

Article

Effects of Rust on Plant Growth and Stoichiometry of *Leymus chinensis* under Different Grazing Intensities in Hulunber Grassland

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Abstract: Grazing is the main utilization of native grassland, and forage fungal disease is one of the limiting factors of grassland productivity. The present research in the Hulunber meadow steppe grassland was conducted to investigate the responses of the dominant plant *Leymus chinensis* (Trin.) to beef cattle grazing, rust, and their interaction influence. Six grazing intensity treatments with three replicates were established. The response of *L. chinensis* to grazing and rust was systematically studied for two consecutive years. The main findings were that grazing and rust had significant effects ($p < 0.05$) on the growth and nutrient elements content of *L. chinensis*. Compared with the 0 cattle ha⁻¹ treatment, the dry matter of *L. chinensis* in the 0.42, 0.63, and 1.67 cattle ha⁻¹ treatments decreased by 42.2%, 90.5%, and 339.5%, respectively. Compared with non-infected plants, dry matter of rust-infected *L. chinensis* plants decreased by 45.6%. The N:C and P:C ratios of rust-infected plants were lower than in non-infected plants, and positively correlated with their relative growth rates. Therefore, we concluded that the growth rate hypothesis still applied in *L. chinensis* under the interactive effects of grazing and disease. Additionally, grazing can alleviate the loss of dry matter caused by disease.

Keywords: dry matter; ecological stoichiometry; grazing intensity; rust; *Leymus chinensis*; Hulunber meadow steppe



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1. Introduction

Grasslands, about 390 million ha, cover 41% of the total land area in China [1]. The Hulunber meadow steppe, located in northeastern China, is one of the largest areas of natural temperate sub-humid meadow steppe grassland in the world, covering an area about 9.97×10^6 km² [2]. It plays a critical role in regional ecological balance and is also an important ecological barrier in north China [3,4]. The most dominant plant species of this grassland is *Leymus chinensis* (Trin.) [5]. *L. chinensis* is a perennial rhizomatous grass with rich nutritional content, high yield, and palatability, and lays the foundation for highly productive livestock husbandry in the Hulunber grassland [4,6].

Large herbivores are key drivers of plant communities and ecosystem functioning in many terrestrial ecosystems as they influence nutrient cycling and availability in soil and plants [7–11]. Livestock grazing is the most prevalent use of grasslands. Grazing herbivores change the vegetation cover and cause soil compaction as a result of ingestion and trampling [4,12–14]. Generally, the response of grassland ecosystems to livestock

disturbance is mediated by various factors, such as local environmental conditions, livestock species, grazing intensity, and plant diseases [9,15,16].

Fungal diseases are one of the main limiting factors affecting grassland productivity, leading to degradation, and can strongly contribute to the rate and magnitude of ecosystem function in grassland [17–19]. In addition, disease also reduces the palatability of grass for livestock [20]. Infection of grasses by some fungi can result in the accumulation of fungal toxins and secondary metabolites that are harmful to grazing animals and even humans [21–23], further restricting the productivity of animal husbandry [21]. It is worth noting that there have been some clues that conservative grazing may be one of the effective ways to control fungal disease in this grassland [24]. However, the influence of grazing on grassland diseases is not a consistent factor with plant diseases. Grazing may reduce the incidence by removing the infected tissues and pathogens on the foliage through ingestion [22], or, in contrast, promote pathogen invasion by forming wounds on plants through ingestion or trampling [25–28]. The transmission and infection of pathogens is also significantly affected by plant species diversity, plant abundance, aboveground biomass, and the functional composition of grassland ecosystems [29–33]. All the above-mentioned factors are directly or indirectly affected by grazing. Therefore, grazing should be regarded as one of the potential available methods to limit the damage caused by plant pathogens in grasslands and forage-based grazing systems.

Ecological stoichiometry describes the balance of energy and nutrients between organisms and their environment and can improve our understanding of impacts of energy and nutrient fluxes, and also balances between plants, microbes, soil, and individual organism growth [34–37]. There are two different mechanisms to explain the effect of grazing on grassland nutrient cycling [9,34,38]. One is that grazing may stimulate soil microbial activity and accelerate nutrient cycling rates by introducing fresh leaves, plant litter, and livestock waste into the soil [39,40]. The other viewpoint suggests that grazing may reduce nutrient cycling rates by increasing plant species with low palatability within the population [9,38,41,42]. Ecological stoichiometry focuses on the chemical elements necessary for all life and is easily generalizable across systems and taxa, which is extremely useful in research on multi-trophic interactions such as infectious diseases on plants [43–45]. The classical paradigm, the growth rate hypothesis, based on the relationships between the N:P in organisms and their growth rate capacity and scaling ecological consequences, has been proved in many systems [35,43]. It allows us to make predictions about the roles of specific elements in diseases, and about the linkage between infectious diseases and nutrient cycles, by uniting them with a common, multimetric currency [46].

Both grazing and disease have significant effects on the productivity of plants [2,47]. However, the mechanism of ecological stoichiometry in response to grassland diseases is rarely reported. How the interaction between grazing and disease affects the stoichiometry of the individual plant is still uncertain. Therefore, a two-year study was carried out in the Hulunber grasslands trial site. *L. chinensis*, the dominant plant species in the local area, was utilized as the experimental species. Through analyzing the growth indexes, disease incidence, and elements content in individual plants, we aimed to explore the ecological stoichiometry response strategies of *L. chinensis* to grazing and disease, as well as their interaction effects. Hopefully, our results will enhance understanding about how to use grazing as one of the methods to control diseases in natural grasslands.

