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Patterns of *Leymus chinensis* in response to grazing exclusion across two steppe habitats in Inner Mongolia: implications for phenotypic plasticity

Respuesta de *Leymus chinensis* a la exclusión del pastoreo en dos hábitats de estepa en Inner Mongolia: implicancias en la plasticidad fenotípica

Shi G[1], ZY Liu[1], T Baoyin[1], J Sun[2], JJ Duan[3], XL Li[3], GF Yang[4]

Abstract. Plant functional traits and their effects on rangeland ecosystem function have received much attention by ecologists. However, the importance of functional traits and the interactive effects of grazing exclusion and climate are poorly understood. This study, therefore, aimed to analyse the response of *Leymus chinensis* functional traits in long-term grazing exclusion in different habitats (rainless typical steppe and rainy meadow steppe). This study showed that although the sensitivity and variability of different traits were similar in two steppe habitats, phenotypic plasticity of *L. chinensis* in meadow steppe was significantly higher than typical steppes. With the increased degree of plasticity, the variability significantly increased and could be fitted by an equation of exponential rise to maximum, and this relationship was unaltered by climate. Additionally, we illustrated that different climate conditions do not alter the patterns of phenotypic plasticity of *L. chinensis* affected by grazing exclusion.

Keywords: Functional traits; Grazing exclusion; Inner Mongolia; Rangeland; Climate.

Resumen. Las características funcionales de la planta, y sus efectos en el funcionamiento de los ecosistemas de pastizales naturales han recibido mucha atención por los ecólogos. Sin embargo, se entiende poco de la importancia de dichas características y los efectos combinados del clima y la exclusión al pastoreo. Este estudio analizó las respuestas funcionales de *Leymus chinensis* en áreas de exclusión al pastoreo durante períodos prolongados en diferentes hábitats: estepas áridas típicas y estepas de pradera lluviosas. Este estudio mostró que aunque la sensitividad y variabilidad de las diferentes características fueron similares en los dos hábitats de estepa, la plasticidad fenotípica de *L. chinensis* en la estepa de pradera fue significativamente mayor que aquella que en la estepa árida. Con el incremento en plasticidad, la variabilidad se incrementó significativamente y se podría describir por una ecuación exponencial hasta el máximo, y esta relación no fue alterada por el clima. Además, mostramos que diferentes condiciones de clima no alteran los modelos de plasticidad fenotípica de *L. chinensis* expuesto a condiciones de clausura al pastoreo.

Palabras clave: Características funcionales; Exclusión al pastoreo; Inner Mongolia; Área de vegetación silvestre; Clima.

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[1] Key Laboratory of Grassland Ecology, College of Ecology and Environment, Inner Mongolia University, Hohhot 010021, China.
[2] College of School of Animal Science and Technology, Qingdao Agricultural University, Qingdao 266109, China.
[3] Key Laboratory of Grassland Ecology and Restoration of Ministry of Agriculture, National Forage Improvement Center, Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot 010010, China.
[4] Grassland Agri-husbandry Research Center, Qingdao Agricultural University, Qingdao 266109, China.
Address correspondence to GF Yang, *e-mail:* yanggf@qau.edu.cn
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INTRODUCTION

In the last 50 years, many of the rangelands, and especially the northern rangeland of Inner Mongolia, China, are degraded, which effects not only their productivity but also their vital environmental services (Li et al., 2016). Human activity, mainly overgrazing, is the primary reason for rangeland degradation all over the world (Wen et al., 2013). In recent years, more researchers have begun to pay close attention to functional trait responses to grazing. Generally speaking, the sensitivity of the plant organ is in its above-ground portion to a greater extent than in its below-ground portion (Guo et al., 2012). In addition, the ecological strategies of functional traits, trade-offs and allometry for example, were adopted in optimising their performance at the end of their growth cycles in grazed rangelands (Acosta-Gallo et al., 2011).

