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Full Length Research Paper

Responses of plant morphology and seed quality to long-term overgrazing in *Leymus chinensis*

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***Leymus chinensis* (Trin.) Tzvel. is a perennial species of Gramineae. It is also a palatable forage; but under severe and prolonged grazing, the individuals of *L. chinensis* become miniaturized and do not immediately revert to normal when grazing stress is removed. To explore the miniaturization mechanism, we compared the difference in the 3-year period 2002 to 2004 between miniaturized *L. chinensi* and normal *L. chinensis* on morphological character and asexual reproduction. The result shows that 1000-seed weight, germination percentage, plant height, fiber root length, height of first ramet, individual biomass and seed weight of single spike were significantly lower ($p < 0.01$) in miniaturized plants, but the tiller number was higher ($p < 0.05$) than the normal ones. Overgrazing was the main factor causing individual miniaturization of *L. chinensis.* We propose that individual miniaturization was a protection strategy to adapt all kind of stress and present a negative feedback mechanism. This study maybe also an important step towards understanding the succession sequences in the restoration of typical steppe and delivering the baseline reference for a sound steppe management plan.**

Key words: *Leymus chinensis*, grazing succession, individual miniaturization, tiller number.

INTRODUCTION

Leymus chinensis is a perennial species of the Graminae family, which is widely distributed in Northern China and the Mongolia. It develops strong rhizomes and adapts to saline, alkaline and dune conditions (Zhou et al., 2002). Due to its high productivity and high protein content, this species serves as major forage grass in Northern China and the Mongolian Plateau (Wang et al., 2004; Ripley et al., 1996). *L. chinensis* can be a candidate grass for the esta-blishment or renewal of artificial grassland (Bai et al., 2009). Most studies of *L. chinensis* have been focused on genetic resource, biodiversity, physiology and reproducibility (Liu et al., 2004; Zhao et al., 2008; Wang et al., 2008). Up to now, the effects of grazing have been well documented for many types of grassland (Austin et al., 1981; Sala et al., 1986; Shang et al., 2003). With increased grazing intensity, foliage cover and above-ground and belowground biomass decreased the structure of plant community changes from complex to simple. Soil volume weight, pH values, soil water content, soil organic matter and the content of nitrogen, phosphorous, potassium and calcium in soil surface (0 to 10 cm) also decrease. Grazing by livestock reduced both asexual and sexual reproductive channels. With the increase of grazing intensity, the densities of asexual shoots and rhizome tillers, seed biomass, number of seeds per inflorescence and sexual shoot ratio decreased significantly, especially after heavy grazing (Wang and Ripley, 2000). Overgrazing also can result in morphological variations of *L. chinensis* (Zhao et al., 2009). However, the characters of morphological variations and mechanism of morphological responses remains unclear. This study was designed to investigate morphological variations of *L. chinensis* under over-grazing. We attempted to explain degradation succession mechanism and provided some bases for restoration of grassland.

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MATERIALS AND METHODS

Region and site description

The experiment was carried out in Guyuan County, HeBei province.

Table 1. The morphological traits of normal *L. chinense* and miniaturized *L. chinense.*

Item / Species	Plant height	Ear length	Fibre length	Rhizome length	Inter-node rhizome length	Height of first ramet
Normal *L. chinense*	58.9**	5.14	8.52**	9.33*	1.52	32.11**
Miniaturized *L. chinense*	16.83**	4.23	4.61**	6.09*	1.424	4.42**

*0.01 < P < 0.05; **0.001 < P < 0.01.

The sites are located between latitudes 41°46′and 42°36′N and longitudes 115°51′and 116°54′E, situated across the southern edge of Hunshandake Sandland. The topography is dominated by low foothills at an elevation of 1536 m. The main soil type is chestnut soil, accounting for 70% of the total area and other soil types are Aeolian sandy soil, meadow soil and chernozem. This area has a continental monsoon climate, semiarid and typical of a middle temperate zone, with an annual mean air temperature of 1.3°C and a frost-free period of about 106 days. The accumulated temperature of ≥10 is 1960°C and the mean temperature of the warmest month (July) is 17.8°C, while that of the coldest month (January) is -18.3°C. Annual mean precipitation is 385.5 mm, while mean potential evaporation is 1748.0 mm. Euroasian typical steppe is the basic grassland type in this region, which is dominated by *L. chinensis, Stipa* spp., *Agropyron cristatum, Artemisia frigida* and *Cleistogenes squarrosa*, accompanied by secondary forests, thickets and sandy vegetation communities.

