



Article Irrigation and Nitrogen Application Promote Population Density through Altered Bud Bank Size and Components in Leymus chinensis

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Abstract: The bud bank of perennial grasses is a controlling factor in population dynamics and is estrongly affected by soil water and nitrogen status. To explore how the bud bank size and its components affect shoot population density under different soil moisture and nitrogen contents in spring and autumn. A three-full-factorial field experiment with factors of treatment timing (spring and autumn), nitrogen rate (control and $10 \text{ g N m}^{-2} \text{ yr}^{-1}$) and irrigation rate (control and +40 mm rainfall) was conducted in Leymus chinensis (a C₃ plant) in the northeast of China. The number of two types of buds (axillary shoot bud and rhizome bud), shoot population density, soil properties and rhizome traits (rhizome length and rhizome number) were determined to explore what and how changes in bud bank composition influences shoot population density in spring and autumn. The results showed that: (1) Regardless of the irrigation and nitrogen application timing, the simultaneous irrigation and nitrogen application significantly increased the number of two types of buds and promoted the shoot population density in spring and autumn by 56.75% and 47.74%, respectively. (2) The bud bank was dominated by rhizome buds in spring under control and dominated by axillary shoot buds under the combined irrigation and nitrogen treatment in autumn. Axillary shoot bud was the determining component in the population density increases in both spring and autumn, which were significantly associated with soil available phosphorus, available nitrogen and rhizome length and number. In summary, the number of different buds was strongly impacted by irrigation and nitrogen application at the crucial bud-bank formation stage. Agronomically, the forage yield could be largely increased through the increase in the axillary shoot bud density by using irrigation and nitrogen application in L. chinensis and other rhizomatous perennial grasses.

Keywords: *Leymus chinensis*; bud bank; population density; seasonal changes; irrigation and nitrogen; rhizome

1. Introduction

The productivity of natural and artificial grassland ecosystems in arid and semi-arid areas is driven by water and nitrogen [1,2]. In grasslands dominated by perennials, the aboveground productivity depends on changes in population density or the biomass of individuals or changes in both of these parameters simultaneously. Studies have indicated that population density is significantly correlated with the underground bud bank density in perennial grasses [3–5], where 99% of the stems originate from underground bud banks [6]. In turn, increased precipitation can enhance the number of buds and the outgrowth [7–9], while low soil moisture that occurs during the peak period of bud production has a major effect on the size of the bud bank, which will also affect the community density of grasslands [5]. The buds in some clonal plants may have resulted in a number



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). of buds not showing significant effects from drought in that year [10]. In a plant community, the buds of different species respond differently to drought, and the response of the dominant species' bud bank is the driver of the community response [11,12]. For example, the community dominated by C_3 plants maintains a relatively stable bud bank size in a year, whereas the changes in the bud bank size in the community that is dominated by the C_4 plants is largely different with C_3 plants [13,14]. Thus, exploration of the response of the bud bank of plant population to changes in soil moisture and soil nutrients is very important to better understand the changes in population density as well as productivity.

L. chinensis (Trin.) Tzvel. is a C₃ rhizomatous perennial clonal plant, with high tolerance of saline–alkaline and drought conditions, and it is widely distributed across the eastern Eurasian steppes [15]. The L. chinensis bud bank can be divided into two types: axillary shoot bud and rhizome bud (including axillary rhizome bud, vertical apical rhizome bud and horizontal apical rhizome bud). Most of the axillary shoot buds (at the basal nodes of the parent shoot) develop into axillary shoots or become apical rhizome buds, new shoots can develop from rhizome apical or axillary buds (Figure 1). It has a complex rhizome system and strong penetration, which can quickly spread into new habits via the rapid growth of rhizomes under conditions with good soil moisture and nutrient matrix. It also has a high yield and high palatability, the annual dry matter yield can reach 4000 kg per ha under natural conditions [15], and its widespread use for grazing and harvesting as hay makes it important for local animal husbandry [16]. L. chinensis reproduces by seeds and buds in natural habitats, but the occurrence of sexual reproduction is low, so the productivity of population is mainly determined by asexual reproduction via axillary shoot buds and rhizome buds in spring and autumn [5]. In Northeast China, the phenological stages of *L. chinensis* are shown in Figure 2. The spring-grown vegetative plant population of L. chinensis has a long growing season and high yield, thus they are usually harvested in the autumn as a hay reserve. The newly produced vegetative plant population in autumn is soft and palatability, and generally used for grazing. Similar to most clonal plants, different types of buds of *L. chinensis* have different population functions and different effects on plant strategies [17].