In practice, grazing exclusion has been commonly adopted to restore such ecosystems all over the world (Han et al., 2008). A growing body of empirical evidence suggests that biodiversity and ecosystem function in rangeland ecosystems typically increase with prolonged grazing exclusion (Rusch & Oesterheld, 1997; Liu et al., 2011). The response of individual species to protection from grazing was productivity dependent, with plant size playing a central role (Osem et al., 2004). Whereas plant functional traits in rangeland ecosystems have received much attention, the importance of functional traits and the interactive effects of grazing exclusion and biome are poorly understood. This question is important as it relates to our understanding of the mechanisms that underlie the functional traits–ecosystem function relationship under the disturbance of grazing and climate (Reich, 2014).

Here we provided two groups of long-term (a continuous 17-year) grazing exclusion experiments across a typical steppe and a meadow steppe. We examined the effects of grazing exclusion on *Leymus chinensis*, a dominant and high palatability grass in the northern rangelands of China, and its functional traits in two different field habitats. We try to address the question of patterns of functional traits in response to grazing exclusion in the two rangeland types (i.e., typical steppe *versus* meadow steppe) under different climate conditions.

MATERIALS AND METHODS

Study site. Two long-term field rangeland research stations with different climatic conditions were selected to represent rainless typical steppe and rainy meadow steppe habitats, respectively. These field stations were located in Xilinhot city (43° 02′ N - 44° 52′ N, 115° 13′ E - 117° 06′ E) and Hailar city (49° 05′ N - 19° 27′ N, 119° 30′ E - 120° 35′ E) in Inner Mongolia, China. The two sites were extremely different in climate (e.g., temperature, precipitation) and vegetation conditions; however, one of their dominant species was *L. chinensis*. In Xilinhot, the semi-arid continental climate is characterised by a mean annual precipitation of 266.4 mm and a mean annual temperature of 2.9 °C (1980 to 2012) (Li et al., 2015). In Hailar, the mean annual air temperature is –0.53 °C. The mean annual precipitation was approximately 349.6 mm (1980 to 2012) (Yan et al., 2015).

Experimental design and sampling. Near the long-term grazing plot, grazing exclusion (un-grazed) plots were simultaneously established in 1997 by IMGERS and HGEORS for long-term ecological observation and research in the typical steppe and meadow steppe habitats, respectively. The grazing plot of IMGERS, more than 200 ha in area, was situated adjacent to the grazing exclusion plots which were grazed by more than 600 sheep and goats all year round for more than 50 years. Similarly, public pasture was over-used by sheep and goats of many herds in grazing plots of HGEORS.

Five 20 m × 20 m replicate plots were randomly established along a transect line, and the paired sampling method was used within the long-term grazed and grazing excluded treatments. Three 1 m × 1 m subplots were established in each replicate plot for field investigations and samplings. On grazing plots temporary movable exclusion cages were set-up at each sampling point before the growing season in early April, 2013. The field sampling was carried out during 15 to 20 August, 2013, corresponding to annual peak-standing biomass. Three *L. chinensis* individuals were selected randomly in every 1 m × 1 m quadrat. Phenotypic traits of *L. chinensis* were measured in a shaded laboratory. Then, all plant samples were oven-dried at 65 °C for at least 48 h and weighed. Table S1 details the 16 functional traits of *L. chinensis* included in this research.

Statistical analysis. Significant differences in the plant traits between the un-grazed and grazed plots were assessed using paired t-tests. The variability of *L. chinensis* traits was calculated using $CV = SD / M$ (CV: coefficient of variation; SD: standard deviation; M: mean value). The degrees of responses to grazing exclusion were analysed using plasticity index $PI = (FU - FG) / FG$ (FU: un-grazed habitats; FG: grazed habitats) (Couso & Fernández, 2012). A principal component analysis on correlation matrix (PC) was performed to determine relations among the 17 functional traits and the effect of grazing and steppe habitats on these traits (Fort et al., 2015). All statistical analyses were performed to determine the significance of treatment means at $P<0.05$ and $P<0.01$ using SPSS 19.0 statistical software. Statistical graphs were prepared using Sigmaplot® version 12.0.