2. Materials and Methods

2.1. Study Site

This study site is located at the Hulunber Grassland Ecosystem Station of the Chinese Academy of Agricultural Sciences, which is in the northeast Inner Mongolia grassland, a central part of the Hulunber meadow steppe, China (N 49°19'21"~49°20'10", E 119°56'31"~119°57'51"; elevation varies from 666 m~680 m) [4]. The climate of this location is temperate semiarid continental, with an annual average of 110 frost-free days. The mean annual temperature range is −5 °C~−2 °C and with an annual precipitation

range of 350 mm~400 mm, with more than 80% falling from July to August [5]. The precipitation was about 280 mm~320 mm during the growing season in 2015 and 2016. The type of the soil is kastanozems according to FAO/UNESCO System of Soil Classification. The vegetation is characterized as typical meadow steppe that is dominated by *L. chinensis*, *Stipa baicalensis* (Roshev.), and *Carex duriuscula* (C.A. Mey), other species are *Galium verum* (L.), *Bupleurum scorzonerifolium* (Willd.) and *Filifolium sibiricum* (L.). The grassland usually regrows in early May and starts to wither in late September. It is suitable for cattle grazing only from June to October, limited by the short growing season.

2.2. Experimental Design

The grassland area was divided into 18 paddocks, each of 5 ha (300 m × 167 m). Six levels of cattle grazing intensity were set as follows: 0.00, 0.42, 0.63, 0.83, 1.25, and 1.67 cattle ha⁻¹, corresponding to 0, 2, 3, 4, 6, and 8 adult cattle with 500 kg of live body weight per plot, which were designated G0, G1, G2, G3, G4, and G5, respectively, in a randomized complete block design with three replicates (Appendix A Figure A1). The grazing cattle were kept in plots for 24 h a day over the grazing period and supplied with water from an outside source. This study site has been used since 2009 as summer pastures, with a grazing period from June to September each year.

2.3. Plant Sample Collection and Measurements

During the growing season, tillers of *L. chinensis* were obtained at the end of June, July, and August in 2015 and 2016. One hundred tillers were collected in each plot from rust-infected and also non-infected plants (100 tillers per plot × 6 grazing intensities × 3 replicates). Tillers that had 30–50% of the leaf area covered by uredinia and telia were collected as rust-infected samples, while tillers that were without any rust pustules were collected as the non-infected samples [48]. All of the collected tillers were cut near to the soil surface and oven-dried at 65 °C to a constant weight (at least 48 h) to obtain dry matter (DM) content. Then the weighed samples were milled to a fine powder with a particle size <0.2 mm for plant organic carbon (C), plant-total-nitrogen (N), and total phosphorus (P) analysis [48,49]. The percentage of C was analyzed by the Walkley–Black modified acid-dichromate FeSO₄ titration method as described by Nelson and Sommers [50]. Briefly, 0.015 g of milled plant tissue was digested with 5 mL of 1 N K₂Cr₂O₇ and 10 mL of concentrated sulfuric acid at 185 °C for 5 min, followed by titration of the digests with standardized FeSO₄. N and P were determined by a FIAstar 5000 Analyzer (Foss Tecator, Höganäs, Sweden) [9].

We surveyed *L. chinensis* monthly for the presence of rust pathogens in five assigned quadrats (1 m × 1 m) in each of the 18 (5 ha) grazing plots from June to August in 2015 and 2016. We conducted the survey using an 'X' preselected pattern, and the separation distance for adjacent quadrats was at least 100 m. The quadrats were located away from the plot fences to avoid marginal effects. In each survey, we visually examined the aboveground tissues (stems, leaves, inflorescences) of all the *L. chinensis* plants present per quadrat. We also collected 5~10 samples of rust-infected *L. chinensis* to establish the identity of the rust species in the laboratory using both the morphological methods via light microscopy (Olympus CX31, Japan) and molecular methods.

Relative growth rate (μ) was calculated as [34]:

$$\mu = \ln(M_1/M_0)/t \quad (1)$$

where M_1 and M_0 represented the DM of *L. chinensis* at the beginning and at the end of the month, respectively, t for 30 days.

The response ratio was calculated as the ratio of non-infected tillers to rust-infected tillers. The smaller the ratio, the smaller the impact of the rust [51].

2.4. Statistical Analysis

The incidence of rust on *L. chinensis* was calculated as the percentage of rust-infected tillers in each quadrat. The data analyses were performed using the GenStat version 17.1

(VSN International Ltd., Oxford, UK) based on the 2-year (2015 and 2016) average. One way analysis of variance (ANOVA) with Tukey's tests was used to evaluate the effects of grazing intensity on dry matter, relative growth rate, C, N, and P content (%), and C:N:P stoichiometry of non-infected and rust-infected *L. chinensis*, respectively. Generalized linear mixed models were used to test the significance of variation in dry matter, relative growth rate, C, N, and P content (%), and C:N:P stoichiometry, with sample date and grazing intensity as main effects. Individual comparisons of dry matter, C, N, and P content (%), C:N:P stoichiometry and relative growth rate between non-infected and rust-infected tillers were performed using independent sample *t*-tests at $p < 0.05$.

