



ORIGINAL ARTICLE

Heavy grazing effects on stem elongation and internode allometry: Insights from a natural pasture grass

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Funding information

National Natural Science Foundation of China, Grant/Award Number: 31702161 and 31860681; Natural Science Foundation of Inner Mongolia, Grant/Award Number: 2017MS0317; China Agriculture Research System, Grant/Award Number: CARS-34

Abstract

Limited plant stem elongation is hypothesized to be the primary cause of decreased plant growth in response to long-term heavy grazing (LHG). However, it remains largely unknown how LHG-induced changes in stem elongation are mediated by internode traits. Accordingly, we experimentally investigated the effects of LHG (defined as twice the stocking rate recommended by the local government) on plant stem elongation and internode allometry in *Leymus chinensis* in Xilinhot, Inner Mongolia, China. Plant size and internode traits, including internode length (IL), internode diameter (ID), and internode number (IN), from plant individuals were measured according to their phytomer position from base to apex. The results revealed that IN and their distributions were changed in response to LHG. Although LHG negatively affected IL and ID of each *L. chinensis* internode from base to apex and eventually stem length, the plasticity index of IL and ID increased initially and then decreased from the lowermost to the uppermost internode in response to LHG. These contrasting responses of internode traits were result of allometry between internode traits determined by both LHG and internode position. Ultimately, IL and IN were identified to be key traits influencing stem length reduction under LHG conditions and explaining 52.51% and 47.49% of variation in stem length, respectively. Overall, our findings suggest that the limitation of *L. chinensis* stem elongation induced by LHG is mediated by the plasticity and allometry of internode traits. This result emphasizes the sensitivity of individual plants to LHG and is potentially valuable for optimal grassland management.

KEYWORDS

grazing, internode elongation, plant-animal interactions, rhizomatous grass

1 | INTRODUCTION

Grazing by large herbivores is the primary economic use of natural pastures worldwide, especially in the semi-arid steppe regions of Inner Mongolia, China (Bai et al., 2012; Haynes et al., 2014). Identifying the factors that determine individual characteristics of natural pasture plant species is important for reducing uncertainty in ecosystem function changes induced by grazing (Díaz, Noy-Meir, &

Cabido, 2001). Over the past several decades, natural pastures, particularly in Inner Mongolia, have undergone a dramatic increase in livestock number per unit pasture area (Li et al., 2014). Accordingly, the livestock population of Inner Mongolia increased from about 45 million before 1980 to more than 100 million since 2000 onward (Wu, Zhang, Li, & Liang, 2015). This increase in livestock population exceeded the optimum stocking rate threshold for grasslands, i.e. it constituted long-term heavy grazing (LHG). Consequently, this

long-term LHG significantly affects not only productivity but also the vital roles of these ecosystems in essential ecological services such as hydrology, biodiversity, and carbon cycles (Kemp et al., 2013).

Typical steppes, which are dominated by *Leymus chinensis*, cover the majority of the eastern Eurasian temperate grassland at the northern boundary of China (Schönbach et al., 2009). These grasslands are traditionally used as rangeland for both livestock grazing and haymaking in Inner Mongolia (Wu, 1980). *Leymus chinensis* is a native, perennial rhizomatous grass with high palatability and forage value; it is also considered to be one of the most promising grass species for grassland rehabilitation and reconstruction in arid regions of northern China (Huang et al., 2015; Liu & Han, 2008). This grass is economically and ecologically important because it is rich in vitamins, high-quality protein, minerals, and carbohydrates; moreover, it grows rapidly and is highly tolerant of the region's arid conditions (Ma, Yuan, Guo, & Wang, 2014; Ren et al., 2014). Therefore, from the perspectives of both theory and application, this grass has been of keen interest to ecologists and agronomists for the past several decades.

Because *L. chinensis* is a preferred forage species for large herbivores, it undoubtedly sustains higher relative levels of defoliation (Wang, Wang, He, Liu, & Hodgkinson, 2010). Further, *L. chinensis* is very sensitive to LHG both in terms of its individual morphological plasticity and spatial distribution (Zhao, Chen, Han, & Lin, 2009). Previously, our research indicated that LHG caused aboveground biomass declines primarily through a "bottom-up" effect on plant height and stem traits of *L. chinensis* (Li et al., 2015). In addition, LHG promotes an asymmetrical response in different plant functional traits leading to variation in individual plant size (Li et al., 2015). More generally, stems have been suggested to be more sensitive than leaves to LHG conditions (Cruz et al., 2010; Guo et al., 2012). However, it remains largely unknown how changes in *L. chinensis* stem elongation are induced by LHG. The present study expands on the previous work of Li et al. (2015) and investigates the changes in stem elongation induced by LHG as mediated by various internode traits, i.e. internode length (IL), internode diameter (ID), and internode number (IN). For individual plants, differences in leaf position from the lowermost to the uppermost leaf correspond to differences in age and development (Bhakta & Ganjewala, 2009). For example, an increasing defense effect of position on leaf function has been observed from the bases to the apices of plants (Shim, Lee, Ryu, & Kang, 2003). Similarly, many studies have reported that photosynthesis differs dramatically from the base to the apex of plants in various species owing to differences among developmental stages (Gonzalez-Real & Baille, 2000; Hejrnák, Hniličková, & Hnilička, 2014; Mooney, Field, Gulmon, & Bazzaz, 1981). However, there is a dearth of information on the effects of LHG on internode characteristics among different leaf positions of ecologically and economically important grassland plants such as the dominant *L. chinensis* of typical Eurasian steppes.