Two field sites in similar ecological condition were selected for this research. The first was a long-term experimental site, which has been fenced since 1992 and under grazing ban for 11 years in 2002. The site was dominated by *L. chinensis* and *S. grandis.* The second sampling site (for miniaturized *L. chinensis*) was near with enclosed grassland and has been grazed by livestock for a long-time and the plant community was severely degraded. Vegetation cover was only 31.2% and the dominant species were *A. frigida*, *C. squarrosa*.

Methods

From 2002 to 2004, we have investigated the two sampling sites at mature period of *L. chinensis*. Miniaturized *L. chinensis* plants were sampled randomly using soil cuboid (0.25 × 0.25 × 0.25 m), which was kept above-ground and below-ground parts intact, with 25 to 30 replicates at experimental plots. The tiller height, length of ear head, number of tillers and the length of fibrous roots and length of rhizomes of *L. chinensis* were recorded separately after washing with water (using a flotation technique). Above ground and below ground biomass were measured by electronic balance. Seed collection from both treatments was carried out on 6 Sept every year, at that time seed weight of single spike were measured and recorded.

Germination tests of *L. chinensis* were conducted on 2 November 2003. Each sample of 100 seed was replicated 4 times. All seeds were treated by 0.2% potassium nitrate for 7 days before putting into incubators (Sanyo Versatile Environmental Chamber MLR-350H, Sanyo Scientific, USA); temperature of incubator was designated to 30°C from 9:00 to 18:00 and 20°C from 18:00 to 9:00.

Data analysis

In this study, all data were listed in the spread sheet, the mean height, ear length, fibre length, rhizome length, inter-node rhizome length, height of first ramet, biomass and seed weight of single spike, 1000-seeds weight, germination percentage of *L. chinensis* at each site were statistically analyzed with SAS 8.2. Differences between the two *L. chinensis* were tested using ANOVA.

RESULTS

Morphological character

It is obvious that the plant height is the main character of miniaturized *L. chinensis*, there were significant difference between normal and miniaturized *L. chinensis* and the height of miniaturized *L. chinensis* was only 16.83 cm (Table 1). Although, it was not statistically significant, ear length in miniaturized *L. chinensis* was shorter (0.91 cm) compared with normalized plants. There were significant difference in fibre length and rhizome length of the two *L. chinensis*, but inter-node rhizome length was approximately the same. So, roots of miniaturized *L. chinensis* were mainly distributed in the top layers of the soil.

The result shows significantly different between normal *L.chinensis* and miniaturized *L.chinensis* on tiller number (P < 0.05) and average tiller number were 2.78 and 4.22, respectively (Figure 1). The result was opposite to Yang's (1989) and her result showed that tiller number of *L. chinensis* would decline obviously by overgrazing.

Individual biomass and seed weight of single spike

Many characters of individual plant came to characterize individual miniaturization, but the decrease of individual biomass was the most important index to represent individual miniaturization. The above ground individual biomass of miniaturized *L. chinensis* was 0.186 g and was significantly lower than normal *L. chinensis* (Figure 2). There are also significant differences between miniaturized *L. chinensis* and normal *L. chinensis* on underground biomass (P < 0.01), 0.096 and 0.22 g, respectively. Seed weight of single spike was an important index to evaluate the ability of asexual reproduction and had a significant positive correlation with number of floret per spike and seed-setting percentage (Zewdie et al., 2007). Seed weight of single spike of miniaturized *L. chinensis* was 0.12 g and was significantly lower than normalized *L. chinensis* (0.59 g).