Prior to the ANOVA, data were checked for normality and homogeneity, and were transformed whenever necessary to meet the assumption. Means are reported with standard errors. To assess the relationship between the relative growth rates of non-infected and rust-infected plants with the C:N:P stoichiometry, we constructed a series of generalized linear models. The redundancy analysis (RDA) was used to further assess the effects of grazing and rust on the DM and C, N, and P content (%). To quantify the relative importance of the grazing and rust on the DM of *L. chinensis*, we constructed the structural equation model (SEM) based on the known relationship among grazing intensity and the C, N, and P content (%) of *L. chinensis*. The RDA and the structural equation were carried out with the Canoco console 4.5 [52] and RStudio, respectively.

3. Results

3.1. Effects of Grazing Intensity on *L. chinensis* Rust Incidence and Growth

The rust on *L. chinensis* was predominantly caused by *Puccinia elymi* (Westend.), a species that was first reported in Jilin province of China [53]. The specific identification process and pathogenicity testing was as used in our previous studies [16]. The field survey results indicated that the rust incidence tended to significantly ($p < 0.05$) decrease with increasing grazing intensity from June to August. As compared with the G0 treatment, the incidence under the G5 treatment decreased by 89%. The rust incidence was the highest under the G0 and G1 treatments in July. Under the rest of the grazing intensities, incidence on *L. chinensis* was the highest in August (Figure 1).

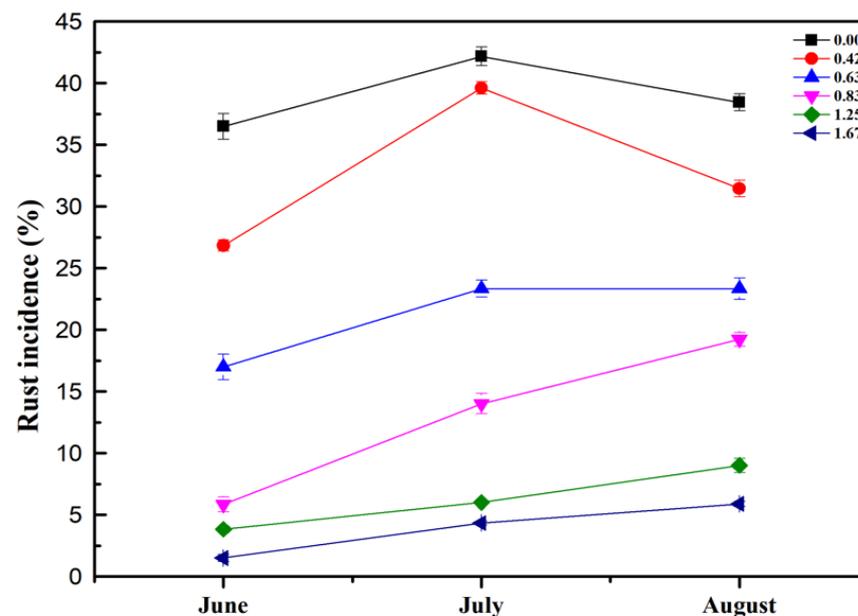


Figure 1. Rust (*Puccinia elymi*) incidence of *Leymus chinensis* under different grazing intensities from June to August based on the 2-year (2015 and 2016) average. Grazing intensities (0, 0.42, 0.63, 0.83, 1.25, and 1.67 cattle ha⁻¹) are indicated by different colors. The presented data are the mean of three replicates and the bars indicate standard errors.

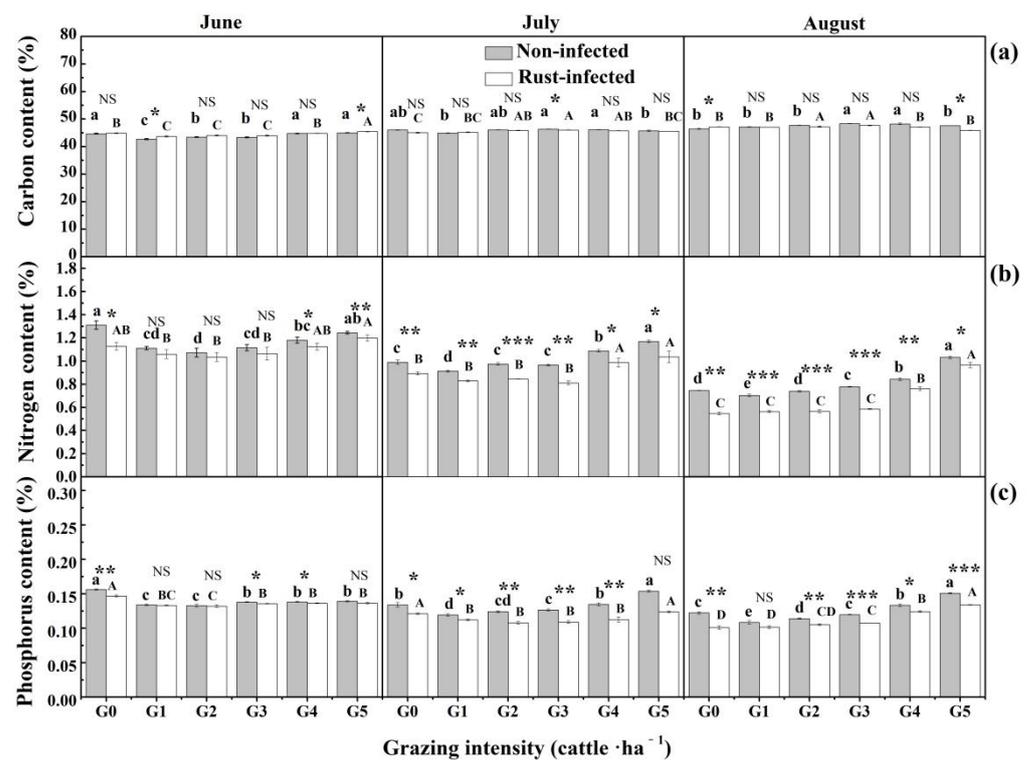


Figure 3. Influence of grazing intensity on the carbon (a), nitrogen (b) and phosphorus contents (%) (c) of non-infected and rust-infected *Leymus chinensis* from June to August based on the 2-year (2015 and 2016) average (error bars denote standard errors). Different lower-case and upper-case indicate significant differences among grazing intensities. The asterisk (*) indicates significant differences between non-infected and rust-infected plants (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$).