Here, we used long-term paired grazed and ungrazed experiments to evaluate the effects of grazing on plant stem elongation

and internode allometry in *L. chinensis*. In particular, we evaluated the contribution of internode traits to changes in stem elongation induced by LHG. We addressed the following three main research questions: (a) How does the LHG-induced dwarf phenotype affect the morphological plasticity of internode traits in *L. chinensis*? (b) How do LHG and leaf position influence allometric scaling between internode length and diameter (ILD) in *L. chinensis* individuals? and (c) How is the LHG-induced limitation of stem elongation mediated by changes in internode traits?

2 | METHODS

2.1 | Study site

Our experimental site is located at Inner Mongolia's Xilingol Grassland Ecosystem Observation and Research Station (43°38'N, 116°42'E, 1,200 m a.s.l.), in southern Xilin, China. This region has a semi-arid continental climate. Long-term meteorological data (1980–2013) show the mean annual temperature is 0.9°C, with mean monthly temperatures ranging from 3.6°C to 19.4°C during the growing season of April to August (Li et al., 2016). The mean annual precipitation is 328.54 mm. Maximum precipitation coincides with the highest temperatures in May, June, July, and August (Li et al., 2015). For perennial plants, e.g. *L. chinensis*, the growing season lasts for approximately 150 days from April to September in most years. The perennial rhizome grass *L. chinensis*, which was selected for this research, dominates the typical steppe communities in the grasslands of northern China (Wang, Gao, Lin, Mu, & Mu, 2010). The soil is classified as haplic calcisols and calcic orthic aridisol by FAO and the US soil classification systems, respectively (Zhang et al., 2014).

2.2 | Experimental design

Our long-term grassland experiments have consisted of paired plots of grasslands heavily grazed by sheep and of grasslands with continuous enclosure (i.e. ungrazed areas) since 1983. The grazed plots are about 200 ha in area and are located adjacent to the enclosure plots (approximately 25 ha in area). The continuous heavy grazing plots have been grazed by approximately 600 sheep and goats year-round for more than 30 years at a stocking rate of approximately 3 sheep per ha. This rate is significantly higher than the local stocking rate of 1.5 sheep per ha, which is necessary for maintenance of the grass-livestock balance recommended by the local government (Li et al., 2015).

2.3 | Sampling and measurement

The field site was subject to pseudo-replication, which is often reported for many chronosequence ecological studies (Blois, Williams, Fitzpatrick, Jackson, & Ferrier, 2013; Lü et al., 2014; Walker, Wardle, Bardgett, & Clarkson, 2010). Under this situation, a paired sampling method is recommended for field sampling. In this study, 15 replicate 20 × 20 m plots were established as pairs within the long-term

TABLE 1 Effects of long-term heavy grazing on internode morphological traits of *Leymus chinensis* individuals

Internode traits	Ungrazed		Grazed		F-value	p-value
	Mean	SE	Mean	SE		
Internode number	6.56	0.15	4.73	0.17	64.51	<0.001
Internode length (cm)	5.83	0.09	2.88	0.08	574.29	<0.001
Internode diameter (mm)	1.05	0.02	0.87	0.02	50.16	<0.001
Internode length to diameter ratios (cm/mm)	5.50	0.12	3.34	0.09	219.26	<0.001
Stem length (cm)	37.79	0.65	13.80	0.74	595.06	<0.001

grazed and ungrazed treatments along a transect. The plots were randomly placed within 10–15 m of each other along this transect. Three 1 × 1 m subplots were then established in each plot for field investigation and sampling. Temporary movable enclosure cages were set up at each sampling point before the growing season in early April 2014 (Li et al., 2015). We conducted field sampling during August 10–15, 2014, which corresponds to the annual peak growth season. For stem and internode trait measurements, five *L. chinensis* individuals were selected randomly in every 1 × 1 m subplot. Phenotypic traits of *L. chinensis* were measured in the laboratory after the entire above-ground portion of each plant was completely clipped off in the field. Internodes from each *L. chinensis* individual were measured according to their phytomer position from the base to the apex (Coelho, Valério, & Monteiro, 2009; Yang, Auerswald, Bai, Wittmer, & Schnyder, 2011). Phenotypic traits of these *L. chinensis* individuals, including stem length (SL), IL, ID, and IN, were measured with electronic digital calipers (Pérez-Harguindeguy et al., 2013).