RESULTS

All 16 functional traits were significantly positively ($P<0.05$) affected by grazing exclusion not only in meadow steppe but also in typical steppe (Table S2). Grazing exclusion significantly increased the loading score of plant size along the PC1 and PC2 axes (Fig. 1). The sensitivity trait, such as plant height, can be restored approximately 10-fold after 17-years of grazing exclusion. The sluggishness traits, such as leaf number, can be only restored 1.5-fold compared with free-grazing (Fig. S1). The sensitivity of *L. chinensis* functional traits was similar in meadow steppe and typical steppe habitats, which was indicated by the highly significant correlation of plasticity indices and the difference of functional traits between meadow steppe and typical steppe ($P<0.01$, Fig. 2). The phenotypic plasticity of *L. chinensis* in the meadow steppe was higher than that in the typical steppe when affected by grazing exclusion (Fig. 2). The differences in climate conditions did not alter the rank ordering of the variability of functional traits in response to grazing exclusion (Fig. 2). The variation of *L. chinensis* in meadow steppe was higher than that in typical steppe affected by grazing exclusion (Fig. 2). The plasticity and variability of all the 16 plant functional traits and its difference had a significant correlation in meadow steppe and typical steppe. With the increased degree of plasticity, the variability significantly increased and could be fitted by an equation of exponential rise to a maximum ($P<0.05$), and this relationship was unaltered by climate (Fig. 2).

DISCUSSION AND CONCLUSIONS

Grazing exclusion can gradually restore plant individual characteristics (Osem et al., 2004). Our results indicated that grazing exclusion significantly increased the size of *L. chinensis* individuals, not only in meadow steppe but also in typical steppe habitats. Phenotypic plasticity is a comprehensive strategy of plants to acclimate to heterogeneous habitats and is aimed at effectively gaining access to resources (Via et al., 1995; Davidson et al., 2011). These results demonstrated that plants adapted to overgrazing by phenotypic plasticity were a quick reaction mechanism of rangeland plants.

Our results demonstrated that all the *L. chinensis* functional traits had allometry scaling in response to grazing. Some traits, such as stalk biomass, aboveground biomass, and plant height, were sensitivity traits. Yet other traits, such as leaf number, leaf mass per area, leaf biomass, leaf width, and stalk diameter, were sluggishness traits in response to the two disturbances. Hence, the functional traits had an asymmetric response mechanism to abiotic disturbances. Two forms of traits-based plant strategies were reported by previous studies; positive correlation and allometry of different traits (Wesuls et al., 2012), and negative trade-off of different traits (Zheng et al., 2011). Our results demonstrated with the increase of