Figure 1. Plant architecture of *Leymus chinensis*. Shown are the various bud locations and shoots. The figure has been modified from Zhang et al. (2009) [17].



Figure 2. Phenological stages of Leynus chinensis. The figure has been cited from Shi et al. (2017) [15].

The propagation and expansion of *L. chinensis* mainly depend on the axillary shoot buds, which are supplemented by rhizome buds, using a combination of "guerrilla"type (buds and daughter shoots are located at the tip or nodes of the long rhizome) and "phalanx"-type (buds and daughter shoots are located at the basal nodes of the parent shoot) growth strategies [17–20]. This combined growth approach allows it to maintain sufficient plasticity to conversion between the two growth types, and to adjust its growth strategy by adjusting its bud bank composition and clonal configuration when soil moisture or nitrogen conditions changed [19,20]. The output of spring shoots of L. chinensis originate predominantly from the underground buds after overwintering [3,17], a process that is easily affected by available soil moisture and nitrogen content. For example, rhizome buds are more susceptible to changes in soil moisture, and higher soil moisture produces more rhizome buds [5,21]. Usually, increases in moderate soil moisture and nitrogen can promote the production of rhizomes and the output of axillary shoot buds, changing the size of the bud bank [22–25]. Rhizome traits such as length and number can also directly affect the number of buds growing on the rhizomes [5,11], ultimately increasing population density [25–28]. However, rhizomatous clonal plants promote the output of axillary shoot buds at high nitrogen levels and inhibited the production of rhizomes and rhizome buds, causing changes in their growth strategies. Nitrogen addition increases the nutrition of soil matrix and reduces the need for rhizomatous clonal plants to seek nutrients in a heterogeneous environment [29].

The Songnen Plain of Northeast China is a semi-arid area where grassland productivity is dually inhibited by water and nitrogen. Previous studies on bud banks in *L. chinensis* have mostly focused on characteristics and dynamic changes under natural conditions. It is not clear how irrigation and nitrogen applications affect the total bud number and its components of axillary shoot buds and rhizome buds in spring and autumn. Furthermore, it remains unclear what relationships different types of buds have to shoot population density. Here, a field experiment was conducted and aimed to explore how the treatments of spring and autumn irrigation and nitrogen application affect the bud bank size and its

component. We hypothesized that: (1) the irrigation and nitrogen application combinations have a positive influence on the number of the two types of buds and the shoot population density in both spring and autumn; (2) axillary shoot buds are the main components of the underground bud bank in both spring and autumn, and their number is closely related to the shoot population density increase; and (3) the available soil nitrogen content in both spring and autumn has significant effects on axillary shoot buds, whereas soil moisture content and rhizome traits have significant effects on rhizome buds.

2. Materials and Methods

2.1. Study Sites

The field experiment was conducted during the growing season in an artificial grassland located at the Grassland Ecosystem Field Station (123°35′ E, 44°34′ N, 167 m a.s.l.) of Northeast Normal University in the Songnen Grassland of China. The field station has a typical temperate semi-arid monsoon climate with a frost-free period of about 140 days, and a mean annual temperature of 6.3 °C. Average annual precipitation ranges from 280 to 644 mm, with rainfall occurring mainly from June to August (averaged values from 2002 to 2019). The area is characterized by meadow soil with saline–alkaline properties. The *L. chinensis* grassland was established with seed in 2016 and was fenced before planting to avoid animal grazing and human interference. The experiment site had a uniform density and high purity. This research examined a native plant species that does not require any special permit. Research permission was obtained for this project from the Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University.