2.4 | Statistical analyses

The IL and ID were measured for all internodes of each plant, and the mean of all internodes was determined to characterize the internode traits of each individual plant. Prior to statistical analysis, the mean of each of the plant traits was calculated from measurements of three *L. chinensis* individuals in a single subplot. The responses of functional traits of *L. chinensis* between grazed and ungrazed grassland plots were assessed using the plasticity index (PI) (Li et al., 2016; Olmo, Lopez-Iglesias, & Villar, 2014).

$$PI = \frac{FU - FD}{FD} \quad (1)$$

where FU is the stem trait in the long-term ungrazed plots and FD is the stem trait in LHG plots.

Allometric relationships were analyzed using the classic allometric model (Li et al., 2016; Warton, Duursma, Falster, & Taskinen, 2012):

$$Y = aX^b \quad (2)$$

where a is the allometric coefficient and b is the allometric exponent. The Equation (2) is usually fitted in the form $\log Y = \log a + b \log X$. An exponent that is significantly different from 1 indicates an allometric (i.e. non-isometric) relationship. Researchers have argued that

model II regressions (e.g. standardized major axis or SMA) are more appropriate than traditional least squares for fitting allometric data and estimating parameters (Niklas and Cobb, 2006); therefore, SMA regression was used for data analysis. For each population, significant differences in the slope from 1 were evaluated (Falster, Warton, & Wright, 2006).

Structural equation modeling (SEM) was performed to analyze hypotheses that may explain the pathways responsible for the indirect effects of LHG on individual-level *L. chinensis* stem elongation reductions influenced by IL and IN (Byrne, 2013). SEMs were developed based on hypothesized relationships between variables and tests of preliminary models. Co-varying factors with non-significant regression weights within the SEMs were not included. The final SEMs were then applied to each of the *L. chinensis* indices. The utility of each functional trait index within the SEM was compared based on the power of the particular model to explain the variation in *L. chinensis* stem elongation (i.e. r^2), measures of model significance and fit (i.e. χ^2 and AIC), and the significance (i.e. significance of regression weights) of the functional trait variables within the model (Li et al., 2016).

In this study, the internode positions of *L. chinensis* individuals were serial data. Thus, the relationships of internode position with IL, ID, PI, allometric slope, and allometric intercept were tested using Spearman's rank correlation coefficients (Gauthier, 2001). The method of principal components analysis (PCA) was performed to determine the overall effect of grazing on internode traits. Significant differences in each of the plant traits between the ungrazed and LHG plots were evaluated by one-way analysis of variance. Correlations among functional traits of *L. chinensis* were analyzed using the Pearson method (Li et al., 2015).

All statistical analyses were performed using SPSS 19.0. Allometric scaling and SEM were performed using the (S)MATR 2.1 and IBM SPSS AMOS 18 software packages, respectively.

3 | RESULTS

3.1 | Internode traits affected by grazing

The internode traits of *L. chinensis* were strongly affected by grazing (Supporting information Figure S1). Grazing significantly decreased IN per *L. chinensis* individual from 6.56 to 4.73, on average ($p < 0.01$, Table 1). The IN distribution of *L. chinensis* populations in ungrazed

and LHG plots was fitted using a Gauss equation (Figure 1). In addition, IN distributions and their skewness and kurtosis were obviously affected by grazing. Further, grazing dramatically increased the variability of IN in *L. chinensis* populations (Figure 1). Moreover, grazing negatively affected mean IL, ID, IL-to-ID ratios, and SL of *L. chinensis* individuals ($p < 0.01$, Table 1).

3.2 | Response of internodes in different positions to grazing

The IL, ID, and IL-to-ID ratios increased initially and then decreased from the lowermost to the uppermost internode (Supporting information Figure S2a). The relationships of these three traits with internode position were effectively fitted using a multiple regression equation ($p < 0.001$, Supporting information Figure S2b). In general, the second or the third internode of an *L. chinensis* individual had the largest size. In addition, the uppermost internode of an *L. chinensis* individual had the smallest size (Figure 2a,b,c). Overall, grazing significantly decreased both IL and ID in each internode from the bottom to the top (Table 2). With grazing, the plasticity index of IL and ID increased initially and then decreased from the lowermost to the uppermost internode (Figure 2a,b). However, the plasticity indices of IL to ID ratios were negatively correlated with internode positions (Figure 2c).

