | −0.15 | −0.20 | −0.37** | 1.00 | | | | | |
| $C/P_{leaf}$ | 0.03 | −0.14 | −0.19 | 0.64** | 1.00 | | | | |
| $N/P_{leaf}$ | −0.12 | 0.12 | 0.23* | −0.55** | 0.28** | 1.00 | | | |
| $C/N_{whole}$ | 0.75** | 0.37** | −0.53** | 0.66** | 0.45** | −0.31** | 1.00 | | |
| $C/P_{whole}$ | 0.47** | 0.62** | 0.08 | 0.14 | 0.62** | 0.51** | 0.55** | 1.00 | |
| $N/P_{whole}$ | −0.32** | 0.23* | 0.65** | −0.55** | 0.16 | 0.85** | −0.51** | 0.43** | 1.00 |

(Cease *et al.* 2012). In the contrast, our N concentration data from *L. chinensis* were sampled from a grassland that was only grazed for five years. The response of soil properties had a lagging effect when comparing standing vegetation to continuous grazing disturbance in a grassland ecosystem (Jia *et al.* 2007). Therefore, in the initial stage of degradation caused by short-term grazing, soil nutrients were not significantly changed compared with the ungrazed habitat (Yan *et al.* 2014). Hence in our research, C, N, and P concentrations increased according to grazing intensity when the grazing disturbance was continuous only five years, but this finding differs from results observed following in long-term grazing studies.

The variation in C, N, and P concentrations increased gradually along the increasing grazing intensity gradient from 0.00 to 0.92 AU ha$^{-1}$. This finding does not support our hypothesis that nutrient concentrations in plant tissues would be quickly affected by large herbivores when changing from an ungrazed to a grazed environment. On the contrary, our results suggest that plant nutrient usage strategies are gradually affected by grazing intensity, which may be related to the three previously discussed hypotheses: the growth dilution hypothesis, the increasing metabolism hypothesis, and the nutrient competition hypothesis. The growth dilution hypothesis predicts that if the increase in the accumulation of leaf biomass is more than the increase in nutrient acquisition under grazing exclusion, then nutrient concentrations will decrease (Bai *et al.* 2012; Shi *et al.* 2013). The increasing metabolism hypothesis predicts that the increase in C, N, and P concentrations would enhance the number and activity of photosynthetic enzymes and improve the photosynthetic rate when plants are grown under grazing conditions (Scogings *et al.* 2014). Moreover, the improvement of plant secondary metabolism in order to adapt to the stress as a result of grazing by large herbivores may increase the nutrient requirements *via* the pathways of enhancing nutrient absorption efficiency (Li *et al.* 2014) and increasing new tissues (Scogings *et al.* 2013). The nutrient competition hypothesis assumes that there are no significant changes in the soil nutrient content during the initial stage of grassland degradation (Lü *et al.* 2014; Veen *et al.* 2014). Therefore, competition for soil nutrients between grassland plants becomes less significant than in non-grazed habitats, thereby promoting an increase in nutrient concentrations.

### 3.2. Tradeoffs among biomass accumulations and C, N, and P contents

Our results indicated that C, N, and P contents per *L. chinensis* individual were significantly negatively correlated with C, N and P concentrations in the whole plant. Therefore, it is likely that there was a significant tradeoff relationship between nutrient accumulation and concentration in *L. chinensis* individuals. Previous studies have reported that a negative relationship between forage quantity and quality exists in 177 sites distributed in all the major grassland types across China (Shi *et al.* 2013). Specifically, higher forage quantity means more crude fiber but less ether extract and crude protein (Shi *et al.* 2013). Our results supported the results of Shi *et al.* (2013) to some extent, but we have also provided new insights into the effect of nutrient enrichment in *L. chinensis* at an individual level in response to grazing by herbivores in a meadow steppe.

The observed decrease in content per *L. chinensis* individual is likely to have arisen from the miniaturization of plants in the grazing habitat. Our results also indicated that grazing had a significant effect on *L. chinensis* leaf and stem functional traits. Plant individuals in grazed habitats became smaller compared with *L. chinensis* in the ungrazed grassland habitats. Furthermore, the miniaturization of phenotypic traits was derived from the decline in the aboveground biomass in plant individuals subjected to grazing (Shi *et al.* 2013). Thus, the amount of accumulation per *L. chinensis* individual dramatically decreased. Additionally, the tradeoff between nutrient accumulation and concentration may be related to the processes of distribution of elemental nutrients during plant growth. Previous studies indicated that higher productivity in a plant community may have a diluting effect on nutrient concentrations (White and Wight 1984; He *et al.* 2006) because when the mechanical tissues grow, dry mat-

ter accumulates and the proportion of physiologically active non-mechanical tissues gradually declines.