2.2. Experimental Design

The experiment was replicated in 2018 and 2019. In the second experimental year, the 2018 experiment, replicated using new different grasslands, aimed to obtain robust results. Note that the 2018 and 2019 blocks were independent of each other. A fully factorial experiment with three factors, treatment timing (spring (early May) and autumn (early August), respectively), nitrogen rate (N: $0 \text{ g N m}^{-2} \text{ yr}^{-1}$ and $10 \text{ g N m}^{-2} \text{ yr}^{-1}$, respectively) and irrigation rate (I: +0 mm rainfall and +40 mm rainfall, respectively), for a total of eight treatment combinations (0 mm rainfall indicate natural rainfall) was designed. Four replicates per treatment combination per year were established, for a total of 64 plots. All treatment combinations were arranged with a randomized method in the field. The average growing season rainfall in 2018 and 2019 was 404.7 mm and 395.0 mm, respectively (Figure A1 in Appendix A). The area of the plot was $7 \times 8 \text{ m}^{-2}$, and the distance between two adjacent plots was 1 m. The experimental grassland was established by hand-sow seed in 2016 with 24 kg seed per ha. Later, the weed was hand removed. After two years of natural growth, the L. chinensis density was very uniform and no other species were detected. In this study, 10 g N m⁻² yr⁻¹ of nitrogen (46% urea nitrogen content) was uniformly applied to the nitrogen application plots during early May and early August before irrigation (from our previous experiments, early May and early August are the critical periods in L. chinensis for the output of buds and population formation in both spring and autumn, respectively). The amount of nitrogen applied was based on the current agronomic management of L. chinensis artificial grassland by farmers. Before the artificial grassland was planted, the watering PVC pipes are deeply buried in the ground (to prevent the water pipes from freezing and cracking in the severe cold in winter). In order to prevent surface water runoff and ensure the effectiveness of watering, the rotating sprinklers were used for uniform watering. The irrigation treatments used pre-laid sprinklers (the irrigation output per unit time of each sprinkler was quantified and the time required for experimental irrigation was calculated before the experimental treatment). The application date of the irrigation-only and nitrogen-only treatments were the same. The combined nitrogen and irrigation treatment was established at the same time, with the nitrogen application method being identical to the nitrogen-only treatment. All treatments were performed on the same day in each timing of spring and autumn, respectively.

2.3. Data Collection

Two weeks after the irrigation and nitrogen were applied, the underground bud bank was sampled. Subsequently, three $0.5 \text{ m} \times 0.5 \text{ m}$ sampling frames were selected and the below-ground organs were dug out (0–30 cm depth) in the central area of each plot to avoid marginal impact. After that, the underground tissues were carefully separated from the soil, trying to avoid damaging buds and rhizomes. The material was then taken back immediately to the field laboratory and the number of buds was quantified (buds with a length of ≥ 1 mm were measured, including small white buds and the longer purple-red buds). Each plot was subsampled three times, and these three subsamples were combined to calculate single mean values for the plots. A soil drill with a diameter of 5 cm was used to take soil samples at depths of 0–10, 10–20 and 20–30 cm from each plot to measure soil N and P. The five-point method was used to measure the soil moisture content of each plot using a TRIME-PICO 32 moisture sensor (IMKO, Ettlingen, Germany). Soil total nitrogen and total phosphorus content were analyzed with an automatic discontinuous chemical analyzer (Discrete Auto Analyzer, SmartChem 450, AMSAlliance, Latium, Italy), available nitrogen content was determined with an alkaline solution diffusion method and the available phosphorus content was determined with a molybdenum antimony colorimetric method.

For the total aboveground shoot population density, rhizome number and rhizome length, the spring and autumn measurements were undertaken in late July and October, respectively. The parent shoot population produce from April buds of L. chinensis was stable in late July (when hay can be harvested), and the daughter shoot population produce from August buds was stable in late October (the end of *L. chinensis* growing season). Before sampling the rhizomes in July and October, three 0.5 m imes 0.5 m subplots were randomly selected in each plot, and the sum of parent shoot density and daughter shoot density of the aboveground population in the plots was counted as the shoot population density. The shoot population density at sampling time minus the shoot population density at treatment time was used to present the population density increase. For the rhizome number and rhizome length, three $0.5 \text{ m} \times 0.5 \text{ m} \times 0.3 \text{ m}$ size soil samples from each plot were randomly dug out. The rhizomes were then carefully separated from the soil, and the total rhizome number, total rhizome length and total tiller number were counted. Rhizome length was from the point of attachment on the parent shoot to the tip of the rhizome (the number of rhizome internodes ≥ 1 No. was used to distinguish buds and rhizomes). After that, the ratio of the total rhizome number or total rhizome length to total tiller number was used to represent the rhizome number per tiller and the rhizome length per tiller.