3.3 | Allometric scaling of ILD

Allometric scaling of ILD was significantly different to 1 ($P_{\text{slope}} < 0.01$) (Figure 3a,b). Specifically, ILD relationships (log-log transformed) were significant at every internode position in the two ungrazed and LHG sets of plots (Supporting information Figure S3). In the first two internodes from the bottom, the slopes of ILD allometric scaling were significantly less than one not only in ungrazed habitats but also in LHG habitats ($P_{\text{slope}} < 0.01$, Table 2). Further, all internodes had common slopes (with the exception of the third internode, $P_{\text{heterogeneity}} < 0.05$) in ungrazed and LHG habitats ($P_{\text{heterogeneity}} > 0.05$). However, ILD relationships significantly shifted

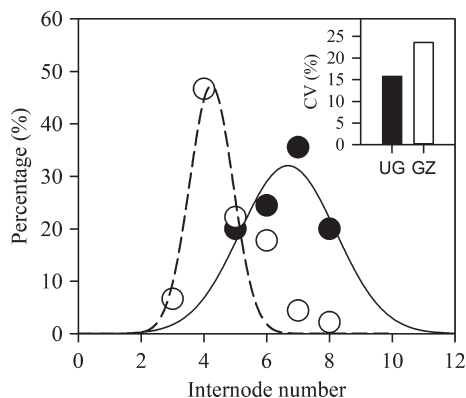


FIGURE 1 Effects of grazing on internode number distribution of *Leymus chinensis* stems in ungrazed (●) and grazed (○) plots. Internode number distributions were fit with a Gauss equation

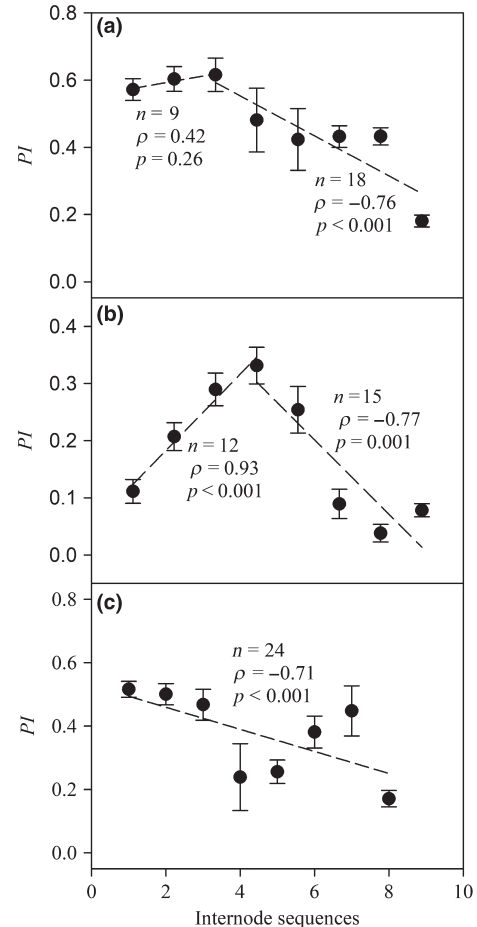


FIGURE 2 The relationships between plasticity indices (PI) of internode length (a), internode diameter (b), and the internode length to diameter ratio (c) with internode positions affected by grazing. Spearman rank correlation ρ values and p -values are presented

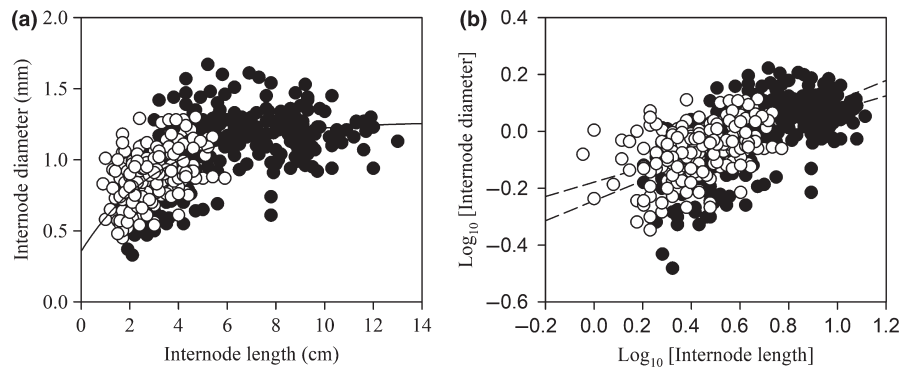
along the common slope of the two habitats ($p < 0.05$, Table 3). The ILD allometric slopes increased with the internode position from the bottom to the top of *L. chinensis* individuals (Figure 4a). The ILD allometric scaling was significant of the first four internodes (slope < 1 , $P_{\text{slope}} < 0.01$, Figure 4b). In addition, they had no common slope for different internode positions ($P_{\text{heterogeneity}} < 0.01$). Based on post hoc multiple comparisons, the slopes among the second, third, and fourth internodes were significantly heterogeneous ($p < 0.05$, Figure 4b).