The C:N ratios of *L. chinensis* increased from G0 to G2, then they decreased from G3 to G5 from June to August. From G3 to G5, reduced C:N ratios were due primarily to increases in N content (Figure 4a). The C:P ratios (Figure 4b) showed similar patterns as the C:N ratios. The C:N and C:P ratios were both the highest in July. However, the N:P ratios did not show specific rules (Figure 4c). In contrast to the C, N, and P content (%) of non-infected and rust-infected plants, the C:N and C:P ratios significantly ($p < 0.05$) increased by 17.3% and 10.2%, respectively, from June to August (Figure 4a, b). There was no significant difference ($p > 0.05$) in the N:P ratios between non-infected and rust-infected plants (Figure 4c).

3.3. The Relationship between Relative Growth Rate and C:N:P Stoichiometry

For non-infected and rust-infected plants, the ratios of N:C and P:C increased with the relative growth rates. The higher growth rates correspond to a higher N:C and P:C ratios (Figure 5).

3.4. The Responses Ratios of *L. chinensis* to Rust

The response ratios of DM fluctuated in June and decreased with the increase of grazing intensity in July and August. The response ratios of N and P were decreased with the increase of grazing intensity in June and August. The response ratio of C was steady under various grazing intensities from June to August (Figure 6).

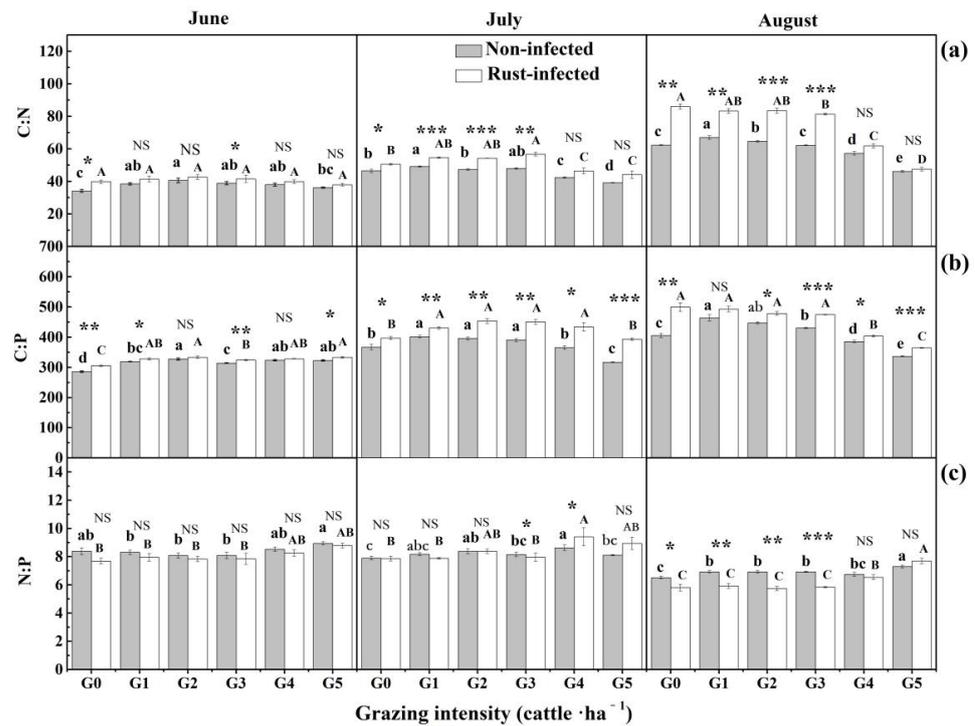


Figure 4. Influence of grazing intensity on the C:N (a), C:P (b), and N:P (c) ratios of non-infected and rust-infected *Leymus chinensis* from June to August based on the 2-year (2015 and 2016) average (error bars denote standard errors). Different lower-case and upper-case indicate significant differences among grazing intensities. The asterisk (*) indicates significant differences between non-infected and rust-infected plants (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$).