### 3.3. Nutrient allocation strategies in response to grazing

Our findings suggest that the effects of grazing on the leaf-to-stem ratio of C concentration increased with grazing intensity along the tested gradient. However, the leaf-to-stem ratios of N and P accumulations significantly decreased with increasing grazing intensity. This implied that the rate of carbon partitioning into the leaves was higher into stems. N and P concentrations did not mimic this variation mechanism in leaves and stems. Grazing increased leaf C and P allocation *via* increasing concentration and accumulation. This result suggested that *L. chinensis* individuals would invest more photosynthetic product in the leaf, and reduce the investment in the stem causing plant miniaturization. In general, the leaves are the most important organs in a plant because they conduct in photosynthesis, transpiration, and nutrient utilization. With the pressure of long-term overgrazing, photosynthesis in plant individuals is limited by herbivore foraging (Poorter *et al.* 2012; Desurmont and Agrawal 2014). Therefore, it is possible that increasing the leaf-to-stem ratios of C, N, and P is a strategy that enhances photosynthesis in a plant when subjected to herbivore foraging pressure. Additionally, the soil microenvironment and plant-soil feedback relationship are affected by grazing, which can influence plant growth and development. Grazing-induced soil-legacy effects have an impact primarily on plant biomass allocation patterns (Veen *et al.* 2014). Therefore, the increase we observed in the leaf-to-stem ratios of C, N, and P with plant miniaturization may be a mechanism for the adaption to grazing-induced changes in plant-soil feedback.

### 3.4. Compensation effect of nutrient use efficiency between the stem and leaves

In general, the C:P and C:N ratios can be a predictive indicator of nitrogen- and phosphorus-use efficiency in many ecosystems (Güsewell 2004; McGroddy *et al.* 2004). Increased nutrient use efficiency in plants is known to be an important strategy for adaptation to nutrient-poor habitats (Cordell *et al.* 2001). According to previous research, there are significant tradeoffs between nitrogen- and water-use efficiency in the dominant species found in semiarid grasslands (Gong *et al.* 2011). Specifically, in nutrient-limited environments, nutrient use can become more efficient in plants (Cordell *et al.* 2001). In our study, we did not detect a grazing-induced increase in either the C:P or C:N ratio in the aboveground portion of the plant. In contrast with the

characteristics of nutrient-poor habitats, we found that the C:P and C:N ratios were significantly correlated with the grazing gradient. It is likely that no significant nutrient-limited characteristics emerged in the five years of short-term grazing. Therefore, the changes in nutrient strategy observed in *L. chinensis* individuals were primarily regulated by the interactions between the plant and the herbivore, but not by the feedback of grassland soil.

The observed decrease in the C:P and C:N ratios along the grazing gradient may be linked to induced defense metabolism. Secondary metabolite content, which is regulated by plants *via* physiological and biochemical pathways to enhance their defense against herbivore foraging, significantly increased when a plant subsisted in a nutrient-poor habitat or adverse conditions (Scogings *et al.* 2013). Previous studies also indicated that a nutrient-poor habitat could decrease nutrient use efficiency in plants, which in low productivity conditions is affected by an increase in secondary metabolism. Therefore, there may be a tradeoff between secondary metabolism and nutrient use efficiency in plants.

Our results suggest that grazing decreased stem N and P usage efficiency in *L. chinensis*. In contrast, the ratios of C/P and C/N in leaves significantly increased with increasing grazing intensity; therefore, our results imply that there may be a compensatory nutrient-use-efficiency effect between the stems and leaves in response to grazing. At the individual level, the C:P and C:N ratios were significantly negatively correlated with grazing pressures, and the stem of *L. chinensis* primarily drove this process. However, the leaves of *L. chinensis* were a compensatory factor in the decrease of nutrient use efficiency in the aboveground portion because the plant tended to allocate more carbon into the leaves, perhaps to increase its adaptive capacity in a grazing habitat.