3.4 | Pathways of *L. chinensis* stem-length reduction

The IL and IN are the two major factors which contributed to the stem length. We detected a tradeoff between IL and IN in the ungrazed grasslands ($p < 0.01$, Figure 5a). However, coordinated variation between them was found in the LHG grasslands ($p = 0.06$, Figure 5b) and the combined datasets ($p < 0.05$, Figure 5c). Moreover, IL and IN were positively correlated with stem length ($p < 0.01$, Figure 5d). The IL is a key trait in driving changes in stem elongation under LHG (Figure 5e). Furthermore, our results demonstrate that grazing indirectly affects SL through a “bottom-up” effect on IL and IN (Figure 5f).

TABLE 2 *Leymus chinensis* internode morphological traits at different internode positions in response to long-term overgrazing

Internode position	Internode length		Internode diameter		Internode length to diameter ratio	
	F-value	p-value	F-value	p-value	F-value	p-value
Internode 1	80.95	<0.001	23.14	<0.001	63.69	<0.001
Internode 2	566.03	<0.001	73.39	<0.001	235.68	<0.001
Internode 3	420.44	<0.001	125.52	<0.001	152.23	<0.001
Internode 4	105.85	<0.001	96.69	<0.001	20.96	<0.001
Internode 5	29.61	<0.001	14.27	<0.001	8.77	0.004
Internode 6	15.79	<0.001	0.32	0.571	5.98	0.018

**FIGURE 3** The relationship between internode length and internode width of *Leymus chinensis*. (a) The relationship fit by the equation describing an exponential rise to a maximum ($R^2 = 0.43$, $p < 0.001$). (b) Effects of grazing on allometric scaling of internode length and internode width (log-log transformed). The ungrazed group fit by standardized major axis (SMA): $R^2 = 0.33$ ($p < 0.001$), and slope of 0.51 (0.46, 0.56), $P_{\text{slope}} < 0.001$; grazed group fit by SMA: $R^2 = 0.19$ ($p < 0.001$), and slope of 0.57 (0.50, 0.64), $P_{\text{slope}} < 0.001$. The slopes did not differ significantly between the grazed group and the ungrazed group ($p = 0.16$). The common slope was 0.53 (0.49, 0.57). There was a significant shift along the common slope ($p < 0.001$). ●, ungrazed; ○, grazed. Solid and dashed lines represent linear fits of ungrazed and grazed groups, respectively**TABLE 3** Standardized major axis (SMA) regression slopes and their confidence intervals for log-log transformed relationships between internode length and diameter of *Leymus chinensis* internodes in grazed and ungrazed groups

Internode positions	Group	R^2	Slope (CI 95%)	Intercept (CI 95%)	Common slope (CI 95%)	Shift?
Internode 1	Ungrazed	0.04 ^{NS}	0.25** (0.18, 0.33)	-0.14** (-0.20, -0.08)	0.28 ^{NS} (0.23, 0.35)	Yes
	Grazed	0.02 ^{NS}	0.32** (0.24, 0.43)	-0.14** (-0.18, -0.09)		
Internode 2	Ungrazed	0.00 ^{NS}	0.74* (0.55, 1.01)	-0.66** (-0.88, -0.43)	0.70 ^{NS} (0.57, 0.87)	Yes
	Grazed	0.04 ^{NS}	0.67* (0.50, 0.90)	-0.41** (-0.53, -0.30)		
Internode 3	Ungrazed	0.01 ^{NS}	-0.73* (-0.98, -0.54)	0.73** (0.53, 0.93)	0.66 ^{NS} (0.55, 0.80)	Yes
	Grazed	0.30**	0.62** (0.48, 0.80)	-0.35** (-0.42, -0.27)		
Internode 4	Ungrazed	0.16*	0.79 ^{NS} (0.60, 1.05)	-0.49** (-0.65, -0.33)	0.66 ^{NS} (0.54, 0.80)	Yes
	Grazed	0.31**	0.56** (0.43, 0.72)	-0.34** (-0.40, -0.27)		
Internode 5	Ungrazed	0.22**	0.94 ^{NS} (0.72, 1.24)	-0.58** (-0.74, -0.42)	0.80*	
	Grazed	0.32*	0.59** (0.40, 0.86)	-0.34** (-0.44, -0.25)		
Internode 6	Ungrazed	0.43**	1.02 ^{NS} (0.79, 1.33)	-0.60** (-0.73, -0.46)	1.07 ^{NS} (0.83, 1.36)	Yes
	Grazed	0.13 ^{NS}	1.35 ^{NS} (0.70, 2.60)	-0.55** (-0.87, -0.23)		

Note. The table shows 95% confidence intervals (CI) of SMA slopes, intercepts and common slopes. In several bivariate cases, SMA tests for common slopes revealed no significant differences between the two groups (i.e. $p > 0.05$). In such cases, common slopes for the bivariate relationships are shown, with the CI of the common slopes provided. In addition, significant shifts along a common slope are indicated. Symbols: ** $p < 0.01$; * $p < 0.05$; ^{NS} $p > 0.05$.