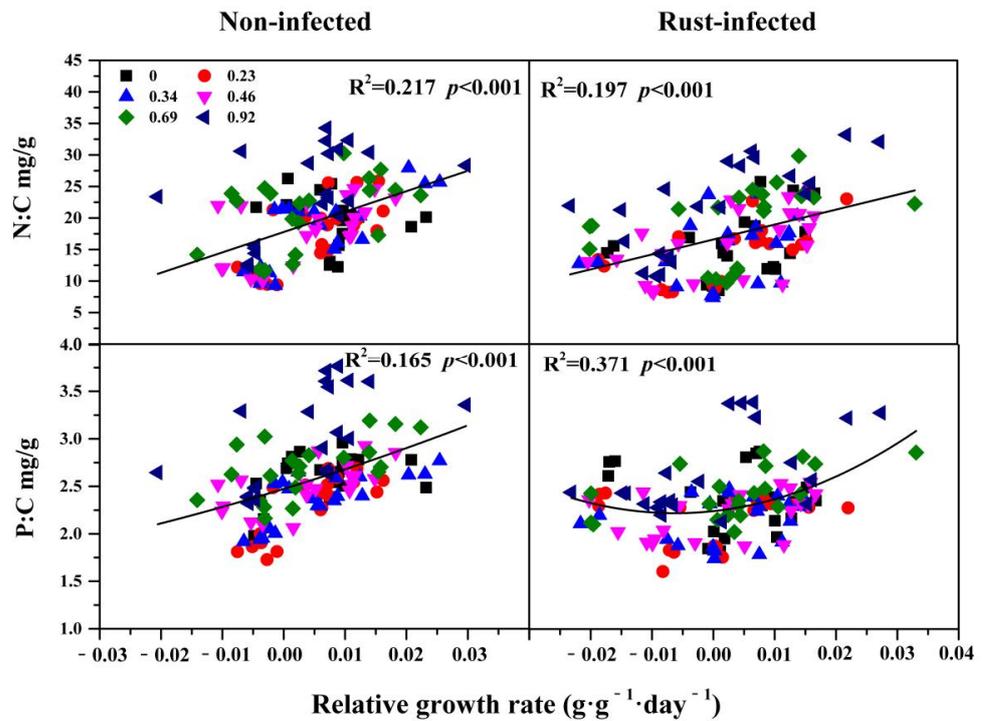


Figure 5. The relationship between relative growth rate and C:N:P stoichiometry of *Leymus chinensis* (non-infected and rust-infected plants) under different grazing intensities. Grazing intensities (0, 0.42, 0.63, 0.83, 1.25, and 1.67 cattle ha⁻¹) are indicated by different colors.

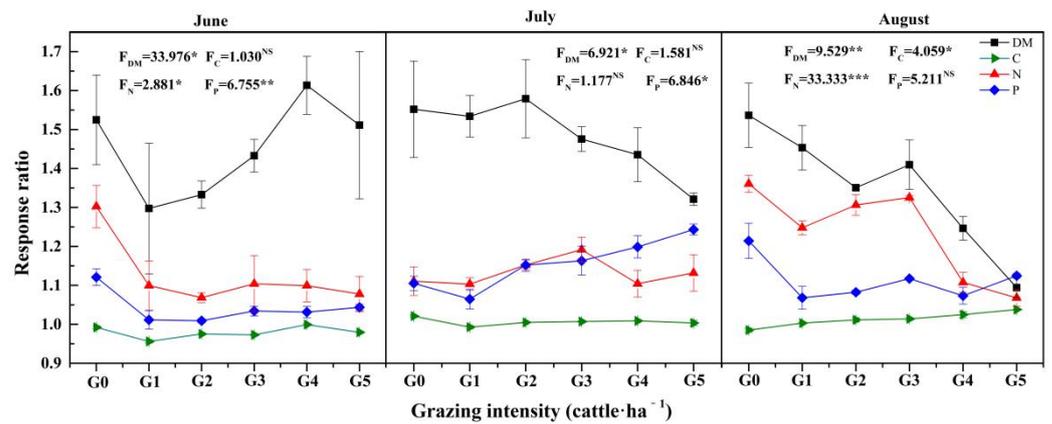


Figure 6. The differences of dry matter (DM), carbon (C), nitrogen (N), and phosphorus (P) contents response ratios between non-infected and rust-infected *Leymus chinensis* under different grazing intensities. Significant differences among grazing intensities are reported from ANOVA as ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, $p > 0.05$ and F-values (F) are given.

3.5. The Interactions of Grazing and Rust to *L. chinensis*

The DM, N, and P content (%), and the C:N and C:P ratios were significantly affected by rust ($p < 0.05$). The DM, relative growth rate, C, N, and P content (%), and the C:N and C:P ratios were significantly affected by grazing intensities ($p < 0.05$). All of the measurements were significantly affected by sample date ($p < 0.05$) and there were only three-way interaction effects of sample date, rust, and grazing intensity on the DM (Table 1).

Table 1. General linear mixed-effects model results for the effects of sample date (SD), grazing intensity (GI), and rust (RU) on dry matter (DM $\text{g}\cdot\text{tiller}^{-1}$), relative growth rate (RW $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$), carbon, nitrogen, and phosphorus contents (%), and C: N: P stoichiometry of *Leymus chinensis*. F-values (F) and p -values (p) are given. Significant p -values ($p < 0.05$) in bold.