2.4. Data Analysis

The interaction of year factor with treatment timing, nitrogen addition and irrigation before data analysis was tested. The results showed that the year factor and its interactions with other factors had no significant effect. Due to this reason, the year factor was not considered and only a three-way ANOVA was carried out. The effects of the three main factors on the total number of buds, axillary shoot buds and rhizome buds and the shoot population density were obtained using a three-way ANOVA via the general linear model (GLM). A normal distribution test on the data was performed before analysis, and the data conform to a normal distribution. Following this, a three-way ANOVA was applied to analyze differences in the shoot population density, bud number, bud number percentage, the soil environment (including soil moisture, total nitrogen, total phosphorus, available nitrogen, available phosphorus) and the plant traits (including population density increase, rhizome number, rhizome length) of L. chinensis. Multiple comparisons of group means between treatments were performed with an LSD post hoc test on different dependents. The independent sample *T*-test was applied to analyze differences in the numbers or proportions of the two types of buds in the same treatment. The general linear regression analysis was used to analyze the relationship between population density increase and bud number. Using CANOCO 4.5 software (Microcomputer Power, Ithaca, NY, US) a

redundancy analysis (RDA) was performed to examine the correlations between the belowground bud bank and soil environment (SE) and plant traits (PTs) in the different treatment timings. Then, the 'explanatory power' was used to indicate how much bud variation was explained by the environmental variables to determine the main factors affecting changes in the bud bank in different treatment timings. All data were analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software and figures were plotted with SigmaPlot 12.5, with the results expressed as mean \pm standard error. Differences were considered statistically significant at *p* values of 0.05.

3. Results

3.1. Shoot Population Density and Bud Bank and Rhizome Traits

The number of total buds and axillary shoot buds was significantly affected by the main factors of treatment timing (T), nitrogen (N), irrigation (I) and the interactions of $T \times N$, $T \times I$, $T \times N \times I$. The number of rhizome buds was significantly affected by the main factors of T and N, the interactions of $T \times N$ and $T \times I$. The shoot population density was only significantly affected by the main factors of T, N and I (Table 1).

Table 1. Combined ANOVA evaluating effects of treatment timing (T), nitrogen (N), irrigation (I) and their interactions on bud number and shoot population density. The data were collected in 2018 and 2019.

Source	df	Total		ASB		RB		PD	
		f	р	f	р	f	р	f	р
Т	1	76.32	0.000 ***	855.47	0.000 ***	167.86	0.000 ***	241.77	0.000 ***
Ν	1	92.26	0.000 ***	123.00	0.000 ***	15.14	0.000 ***	109.85	0.000 ***
Ι	1	78.74	0.000 ***	193.37	0.000 ***	0.20	ns	49.49	0.000 ***
$\mathbf{T} imes \mathbf{N}$	1	12.44	0.011 *	37.01	0.000 ***	18.58	0.000 ***	1.90	ns
$\mathbf{T} \times \mathbf{I}$	1	12.44	0.001 ***	92.16	0.000 ***	11.08	0.002 **	0.62	ns
N imes I	1	0.74	ns	11.99	0.001 ***	3.16	ns	1.84	ns
$T \times N \times I$	1	6.81	0.012 *	11.59	0.001 ***	0.52	ns	0.43	ns
Total	64								

* p < 0.05, ** p < 0.01, *** p < 0.001; ns, no significant effect. Total, total bud number; ASB, axillary shoot bud number; RB, rhizome bud number; PD, shoot population density; T, treatment timing; N, nitrogen application; I, irrigation.

The irrigation and nitrogen application at different times in spring and autumn significantly increased the corresponding shoot population density (including aboveground parent shoots and daughter shoots). Compared with the control, the single nitrogen application increased density by 41.75% and 30.45%, the irrigation treatments increased density by 30.75% and 21.21% and the irrigation and nitrogen application combination increased density by 56.75% and 47.74% in spring and autumn, respectively (Figure 3a). The total bud number in spring and autumn was significantly affected by the combined irrigation and nitrogen application (p < 0.05). Nitrogen application increased the total bud number in spring and autumn by 44.96% and 21.97%, irrigation increased it by 29.84% and 28.86% and the number of buds was the largest when irrigation and nitrogen were applied simultaneously, increasing by 60.08% and 73.27%, respectively (Figure 3b). In spring, the number and composition of bud banks in the control were dominated by rhizome buds. Simultaneous treatments of irrigation and nitrogen significantly increased rhizome buds (increased by 46.3%) and the number of axillary shoot buds increased by 108.14% (Figure 3b). The irrigation and nitrogen application did not change the proportion of the two types of buds, and rhizome buds accounted for more than 70.0% of the total bud bank (Figure 3c). In autumn there was no significant difference in the number and composition of the two types of buds in the control. None of the irrigation and nitrogen applications had any effect on the number of rhizome buds, but significantly increased the number of axillary shoot buds. Specifically, nitrogen application increased the number of axillary shoot buds by 40.29%, irrigation increased it by 65.04% and the combination of irrigation and nitrogen application increased it by 159.02% (Figure 3b). Moreover, the irrigation and nitrogen application significantly increased the proportion of axillary shoot buds (p < 0.05), which rose from 50.72% in the control to 76.02% in the irrigation–nitrogen combined area (Figure 3c).