4 | DISCUSSION AND CONCLUSION

Our previous study demonstrated that the limitation of stem elongation was a key process in the reduction size of individual of *L. chinensis* plants induced by LHG (Li et al., 2015). In the present study, we detected that the process of grazing-induced changes in stem elongation was influenced by changes in IL and IN of individual *L. chinensis* plants. Our findings that LHG dramatically reduces the ultimate individual size, specifically the length of each internode, during the peak-growth season are consistent with the results of previous studies conducted in multiple types of grasslands (James et al., 2001; Koerner, Collins, Blair, Knapp, & Smith, 2014). Theoretically, plant phenotypic plasticity, such as the decrease in stem elongation induced by LHG, is an important mechanism for grazing avoidance in plants that experience grazing (Fu, Thompson, Willms, & Mackay, 2005; McKinney & Fowler, 1991).

In addition, our results showed that the LHG-induced variations in plasticity indices for IL and ID increased initially and then decreased from the lowermost to the uppermost internode, suggesting that the plant would drive the divergence of growth plasticity in different internode positions under LHG conditions. We speculate that these differences are most likely associated with the division of investment in different internode positions, from the lowermost to the uppermost, as shown by data from previous studies (Yang et al., 2011).

Internode traits, including IL and ID, are considered to be a good predictor of the plants response to environmental stresses such as

grazing disturbance. The IL and ID are the two primary determinants of water and nutrient transportation function of grass species (Li et al., 2015). In this research, we detected that the IL to LD ratio of *L. chinensis* decreased significantly under LHG conditions. Our results showed that the plasticity index of this ratio was negatively correlated with internode position from the lowermost to the uppermost. We speculate that this result is most likely related to the allometry of IL and ID influenced both by LHG and internode position. Therefore, in this research, we further documented the linkage between IL and ID both in grazed and ungrazed grasslands. The result of allometric scaling of IL-ID was significant with 1.00, in agreement with our established hypothesis.

Interestingly, we found that allometric scaling between IL and ID was positively correlated with internode position. The internodes at the bottom of *L. chinensis* individuals had higher allometric scaling (slope < 1.00) between IL and ID. This result implied that the internodes at the top of *L. chinensis* individuals were relatively stable under LHG disturbance. Furthermore, the high ecological plasticity of the lower internodes of *L. chinensis* individuals enhanced the ability of plants to respond to grazing conditions. Many previous studies have reported that the functions of lower leaves differ from those of upper leaves (Mooney et al., 1981). Further, these studies provide some explanation of our finding of internode position effects on internode allometry. For example, the carbohydrate concentration, antioxidant properties, photosynthetic capacity, and nutrient content differ dramatically depending on leaf position (Capucho et al., 2010).