Source	df	DM		RW		Carbon		Nitrogen		Phosphorus		C:N		C:P		N:P	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
SD	5	5.52	0.015	41.73	0.023	31.07	0.031	126.57	0.008	17.89	0.053	40.50	0.024	28.61	0.034	27.43	0.035
RU	1	6.71	0.045	3.11	0.251	0.17	0.718	24.71	0.025	6.38	0.111	5.05	0.138	7.60	0.133	0.75	0.449
GI	5	24.54	0.002	61.78	<0.001	4.41	0.065	35.41	0.001	15.54	0.005	15.33	0.005	32.33	0.001	3.60	0.093
SD × RU	4	8.35	0.007	7.15	0.012	5.50	0.024	3.93	0.055	12.64	0.002	17.72	0.001	6.78	0.014	10.74	0.043
SD × GI	25	3.82	0.023	10.76	<0.001	4.21	0.016	7.40	0.020	10.11	0.001	7.61	0.002	6.62	0.003	1.90	0.164
RU × GI	5	6.52	0.006	0.24	0.936	1.02	0.457	1.56	0.258	2.01	0.162	2.23	0.132	0.46	0.800	4.15	0.027
SD × RU × GI	20	3.05	0.001	0.71	0.714	0.53	0.865	0.10	1.000	0.53	0.867	0.10	1.000	0.82	0.612	0.11	1.000

3.6. Factors Influencing DM of *L. chinensis*

The first axis and second axis of the redundancy analysis (RDA) explained 70.1% and 30.1% of the variance, respectively. The total explained value of grazing intensity and rust to DM and the C, N, and P content (%) was 0.85, and the explained value of grazing intensity was 0.76, while the rust was 0.081. According to RDA, grazing was the most important factor to affect the DM of *L. chinensis*. The grazing intensity had a negative effect on dry matter, and a negative effect on the N content (%). The rust had a negative effect on the dry matter, and the C, N, and P content (%) (Figure 7).

The structural equation modeling (SEM) analysis revealed that grazing and rust directly altered the N and P content (%), which further influenced the DM of *L. chinensis*. In contrast, the grazing intensity and rust had no direct influences on the C content. In addition, grazing had a significant ($p < 0.05$) and direct effect on the DM of *L. chinensis* and was the most important pathway for determining the dry matter. The rust also notably affected DM directly, but less than grazing (Figure 8).

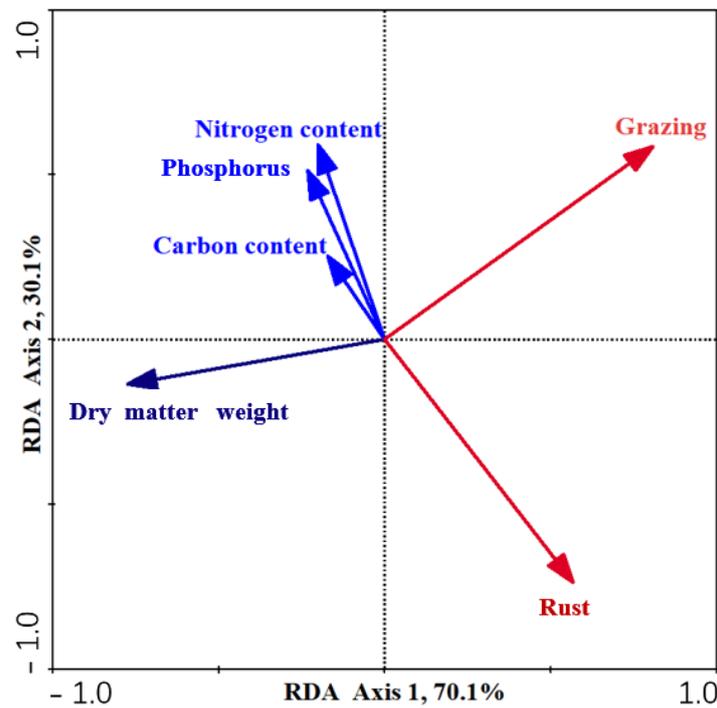


Figure 7. Redundancy analysis (RDA) of the effects of grazing and rust on dry matter, carbon, nitrogen, and phosphorus contents (%) of *Leymus chinensis*, showing the correlational relationships among grazing intensities (0, 0.42, 0.63, 0.83, 1.25, and 1.67 cattle ha⁻¹), disease (rust), dry matter of *L. chinensis*, and the content of elements (C, N, and P). Direction of arrow indicates the correlation between the parameters, and the length of the arrow indicates the magnitude of the association.