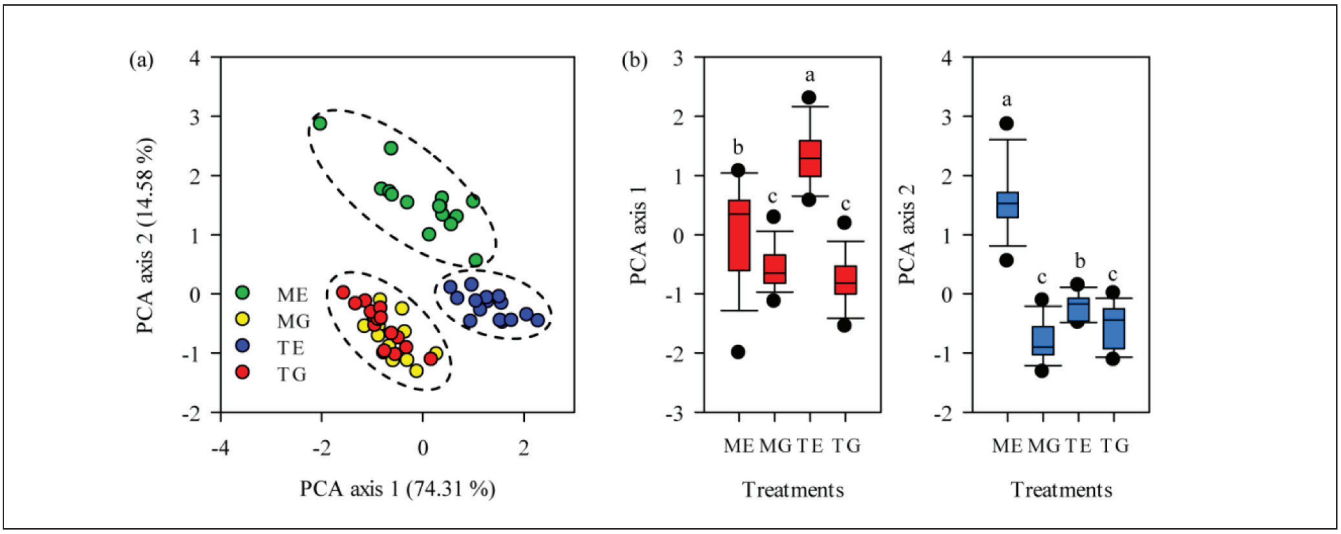

Fig. 1 PCA bi-plot of *Leymus chinensis* functional traits in grazing exclusion and grazing treatments across two steppe habitats based on the variance in 16 functional traits explained by the first (PCA axis 1) and second (PCA axis 2) principal axes. (a) PCA ordination of *L. chinensis* plants from the grazing exclusion and grazing habitats in meadow steppe and typical steppe habitats, respectively. (b) Box plots illustrate the score distribution of *L. chinensis* functional traits from four communities along the two principal axes. Significant differences (at 0.05 level) among the four communities along PCA axis 1, and PCA axis 2 are indicated by different letters. Abbreviations: ME, grazing exclusion in meadow steppe; MG, grazing in meadow steppe; TE, grazing exclusion in typical steppe; TG, grazing in typical steppe.
Fig. 1. Gráfico de Análisis de Componentes Principales (PCA) de las características funcionales de *Leymus chinensis* en los tratamientos de pastoreo y exclusión al pastoreo a través de dos hábitats de estepa basados en la varianza en 16 características funcionales explicadas por el primer (PCA eje 1) y segundo (PCA eje 2) ejes principales. (a) Ordenamiento de PCA de plantas de *L. chinensis* para los hábitats de pastoreo y exclusión al pastoreo en la estepa de pradera y la estepa árida, respectivamente. (b) Gráficos de caja que ilustran la distribución del puntaje de las características funcionales de *L. chinensis* de cuatro comunidades a lo largo de 2 ejes principales. Las diferencias significativas (a un nivel del 5%) entre las 4 comunidades a lo largo del eje 1 del PCA, y del eje 2 del PCA son indicadas por letras diferentes. Abreviaturas: ME, exclusión al pastoreo en la estepa de pradera; MG, pastoreo en la estepa de pradera; TE, exclusión al pastoreo en la estepa árida; TG, pastoreo en la estepa árida.