Different sexual reproduction

The 1000-seeds weight of miniaturized *L. chinensis*

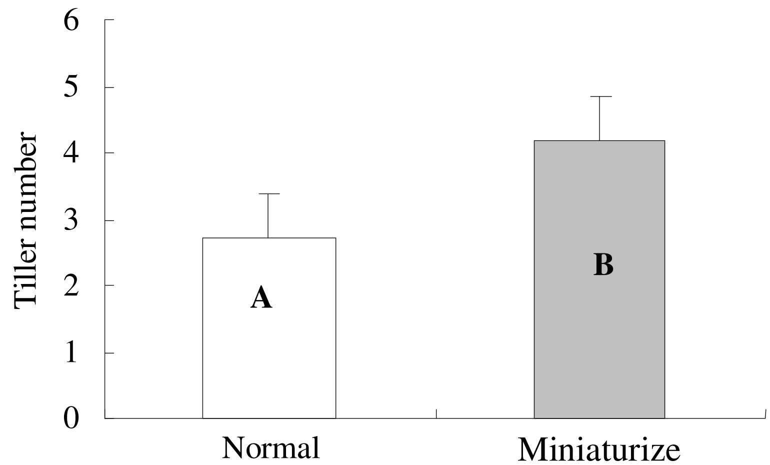

Figure 1. Tiller number of normal (N) and miniaturized (M) *L. chinensis.*

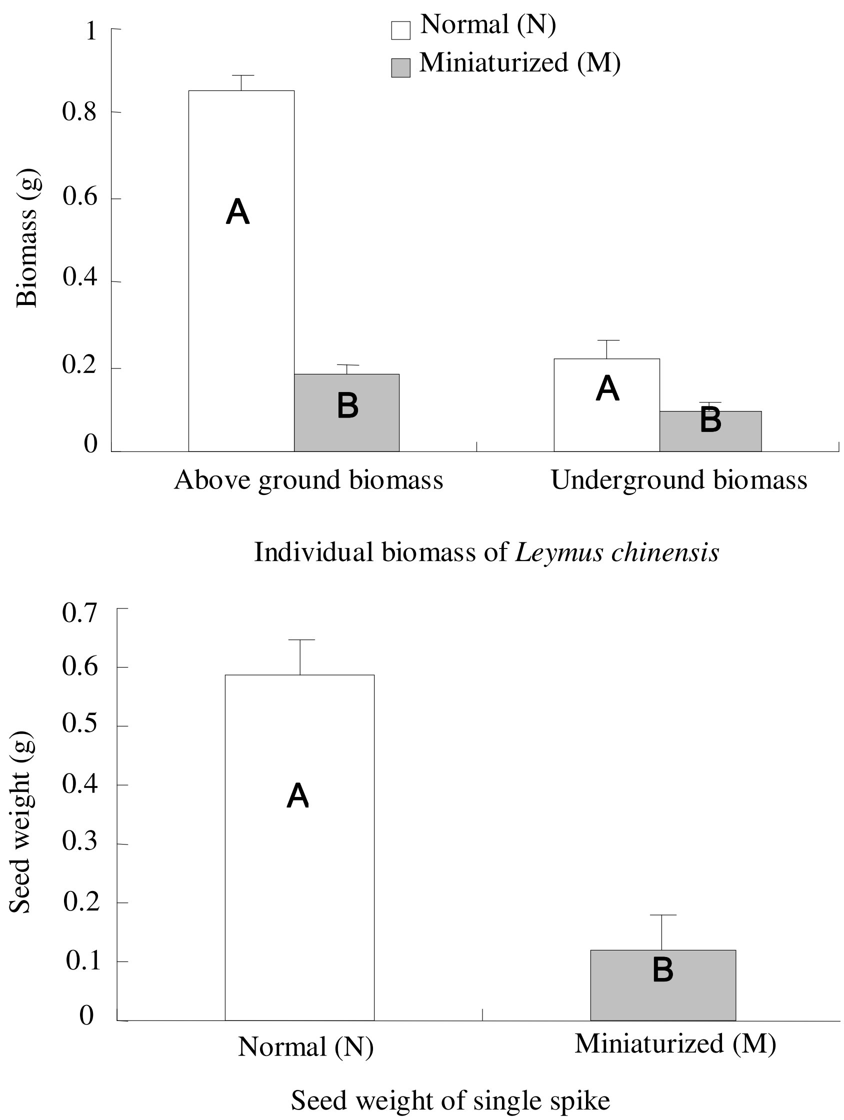

Figure 2. Above ground biomass, underground biomass and seed weight of single spike for normal (N) and miniaturized (M) *L. chinensis.*