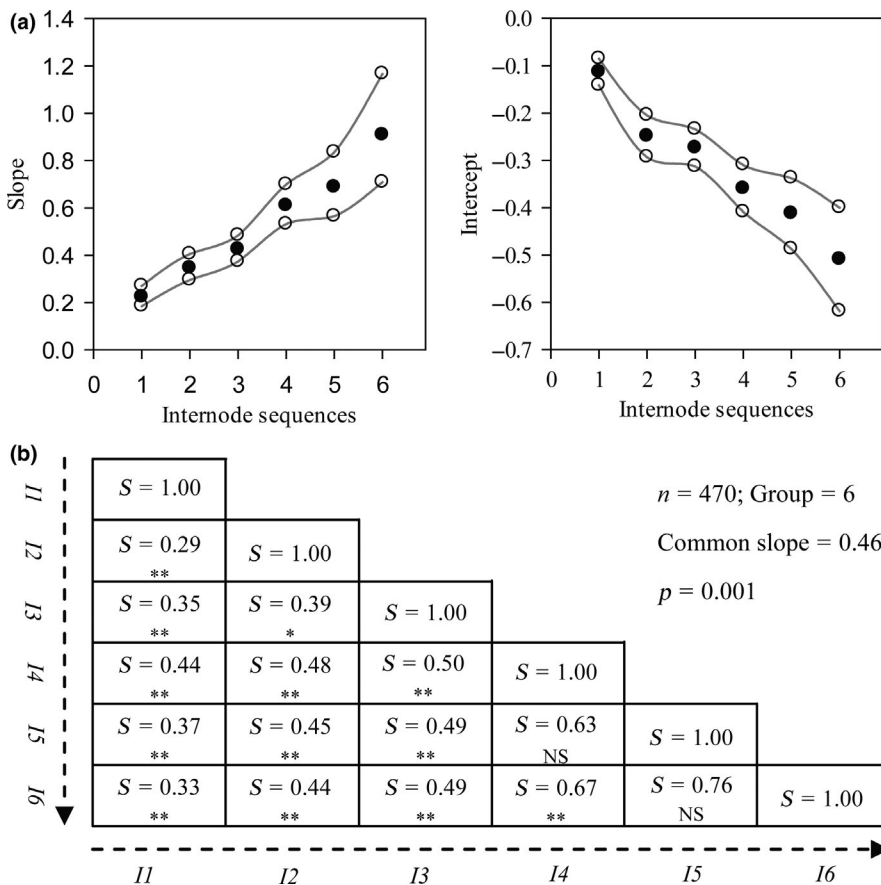


FIGURE 4 The relationship of slope and intercept of stem length-diameter allometric scaling with internode position. (a) Slopes ($p = 0.96$, $p < 0.01$) and intercepts ($p = -0.93$, $p < 0.01$) of internode length-width allometric scaling in response to internode position. ●, slopes or intercepts; ○, 95% confidence interval. (b) Post-hoc multiple comparisons of SMA regression slopes among groups of tests for heterogeneity of slope for internode length-width allometric scaling in different positions. S , common slope. I1-I6 represented the six internode positions in (b). Significant differences in slope among the groups are indicated by ** $p < 0.01$ or * $p < 0.05$