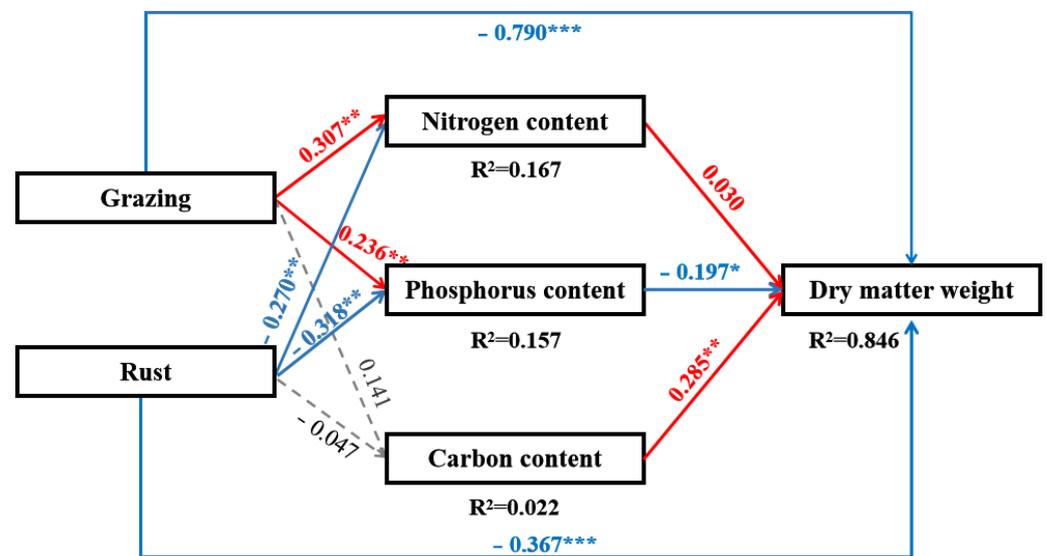


Figure 8. Final results of structural equation modeling (SEM) analysis for the effects of grazing and rust on the dry matter of *Leymus chinensis* in 2015 and 2016. Red arrows, evidence for positive relationships; blue arrows, evidence for negative relationships; gray arrows, insufficient statistical evidence for path coefficients ($p > 0.05$). Width of the arrows shows the strength of the causal relationship, and the number adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship; asterisks indicate statistical significance (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). The proportion of variance explained (R^2) appears alongside each response variable in the model. The final model adequately fitted the data: $\chi^2 = 249.059$, $Df = 4$, $P = 0.104$, $GFI = 0.955$.

4. Discussion

The key findings of the present study showed that grazing intensity and rust infection could negatively affect the biomass accumulation and nutrient element content and affect the ecological stoichiometry of *L. chinensis*. The N:C and P:C ratios were still positively correlated with the relative growth rates of plants. Non-infected *L. chinensis* had higher N:C and P:C ratios than rust-infected plants, which was consistent with the growth rate hypothesis. Meanwhile, almost all response ratios of DM under various grazing intensities were smaller than that in the G0 treatment. The findings of our study indicated that the growth rate hypothesis, based on ecological stoichiometry, still applied in *L. chinensis* under the interactive effects of grazing and disease. Additionally, grazing alleviated the loss of DM caused by disease.

4.1. Effects of Grazing on Rust

Grazing could change the relationship between host plants and pathogenic fungi through direct or indirect influence, thereby affecting the occurrence of grassland diseases. According to another of our studies carried out at the same experimental site, pathogen load on plants was directly affected by grazing through ingestion, and indirectly by shifting the plant community structure and the micro-environment [54]. In the case of this study, the incidence of *L. chinensis* decreased significantly ($p < 0.05$) with increased grazing intensity. Generally, grazing would increase the population of younger tillers [55], and younger plants are generally more susceptible to disease [56]. This is the opposite of our results, which may be because grazing cattle remove not only infected tissues, but also infectious spores when they selectively graze [22]. Ingestion by cattle under higher grazing intensity treatments (G4 and G5) provided ample space for seedlings and young tillers to grow without being severely restricted by abundant surrounding plants. Additionally, grazing decreased the number of old leaves that contained a high load of rust spores. Increasing numbers of young leaves and seedlings of *L. chinensis* grew in an environment where there were fewer infectious propagules and a lower pressure imposed by pathogenic plants in this study. Therefore, the young leaves and seedlings are very likely to have a lower incidence of rust infection. This switch from a predominance of old infected leaves to young leaves is likely a main reason why the rust incidence of *L. chinensis* decreased with increased grazing intensity.

Additionally, the selective ingestion by cattle would also change the species diversity of this grassland. A previous study in this area had verified that the species diversity was increased under the G3 treatment [2]. According to the dilution effect, high plant community diversity could negatively regulate the occurrence of infection [57–59]. Thus, the increase of species diversity in grazing treatments may also have resulted in decreased incidence of *L. chinensis*. This is another potential reason why the incidence of rust is lower in grazing treatments than in non-grazing treatment.

4.2. Effects of Grazing and Disease on DM

The DM of *L. chinensis* decreased gradually with the increase of grazing intensity, which was consistent with prior research based on the community level of Yan et al. [2] carried out in the same experimental site as the present research, and also similar to most grazing experiments carried out in grasslands in other parts of the world [47,60,61]. However, according to the compensation phenomenon, plants have the ability to compensate for the loss in vegetative biomass or reproductive potential, and to alleviate the detrimental effects of herbivores [62], but the result of the present study is different. This may be because *L. chinensis* is a constructive species and is the most favored plant species for cattle grazing in the Hulunber grasslands [2]. Compensatory growth, at least above ground, cannot match the rate of ongoing ingestion of this palatable grass by livestock. As a result, the plants even have negative growth in higher grazing intensities (G4 and G5 treatments). However, a previous study [63] in the same experimental plots showed that the below-ground biomass of *L. chinensis* was the highest in the grazing treatments (G2 and G3) than in the ungrazed

treatment, which may also meet predictions of the compensatory phenomenon. In the present study, the DM of rust-infected plants was smaller than the non-infected plants. It is easy to understand that diseases always damage the plant photosynthesis system, further reducing the biomass accumulation and even resulting in plant death [18,21].

4.3. Effects of Grazing and Disease on Relative Growth Rate and Ecological Stoichiometry

Carbon is considered as a relatively stable framework element, thus grazing and disease did not significantly ($p > 0.05$) affect the C content of *L. chinensis*, which is consistent with the previous study for the above ground plants in this area [64]. N and P are the most important elements for plant growth and affect plant growth and function [65]. The N content in *L. chinensis* increased gradually with the increasing of grazing intensity. This may be because outcomes of grazing livestock include the introduction of more fresh leaves, plant litter, as well as dung and urine, which promote microbial activity in soil, further increase the soil nutrients available to plants, ultimately changing the content and stoichiometry of the plant community [39,40]. On the other hand, pathogen infection would induce N metabolism and transportation [66,67]. Both N and P are acquired via roots from soil [9]. Transpiration is the main force for N and P absorption. Pathogens produce degrading enzymes to damage leaf tissues, negatively affecting photosynthesis and transpiration [20,46,68], further decreasing the absorption ability of rust-infected plants to N and P from soil. This may be the reasons why N and P content was decreased in rust-infected *L. chinensis*.