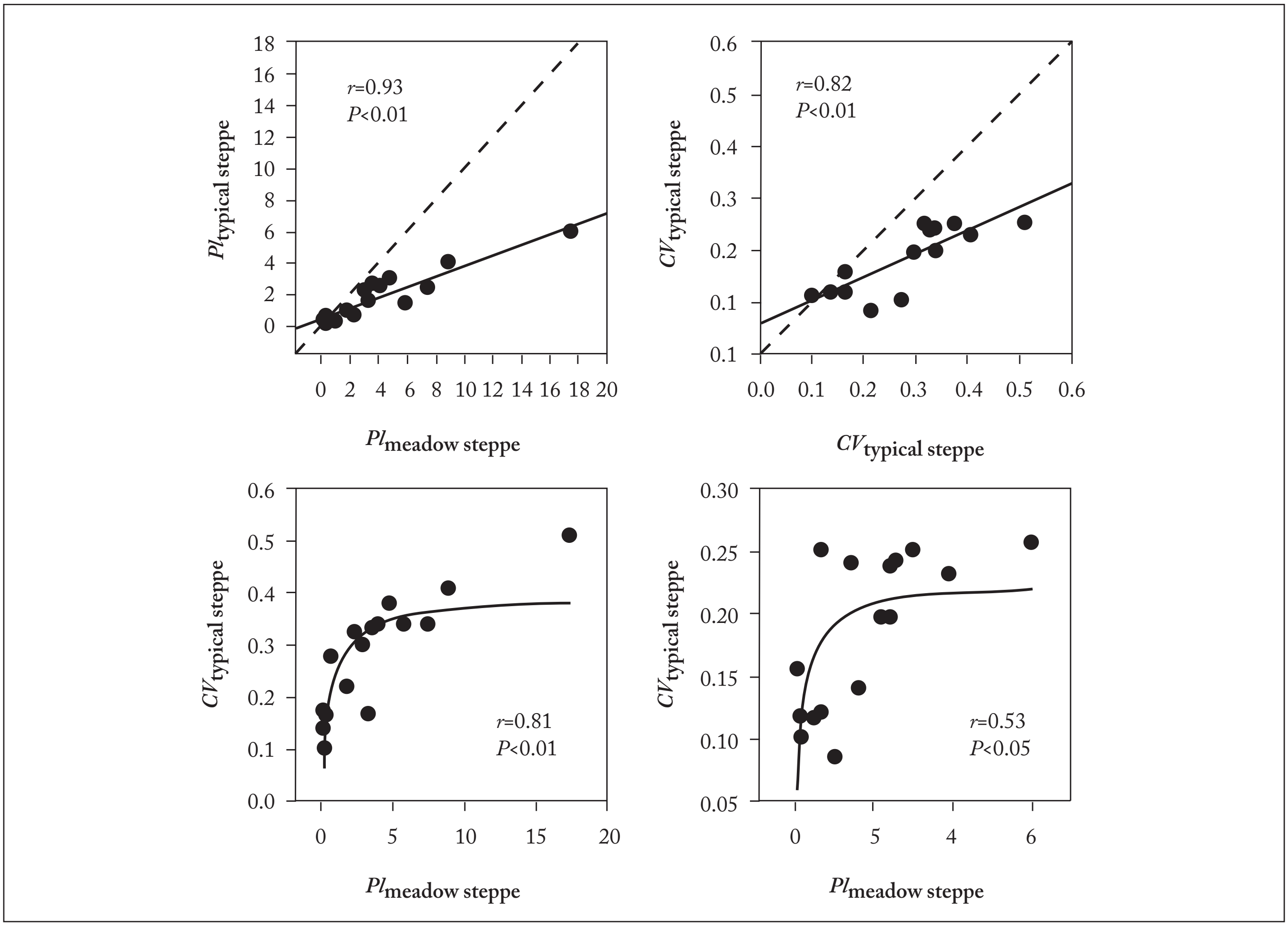

Fig. 2. Comparison of plasticity indices *(PI)* and variability *(CV)* of 16 functional traits of Leymus chinensis between meadow steppe and typical steppe habitats. Dashed lines in the figures represent 1:1 line. Solid lines represent regression curves, which were fit by linear equation and exponential rise to maximum equation.

Fig. 2. Comparación de los índices de plasticidad *(PI)* y variabilidad *(CV)* de 16 características funcionales de Leymus chinensis entre hábitats de una estepa de pradera y una estepa árida. Las líneas cortadas en la figura representan la línea 1:1. Las líneas sólidas representan curvas de regresión, que fueron ajustadas por una ecuación lineal y ascenso exponencial hasta la ecuación máxima.

plant height, there was a significant corresponding increase in the ratios of stalk to leaf biomass. This result suggests that *L. chinensis* individual will invest more of its photosynthetic products in stalks than leaves during the process of a plant returning to a normal plant size after grazing exclusion.