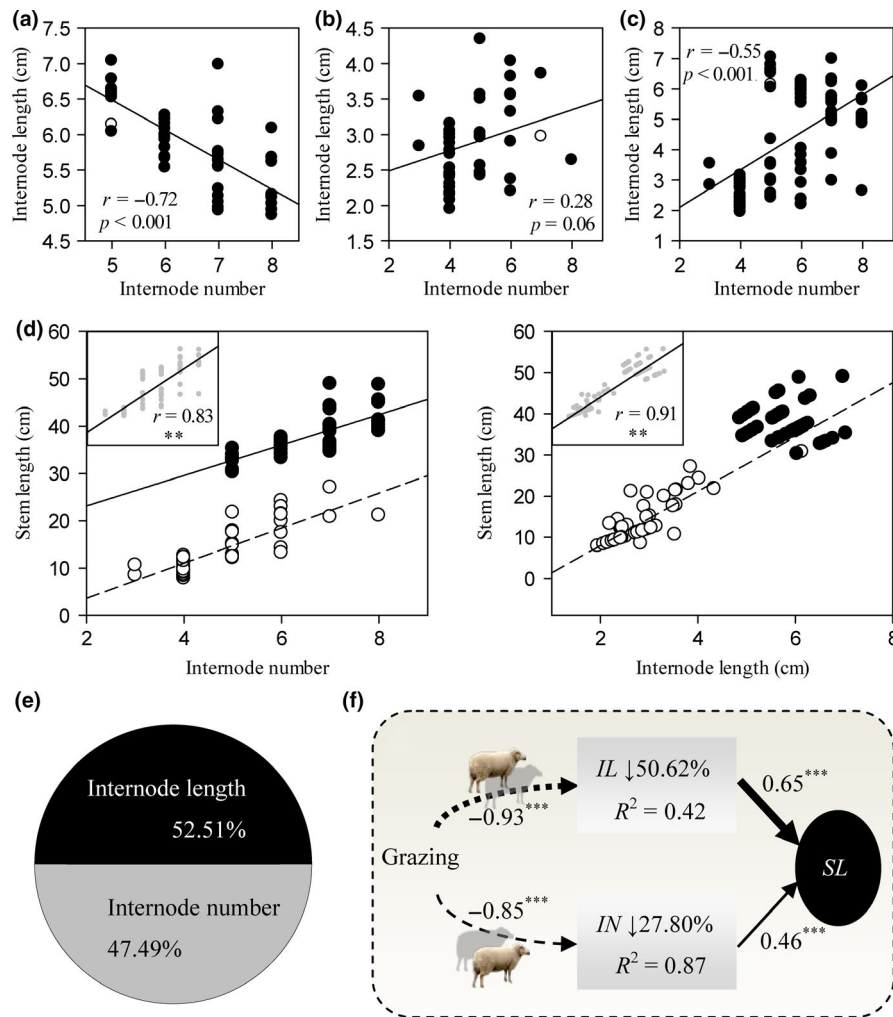


FIGURE 5 Pathways describing *Leymus chinensis* stem-length reduction in semi-arid grasslands induced by overgrazing. The relationships between internode number and mean internode length in the (a) ungrazed plots, (b) long-term heavily grazed plots, and (c) all plots. (d) The relationships of internode number and mean internode length with stem length in the analysis in ungrazed plots (●) and long-term heavily grazed plots (○). (e) The proportion of the stem length variation in *L. chinensis* individuals explained by internode number and mean internode length. (f) Final results of the structural equation modelling (SEM) analysis of the effects of overgrazing on stem length of *L. chinensis* individuals via internode number and internode length pathways. Values associated with arrows represent standardized path coefficients. Solid and dashed arrows represent positive and negative paths in a fitted SEM depicting the impact of variables on stem length reduction. The symbols “↑” and “↓” indicate a significant increase or decrease, respectively, in the response of the variables to grazing. R^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. Symbols: ***, $p < 0.001$. IL, internode length; IN, internode number; SL, stem length [Colour figure can be viewed at wileyonlinelibrary.com]

In general, IL and IN are the two factors that contribute most to the stem length of pasture grass species (Carvalho, Heuvelink, Cascais, & Van Kooten, 2002; Li et al., 2015). Interestingly, we found that IL and IN exhibited contrasting strategies in long-term grazed and ungrazed habitats. There was a significant trade-off between the two factors in the ungrazed grasslands. However, coordinated variation between them was found in the LHG grasslands. These results indicate that grazing affected the strategy of *L. chinensis* stem elongation as mediated by internode functional traits. We speculate that the coupling of IL and IN may be an important strategy in response to grazing. Moreover, our results demonstrated that grazing indirectly affected stem length by a “bottom-up” effect on IL and IN. What is the mechanism underlying

these changes in stem elongation? Previous studies that observed physiological and biochemical changes in plant stems induced by grazing provide some possible answers (Lü et al., 2014; Shen, Wang, & Tang, 2013). The physiological and molecular mechanisms underlying the grazing-induced limitation in stem elongation warrant more detailed studies.

Although ecological theories have provided different explanations for grazing-induced phenotypic plasticity (Fu et al., 2005; McKinney & Fowler, 1991; Verón, Paruelo, & Oesterheld, 2011), there is on-going controversy concerning two main hypotheses for the mechanism of plant morphological plasticity in response to defoliation. The first is the *grazing avoidance hypothesis* which suggests that smaller plants can avoid being consumed

by animals via genetic modification, especially at the epigenetic level (Damhoureyeh & Hartnett, 2002; Fu et al., 2005; McKinney & Fowler, 1991). The second is the *growth limitation hypothesis*, which rejects the genetic modification mechanism and proposes that plant morphological plasticity and trade-offs between different traits are primarily caused by the altering of the soil microenvironment through fertility, hydrology, and soil structure (Bardgett, Streeter, Cole, & Hartley, 2002; McIntire & Hik, 2002; Verón et al., 2011). Many reports have shown that grazing stress can alter the physiochemical properties (e.g. soluble sugar, antioxidant content, and leaf photosynthetic capacity) (Harrison, Evans, & Moore, 2012; Zheng et al., 2011) and gene expression of grassland plants (Flöthe, Molis, Kruse, Weinberger, & John, 2014; Lee, Roche, Donaghy, Thrush, & Sathish, 2010). These physiological, biochemical, and molecular changes can help plants develop adaptive phenotypic changes in response to grazing stress.

In conclusion, our findings provide strong evidence that the limitation of *L. chinensis* stem elongation induced by LHG is mediated by the plasticity and allometry of internode traits. We concluded that LHG significantly decreased IN of *L. chinensis* individuals in a typical steppe of Inner Mongolia. Specifically, IN and its distribution changed dramatically in response to LHG. Although LHG negatively affected IL and ID of each *L. chinensis* internode from the base to apex, and ultimately stem length, the plasticity indices of IL and ID increased initially and then decreased from the lowermost to the uppermost internode in response to LHG. These contrasting responses of internode traits were influenced by the allometry among internode traits which was determined by both LHG and internode position. Moreover, IL and IN were two key traits driving stem length reductions under LHG conditions and ultimately led to a dramatic decrease in individual plant biomass.

ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (31702161 and 31860681), Natural Science Foundation of Inner Mongolia (2017MS0317) and China Agriculture Research System (CARS-34). The long-term project site was maintained by the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Liu Z, Zhang Z, Zhang T, Gao R, Li X. Heavy grazing effects on stem elongation and internode allometry: Insights from a natural pasture grass. *Grass Forage Sci.* 2019;74:427–436. <https://doi.org/10.1111/gfs.12416>