Figure 3. Different treatment effects on the shoot population density (**a**), the number of the two types of buds (**b**) and the percentage bud number (**c**) in different seasons (average of the two years). The capital letters indicate that there were significant differences between total bud number in the different treatments. The lowercase letters indicate that the same type of buds had significant differences in different treatments. * indicates that there were significant differences between two types of buds in the same treatment. Shown are the means ± 1 SE (n = 4). Different letters denote a p < 0.05 difference based on an LSD post hoc multiple comparison test. (CK, control; N, nitrogen application; I, irrigation; NI, irrigation and nitrogen application).

The number and length of rhizomes in spring and autumn were significantly affected by the combined irrigation and nitrogen application (p < 0.05). Nitrogen application increased the number and length of rhizomes in spring by 65.99% and 35.33%, irrigation increased them by 49.99% and 31.60% and the largest number and length of rhizomes occurred with the combined irrigation and nitrogen treatment, which increased by 100.00% and 69.04%, respectively (Figure 4a). Different to spring, the maximum number and length of rhizomes in autumn under the simultaneous irrigation and nitrogen treatment increased by 93.34% and 47.81%, respectively (Figure 4b).



Figure 4. Different treatment effects on the rhizome number (**a**) and rhizome length (**b**) in different seasons. For each independent variable, different lowercase letters or capital letters indicate significant differences between the four treatments in the same season. Shown are the means \pm 1 SE (n = 4). Different letters denote a p < 0.05 difference based on an LSD post hoc multiple comparison test. (CK, control; N, nitrogen application; I, irrigation; NI, irrigation and nitrogen application).

3.2. Relationship between Bud Bank and the Aboveground Population

The increase in shoot population density in late July (as harvested hay) was significantly positively correlated with the total number of buds and the number of both types of buds (p < 0.05) in the spring treatment. The population density increase was significantly positively correlated with the total buds and axillary shoot buds in late October (as grazing green feed) (p < 0.05), but the number of rhizome buds was not significant (p > 0.05) in the autumn treatments (Figure 5).



Figure 5. The linear regression analysis of total bud number (**a**), axillary shoot bud number (**b**) and rhizome bud number (**c**) to population density increase. The same color denotes the same season.

3.3. The Impact of Soil Nutrient Properties and Rhizome Traits on the Bud Bank

The RDA indicated that among different soil and rhizome traits, the explanatory power of total nitrogen to the number of buds in spring and autumn was low and the correlation was not significant. In spring, total phosphorus, available nitrogen, available phosphorus, rhizome number, rhizome length and population density increase had a greater contribution to the total number of *L. chinensis* buds and the two types of buds, and the correlation was significant (p < 0.05), with total phosphorus being a negative correlation and the rest of the parameters being positive (Table 2, Figure 6a). In autumn, the other factors had a greater

contribution to the total buds and axillary shoot buds, and the correlation was significant (p < 0.05), except total nitrogen and total phosphorus. The variables related to the rhizome bud correlation were not significant and the contribution was low (Table 2, Figure 6b).

Table 2. The explanatory power and regression analysis of impact factors on the number of different types of buds in spring and autumn. The explanatory power indicates how much bud bank variation can be explained by controlling factors.