Climate (as a proxy for rainfall, temperature, and etc.), is important for rangelands at the scales of plant traits, individuals, populations, and ecosystems (Díaz & Cabido, 1997; Frenette-Dussault et al., 2013). Plant growth rate of many species is strongly reliant on climate resources in various types of ecosystems (Lavergne et al., 2010). We found that the recovery rate was faster in rainy meadow steppes than in rainless typical steppes. Although the sensitivity and variability of different traits were similar in the two steppe habitats, phenotypic plasticity of *L. chinensis* was significantly higher in a meadow than in a typical steppe habitat. Also, the relationship among the plasticity and variability of plant functional traits was not altered by climate conditions. Therefore, neutral effects of climate on the patterns of phenotypic plasticity and biomass allocation of *L. chinensis* in response to grazing exclusion prevailed in the restoration process of grazed rangelands.

In conclusion, our study illustrated that most of the phenotypic traits of *L. chinensis* were positively affected by grazing exclusion. Although the sensitivity and variability of different traits were similar in the two steppe habitats, phenotypic plasticity of *L. chinensis* in the meadow steppe habitat was significantly higher than that in the typical steppe habitat. The variation of allocation strategy was induced by the allometry of leaf and stalk biomasses across grazing treatments and steppe habitats. We illustrated that differential climate conditions do not alter the patterns of phenotypic plasticity and biomass allocation of *L. chinensis* affected by grazing exclusion.

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APPENDIX

Table S1. The 16 *Leymus chinensis* functional traits included in this research.
Tabla S1. Se listan las 16 características funcionales de determinadas en *Leymus chinensis* en esta investigación.

Functional traits classification	Functional Trait	Abbreviation	Unit
Leaf traits	Leaf number	*LN*	\
	Leaf length	*LL*	cm
	Leaf width	*LW*	mm
	Leaf length / width ratio	*LLW*	cm/mm
	Total leaf area	*TLA*	cm^2
	Averaged leaf area	*ALA*	cm^2
	Total leaf biomass	*TLB*	g
	Averaged leaf biomass	*ALB*	g
	Leaf Mass per Area	*LMA*	g/cm^2
Stalk traits	Stalk length	*SL*	cm
	Stalk diameter	*SD*	mm
	Stalk length / diameter ratio	*SLD*	cm/ mm
	Stalk biomass	*SBM*	g
Whole plant traits	Plant height	*PH*	cm
	Aboveground biomass	*ABM*	g
	Stalk / leaf biomass ratio	*SLB*	\

Table S2. F-ratios and statistical significance of ANOVAs for functional traits of *Leymus chinensis* affected by grazing exclusion in meadow steppe and typical steppe, respectively.
Tabla S2. Valores de F y significancia estadística (valor de P) de ANOVAs para características funcionales de *L. chinensis* afectadas por la exclusión (o no) al pastoreo en la estepa de pradera y en la estepa árida, respectivamente.

Functional traits	Meadow steppe				Typical steppe			
	Ungrazed	Grazed	F	P-value	Ungrazed	Grazed	F	P-value
Leaf number	4.80	4.27	6.40	0.02	5.53	4.80	11.14	0.00
Leaf length	27.06	9.64	354.10	0.00	26.35	13.37	523.62	0.00
Leaf width	6.80	4.61	58.85	0.00	8.90	5.41	259.83	0.00
Leaf length / width ratio	4.05	2.23	90.69	0.00	2.99	2.50	32.18	0.00
Total leaf area	0.43	0.07	364.90	0.00	0.85	0.21	187.81	0.00
Averaged leaf area	0.11	0.02	396.50	0.00	0.15	0.04	372.08	0.00
Total leaf biomass	57.03	12.70	217.77	0.00	81.68	22.56	228.45	0.00
Averaged leaf biomass	11.96	2.97	227.59	0.00	14.77	4.67	352.76	0.00
Leaf Mass per Area	0.01	0.007	16.72	0.00	0.012	0.013	1.50	0.23
Stalk length	45.76	5.41	622.74	0.00	37.69	10.92	455.59	0.00
Stalk diameter	1.98	1.58	23.17	0.00	2.02	1.46	88.12	0.00
Stalk length / diameter ratio	23.58	3.45	282.41	0.00	18.67	7.61	252.32	0.00
Stalk biomass	0.64	0.03	249.08	0.00	0.65	0.09	206.20	0.00
Plant height	73.75	17.09	1075.74	0.00	63.11	23.89	769.14	0.00
Aboveground biomass	1.07	0.11	349.42	0.00	1.50	0.31	221.56	0.00
Stalk / leaf biomass ratio	1.51	0.45	189.52	0.00	0.76	0.45	56.10	0.00