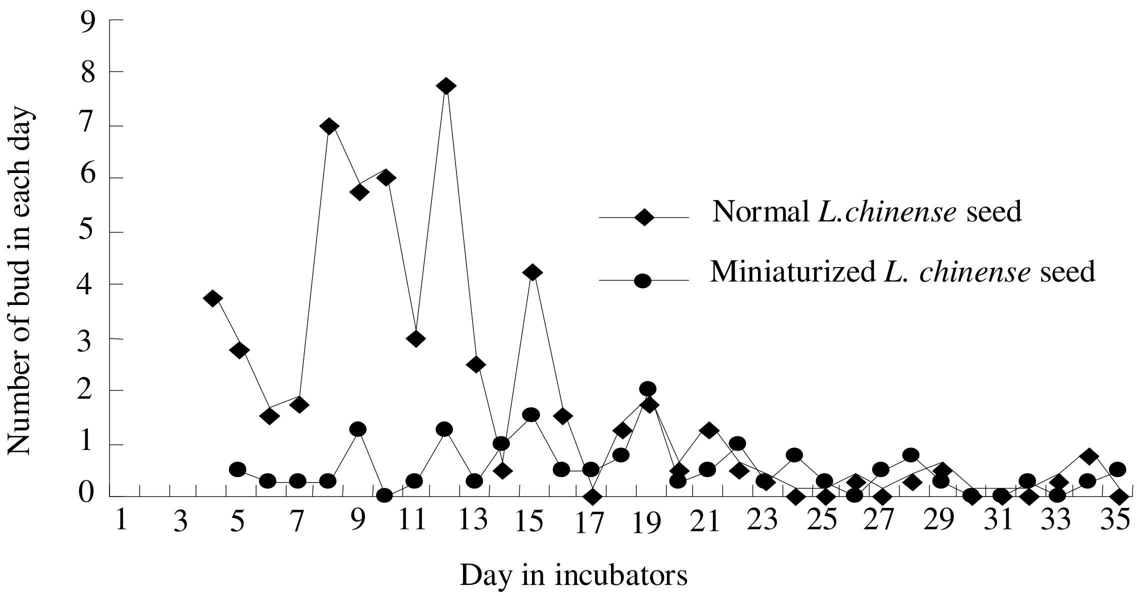

Figure 3. Number germinating on each day of normal and miniaturized *L. chinensis*.

(1.269 g) was significantly lower than from normal *L. chinensis* (Figure 3). As an important index for seed quality, 1000-seeds weight had significant influence on germination percentage, setting percentage and seedling percentage. Therefore, for germination percentage, there were significant differences between normal and miniaturized *L. chinensis*, respectively (55.5 and 16%).

Miniaturized seeds began to germinate at four days, one day later than normal seeds (Figure 4). Germination of normal seed peaked from 14 to 19 November and germination energy reach 12.75% on 15 November. During germination, miniaturized seed showed low germination energy and the top germination energy was only 2%. At same time, we observed that many (about 42.3%) miniaturized seed succumbed to mildew.

DISCUSSION

Overgrazing and rising temperatures are believed to be factors that led to environmental degradation in grassland (Diaz et al., 2007). As a direct factor, grazing had significant effects on the physical and chemical soil characteristics (including nutrient depletion) of the grassland. Poor nutrition leads to *L. chinensis* displaying chlorosis and weak growth. The 1000-seed weight is an important index and closely correlation with germination energy and germination percentage (Zewdie et al., 2007) and miniaturized *L. chinensis* had significantly smaller seed size. Some factors directly influenced the quality of seed, such as light, climate, water and soil fertility, however, the rate of photosynthesis itself is a main factor (Zewdie et al., 2007). Narrowed leaf remarkably reduced photosynthesis of miniaturized *L. chinensis* and less organic matter was gained by plant, carbohydrate reserves were low and seed energy was diminished. Miniaturized *L. chinensis* did continue the reproduction cycle with some seed output. This showed an adaptive coping mechanism (Wang and Ripley, 2000). Miniaturized *L. chinensis* was following an "R" strategy by reproducing in the early stages of degenerated grassland restoration. The shape of *L. chinensis* seed was thin and the color was brown-black, energy of seed was poor and germination percentage decreased to 15.75%. Seed was easy to be invaded by microorganisms, 53.3% seeds were affected with mildew.

Comparing with normal *L. chinensis*, the aboveground and underground biomass of individual miniaturized *L. chinensis* was significantly reduced. We considered that individual miniaturization was a coping mechanism resulting in decreased community productivity during the degradation succession, under prolonged and severe overgrazing. The worsening ecological environment resulted in the decline of seed weight of a single spike and was reflected in the reduced ability for sexual reproduction and ultimately low recruitment to the population.

Plant height not only reflected the form of grassland community, but also was a quantitative index to show size of individual plants (Hao el al., 1997). The height of miniaturized *L. chinensis* decreased by 71.4% when compared with normal *L. chinensis.* Excessive utilization (90% or more) by livestock was the main reason for *L. chinensis* miniaturization (Wang el al., 1999). It is a negative feedback mechanism and a defense strategy that *L. chinensis* has evolved. As availability of biomass declined grazing animals would ingest less biomass from a smaller individual plant each time. So this plant would have more chance to survive but the chance of encounter