Ecological stoichiometry of living organisms has been important in the understanding of the implications of human disturbance to element cycles [34,69,70]. Previous studies demonstrated that higher N:C and P:C ratios in many heterotrophic organisms were coupled to higher relative growth rates [71,72]. According to our results, the N:C and P:C ratios were significant ($p < 0.05$) and positively correlated with the relative growth rate of *L. chinensis* in the six treatments. It was higher in the G1, G2, and G3 grazing treatments than in the G4 and G5 grazing treatments, which is consistent with the growth rate hypothesis [71,72]. Our findings showed that *L. chinensis* plants under lower grazing intensities had higher growth rates than those under higher grazing intensities. In other words, growth rate hypothesis, based on ecological stoichiometry, still applied in plants under various grazing intensities.

Some of the variation in field measurements of stoichiometric homeostasis was due to parasitism [73]. Therefore, infected hosts will display greater stoichiometric homeostatic variability than non-infected hosts [46]. In our study, the rust also had strong negative impacts on *L. chinensis* by altering the C, N, and P content (%) as well as the C:N:P stoichiometry. The N:C and P:C ratios of rust-infected plants were higher than those in non-infected plants. This finding confirmed that the stoichiometric ratio would not only reflect the plant growth rate but further reflect the health status of plants.

4.4. Grazing Alleviated the Loss of DM Caused by Disease

Grazing and rust substantially altered the DM and ecological stoichiometry of *L. chinensis* in the present study. The value of the response ratio could reflect the strength of influence of disease on DM [51]. The larger the value, the greater the impact. Almost all response ratios of DM under various grazing intensities were smaller than that in the G0 treatment, which means grazing alleviated the loss of DM caused by disease. Disease caused the primary loss in production of host plants by damaging the plant photosynthesis system, reducing the content of elements [18,21]. However, grazing increased the content of elements in plants [74]. SEM showed the same result, and this may be one of the potential reasons why grazing could alleviate the loss of DM caused by disease.

Our study provides a stoichiometric approach to understanding the interaction between grass and fungal disease under various grazing intensities, which provides new knowledge linking variation in host and livestock to patterns in the emergence and distri-

bution of fungal pathogens and provides better understanding and prediction of grazing-diseases interactions.

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Appendix A

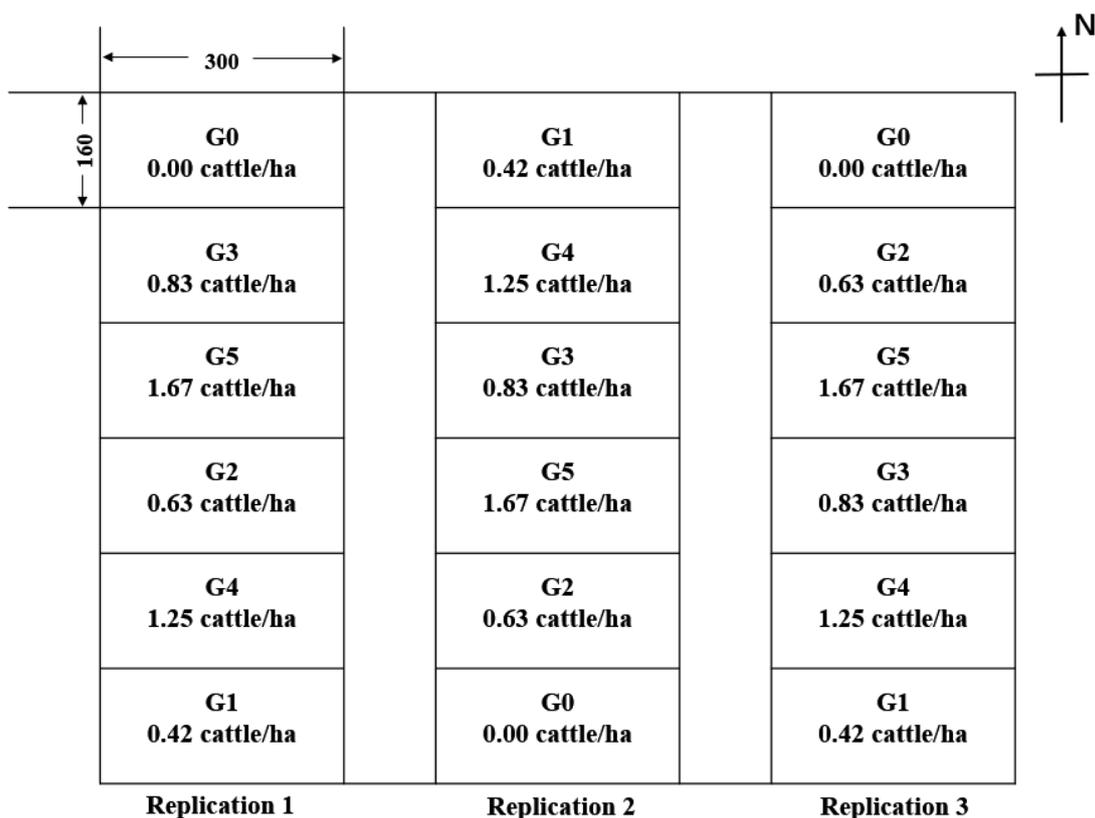


Figure A1. Sketch map of the experimental site.

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