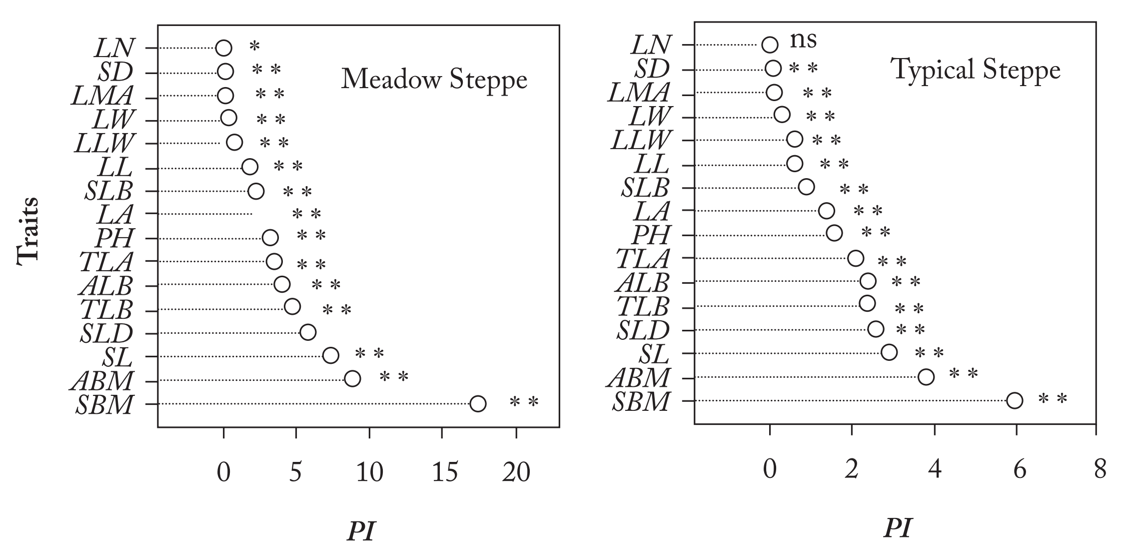

Fig. S1. Plasticity indices of *Leymus chinensis* in response to grazing exclusion in meadow steppe and typical steppe. LN, Leaf number; LL, Leaf length; LW, Leaf width; LLW, Leaf length/width ratio; TLA, Total leaf area; ALA, Averaged leaf area; TLB, Total leaf biomass; ALB, Averaged leaf biomass; LMA, Leaf Mass per Area; SL, Stalk length; SD, Stalk diameter; SLD, Stalk length/diameter ratio; SBM, Stalk biomass; PH, Plant height; ABM, Aboveground biomass; SLB, Stalk/leaf biomass ratio . **, P<0.01; *, P<0.05; ns, P>0.05.

Fig. S1. Indices de plasticidad de *Leymus chinensis* en respuesta a la exclusión al pastoreo en las estepas de pradera y árida. LN, número de hojas; LL, longitud de hoja; LW, ancho de hoja; LLW, relación longitud de hoja/ancho de hoja; TLA, área foliar total; ALA, área foliar promedio; TLB, biomasa foliar total; ALB, biomasa foliar promedio; LMA, peso foliar por área; SL, longitud de tallo; SD, diámetro del tallo; SLD, relación longitud/diámetro de tallo; SBM, biomasa del tallo; PH, altura de la planta; ABM, biomasa aérea; SLB, relación de biomasa tallo/hoja. **, P<0,01; *, P<0,05; ns, P>0,05.