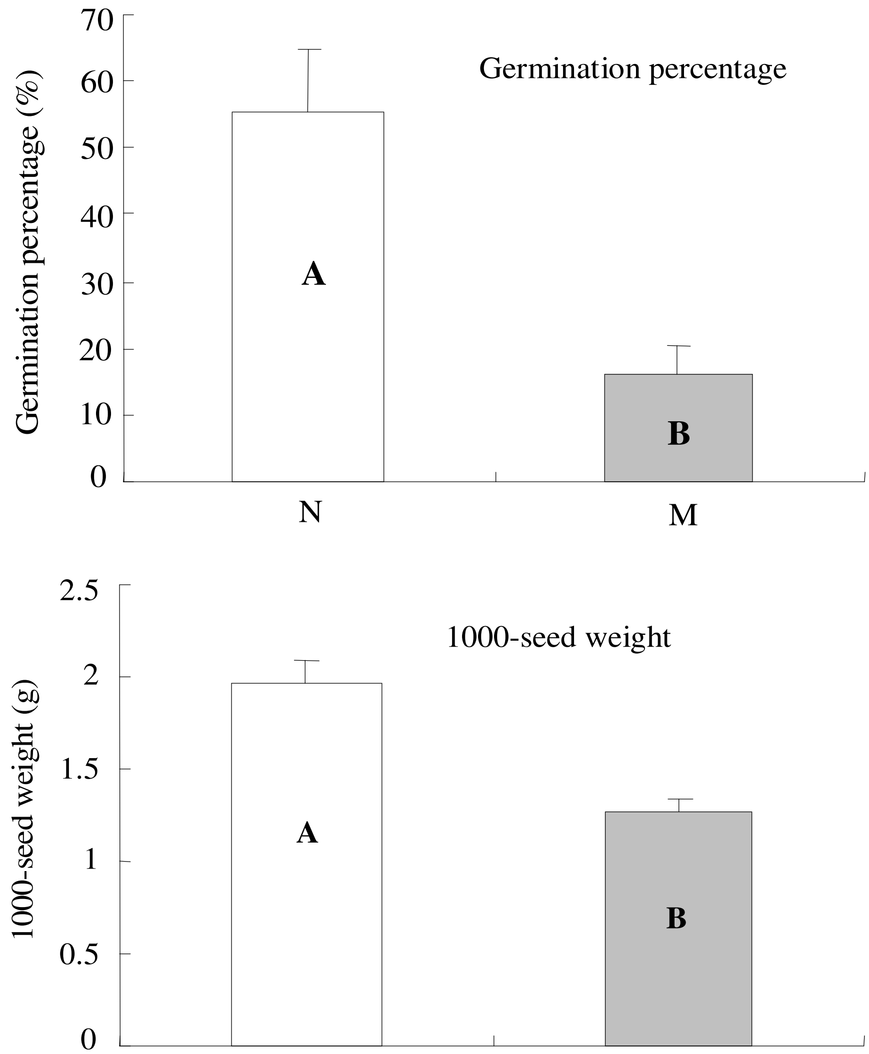

Figure 4. Germination percentage and 1000-seeds weight of normal (N) and miniaturized (M) *L. chinensis.*

with a grazing animal would be greater as duration and intensity of grazing increased.

Roots of miniaturized *L. chinensis* were restricted to the topsoil (0 to 6 cm) and hence, their opportunity to scavenge water and nutrients was greatly diminished. Root pruning in heavily grazed grasses is a major factor contributing to land degradation (Gao et al., 2008) and the phenomenon of "man-made drought" (Yuan et al., 2006). Some studies indicated that the decline of photosynthesis and the decrease of organic matter and energy resulted in roots weakly developing (Mapfumo el al., 2002). But animal trampling was an important factor. Animal trampling, especially after rain, compact the topsoil, increases soil bulk density and reduces soil porosity and water infiltration (Cui et al., 2005). There are secondary impacts, changes in microclimate and community composition and effects on soil processes, including nutrient dynamics (Xu and Zhou, 2005; Xu et al., 2004; Ritchie et al., 1998), such as changes in physical soil characteristics of the grassland significantly affected the growth of roots of *L. chinensis*.

Some studies indicated that moderate grazing could stimulate *L. chinensis* to sprout bud and shoot, but overgrazing would restrict tillers (Wang el al., 2004). In rangeland degradation, tiller number of *L. chinensis* would significantly decreased (Shi and Guo, 2006). Nevertheless, our study shows that tiller number of miniaturized *L. chinensis* increased significantly ($p < 0.05$) when compared with normalized *L. chinensis* and enhanced by 35.7%. We supposed this due to the biological character of *L. chinensis*. Tiller production is the principal reproductive method of *L. chinensis* (Wang et al., 2004).

We assumed that increasing grazing intensity stimulated *L. chinensis* to adapt and defend all kind of stress and individual miniaturization was an effective strategy.

Up to now, we only studied morphological characters; furthermore, we expect to make clear that if the miniaturization of *L. chinensis* had some changes in gene. Understanding the mechanism of the miniaturization of *L. chinensis*, will help reveal the complexity and profoundity of community succession in Eurasian typical steppe.

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