### 3.5. N *vs*. P limitation in *L. chinensis* along a grazing gradient

Nutrient limitation in grassland plants in a plant-herbivore interaction system has long been a controversial topic (Mamolos *et al.* 2005). The N:P ratio of ecological stoichiometry is often adopted to estimate nutrient limiting factors in plants. Previous studies reveal that an N:P ratio>16 indicates P limitation at a community level, while a N:P ratio<14 is indicative of N limitation (Koerselman and Meuleman 1996). In this study, we demonstrated that grazing dramatically increases N limitation in *L. chinensis* individuals in the meadow steppe in the Hailaer region, as evidenced by the decreased N:P ratio. Grazing significantly decreased the N:P ratio of *L. chinensis* at the individual levels. Consistent with our findings, Zheng *et al.* (2012) reported that grazing dramatically decreased the N:P ratio of *L. chinensis* in

leaves. In contrast, Bai *et al.* (2012) found that grazing potentially increased the N:P ratio in aboveground tissue in some species at the functional group scale in the semiarid grassland in the Xilin River Basin, Inner Mongolia, China. Therefore, the grazing-induced change in the N:P ratio appears to differ between plant species in plant-herbivore interaction systems in semi-arid grasslands. Additionally, we found that the N:P ratio was <12 at the grazing exclusion sites, a value lower than the threshold of 14:1 for N limitation. Therefore, increased grazing intensity affected the N-limited condition. Hence, it appears that the variable direction of stoichiometry is affected by the initial state of the plant-soil interaction system.

# 4. Conclusion

In conclusion, our results indicated that as the height of *L. chinensis* plants decreased with an increase in the grazing gradient by larger herbivores, the concentrations of C, N, and P in leaves and stems significantly increased. Affected by grazing, the accumulations of C, N, and P per individual were significantly negatively correlated with the concentration of aboveground tissues, suggesting there was a tradeoff in *L. chinensis* between nutrient accumulation and concentration of *L. chinensis* at the individual scale. The leaf-to-stem ratios of C, N, and P contents increased with grazing intensity, indicating a tradeoff in nutrient allocation and plant size at the individual plant level. Grazing intensities were negatively correlated with the ratios of C/N and C/P in the stem; however, these ratios in leaves significantly increased with grazing intensity. Our findings suggest that *L. chinensis* in the meadow steppe adapts to grazing disturbance through tradeoffs between plant size and nutrient use strategies. Moreover, our results imply that grazing produces a compensatory effect on nutrient use efficiency between the stems and leaves of *L. chinensis*.

# 5. Materials and methods

## 5.1. Study site

A native meadow steppe was selected at the Hulun Buir Grassland Ecosystem Observation and Research Station (HGEORS) of the Chinese Academy of Agricultural Sciences, which is located at the Xiertala Farm, Hailar, in the center of the Hulun Buir meadow steppe in the Inner Mongolian Autonomous Region, China. This field site is situated in the eastern part of the Eurasian steppe, in northeastern China (latitude 49°19′N, longitude 120°02′E, altitude 628 m; Fig. 9-A). Mean annual air temperature in this area is −3 to −1°C. The mean precipitation during the summer from May to September from 1980–2012 was approximately 350 mm.

The mean length of the growing season is approximately 150 days. Soil in the experimental sites is characterized as chestnut (Chinese classification; soil texture: 42% sand, 35% silt, and 23% clay), with low nutrient availability in the surface layer (Yan *et al.* 2015). Soil bulk density is 1.37 g cm$^{-3}$ and the pH is 7.7 (for the top 10 cm). While the native meadow steppe is dominated by *L. chinensis*, other prevalent grasses include *Stipa baicalensis*, *Cleistogenes squarrosa*, and *Carex duriuscula* (Ma *et al.* 2012).

## 5.2. Experimental design

Grazing experiment facilities were established in the meadow steppe dominated by *L. chinensis* in 2009 with five stocking rates and one control plot (Yan *et al.* 2015). Stocking rates were set as 0, 0.23, 0.34, 0.46, 0.69, and 0.92 AU ha$^{-1}$, where 1.00 AU=500 kg of adult cattle, with three replicates for each stocking rate and each replicate occupying a 5 ha paddock. There were 18 randomly distributed plots in total that were located over a homogeneous area of 90 ha (Fig. 9-B). In the plots, zero, two, three, four, six, and eight heads of 250–300 kg young cattle grazed in each replicate plot of the six stocking rate treatments, respectively, with 69 head of cattle grazing in total. Annually, beginning in 2009, continuous grazing lasted for 120 days between June and October. The grazing cattle were kept in the grazing plots day and night, and their drinking water was supplied from an outside water source. Before the plots were fenced off, the site had been under long-term grazing from free-ranging cattle or sheep. In the summer of 2008, a baseline measurement was conducted prior to field treatments, where a 50 m transect in each plot used to investigate vegetation and soil traits. All of the plots in both grazed and ungrazed treatments had the same soil type and similar physiographic conditions (e.g., slope degree, slope direction, topography, and altitude).

## 5.3. Field sampling and chemical analysis

In each grazing plot, five temporary movable ex-closure cages were set up at each sampling point prior to grazing and before the growing season in early April 2013. Five 1 m× 1 m quadrats were established in each plot for field investigation and sampling. The quadrats were at least 20 m apart and the physiographic conditions within the quadrats did not differ.

Field sampling was conducted between August 5th and 10th in 2013, corresponding to annual peak-standing biomass. Three *L. chinensis* individuals were selected randomly in each 1 m×1 m quadrat. Phenotypic functional traits such as plant height were measured in a laboratory after the entire aboveground portion of the individuals was
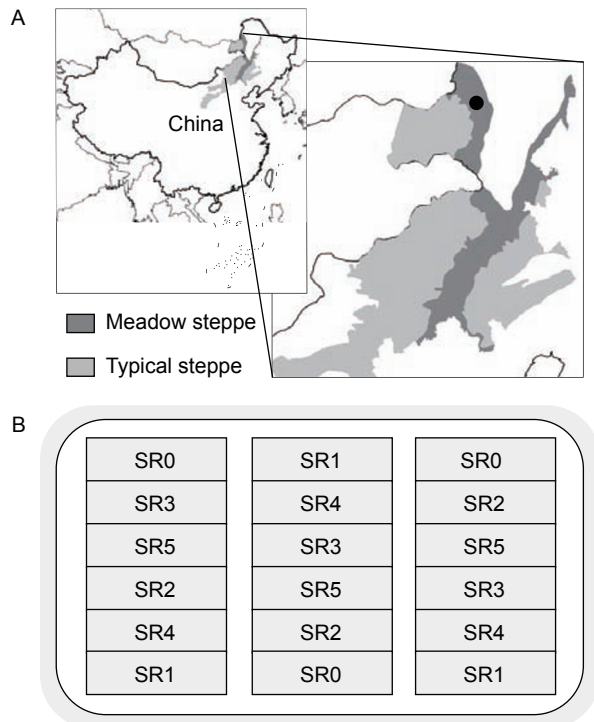
**Fig. 9** Location map of the study area (A) and the layout of cattle grazing with different stocking rates on the experimental plots of *Leymus chinensis* grassland (B). The meadow steppe in Inner Mongolia, China is mainly dominated by *L. chinensis*.

clipped completely in the field. After the measurement of phenotypic traits, the leaves and stems were packaged in individual paper bags. Subsequently, all plant samples were oven-dried at 65°C for at least 48 h. After drying, the mass of the samples was measured, as was leaf and stem biomass.

The leaves and stems of the *L. chinensis* individuals in each quadrat were combined, pulverized using a mechanical mill (Retsch MM 400, Retsch GmbH & Co. KG, Haan, Germany), and passed through a 40-mesh sieve. The total concentration of C in leaves and stems was determined using the $H_2SO_4$-$K_2Cr_2O_7$ oxidation method. Subsamples were digested in $H_2SO_4$-$H_2O_2$ (Bennett *et al.* 2003). Total concentration of N was analyzed using an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden), and total concentrations of P were measured colorimetrically at 880 nm after reaction with molybdenum blue. All stoichiometric ratios of C:N:P were reported as mass ratios (Lü *et al.* 2012).

### 5.4. Statistical analysis

The plant heights of *L. chinensis* individuals in each quadrat were averaged. The Kolmogorov-Smirnov test was used to test for normality in the average plant height and C, N,

and P concentrations in *L. chinensis* leaves and stems. C, N, and P contents in each *L. chinensis* individual were calculated by multiplying the C, N, and P concentrations by the biomass of corresponding tissues. Significant differences in the plant traits between various treatments were evaluated using one-way analysis of variance (ANOVA). The relationships between plant height/grazing intensity and C, N, and P concentrations were tested with Pearson's correlation method. Confirmatory analyses of structural equation modeling (SEM) were applied to quantify the direct and indirect effects of grazing intensities on C, N, and P accumulations. A conceptual model of the hypothetical relationships was created based on prior and theoretical knowledge. Data were fitted to the model using the maximum likelihood estimation method. Adequate model fit was indicated by a nonsignificant chi-squared test ($P$>0.05). All of the statistical analyses were performed using SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA), with $P$<0.05 set as the level of significance. A geographical map of the study area was produced using ArcGIS 10.1 (ESRI, Redlands, California, USA). SEM analysis was performed using AMOS 20.0 software (IBM, SPSS, Armonk, NY, USA).

**Appendix** associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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(Managing editor  ZHANG Juan)