Bud Type	Controlling Factors	D (Sprin		Autumn			
		Parameters	Explanatory Power	R ²	р	Explanatory Power	R ²	p
		TN	0.019	0.13	0.685	< 0.001	0.00	0.998
		TP	0.626	0.80	0.002 **	0.336	0.54	0.071
	SE	AN	0.730	0.85	0.000 ***	0.838	0.91	0.000 ***
		AP	0.735	0.83	0.001 **	0.534	0.73	0.007 **
Bud Type Total		SM	0.162	0.37	0.241	0.543	0.72	0.008 **
	PT	RHN	0.679	0.80	0.002 **	0.557	0.73	0.008 **
		RHL	0.567	0.74	0.006 **	0.754	0.86	0.000 ***
		PI	0.683	0.80	0.001 **	0.817	0.90	0.001 **
	SE	TN	0.001	0.03	0.937	0.014	0.09	0.786
ASB		TP	0.615	0.78	0.003 **	0.305	0.48	0.116
		AN	0.788	0.90	0.000 ***	0.832	0.90	0.000 ***
		AP	0.810	0.85	0.001 **	0.499	0.71	0.009 **
ASB		SM	0.153	0.28	0.371	0.644	0.76	0.004 **
ASB		RHN	0.646	0.77	0.003 **	0.542	0.71	0.010 *
	PT	RHL	0.585	0.78	0.003 **	0.788	0.87	0.000 ***
		PI	0.764	0.87	0.010 *	0.808	0.90	0.000 ***
		TN	0.036	0.17	0.588	0.224	0.46	0.136
RB	SE	TP	0.525	0.72	0.008 **	0.014	0.16	0.620
		AN	0.566	0.73	0.007 **	0.044	0.20	0.544
		AP	0.577	0.72	0.008 **	0.011	0.09	0.777
		SM	0.152	0.37	0.234	0.186	0.42	0.171
	PT	RHN	0.570	0.72	0.008 **	0.005	0.07	0.827
		RHL	0.448	0.63	0.027 *	0.086	0.29	0.355
		PI	0.520	0.68	0.001 **	0.053	0.23	0.499

* p < 0.05, ** p < 0.01, *** p < 0.001. Total, total bud number (No·m⁻²); ASB, axillary shoot bud number (No·m⁻²); RB, rhizome bud number (No·m⁻²); SE, soil environment; PT, plant trait; TN, total nitrogen (mg·kg⁻¹); TP, total phosphorus (mg·kg⁻¹); AN, available nitrogen (mg·kg⁻¹); AP, available phosphorus (mg·kg⁻¹); SM, soil moisture content (%); RHN, rhizome number (No·m⁻²); RHL, rhizome length (cm·plant⁻¹); PI, population density increase (No·m⁻²).



Figure 6. Cont.



Figure 6. The redundancy analysis of the relationship of soil environment and plant traits to bud bank density in spring (**a**) and autumn (**b**). The axis represents a linear combination of the environment variables that participated in the sort. Each trait is represented by the mean of sample means in all samples. The red arrows represent environmental variables, and the blue arrows represent species variables. Total, total bud number; ASB, axillary shoot bud number; RB, rhizome bud number; TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; AP, available phosphorus; SM, soil moisture content; RHN, rhizome number; RHL, rhizome length; PI, population density increase.

4. Discussion

4.1. Bud Bank Composition and Seasonal Variation

In the ecosystems of arid and semi-arid regions, water and nitrogen play vital roles in the formation, establishment and dynamics of bud banks [2,22,30,31]. The present study showed that irrigation and nitrogen applications significantly increased the size of the bud bank in L. chinensis and the number of the two bud bank components (axillary shoot buds and rhizome buds) (Figure 3b), and this was related to the increase in the number and length of rhizomes and other organs (Figure 4). Indeed, the growth of buds from the rhizome is known to be influenced by crucial limits to the uptake of resources such as soil moisture and nitrogen [5,32,33]. Here, irrigation and the application of nitrogen released limited soil resources and significantly increased soil moisture and the content of available nitrogen and available phosphorus (Table A1). This in turn facilitated the growth and development of rhizomes, thereby increasing the number of rhizome buds and axillary shoot buds. Rhizome number, rhizome length and population density increases had similar explanatory power as total phosphorus, available nitrogen and available phosphorus for most bud types and in most seasons (Table 2). This is likely due to the fact that buds are produced in the axils of both stem and rhizome nodes. As more tillers and rhizomes grow, the number of buds will naturally increase [34].

Clonal plants adjust resource acquisition strategies through plasticity in clonal configuration and growth strategies while facing changes in environmental circumstances [7,20]. This study found that there were significant differences in the composition of the *L. chinensis* bud bank during different treatment timings. Irrigation and nitrogen application improved the soil environment and plant traits in spring (Table 2, Figure 6a), which increased bud number and the composition of the bud bank became dominated by rhizome buds (Figure 3). Increases in the number of rhizome buds (the expansion capacity of rhizome buds is better than axillary shoot buds) would have facilitated the procurement of water and nutrient resources [7,17]. This reduces the impact on the growth of *L. chinensis* of the frequent spring droughts experienced in the Songnen grassland and avoids competition with the parent shoots. However, these findings are inconsistent with previous studies where the rhizome-type buds of *L. chinensis* have been divided into multiple subtypes [17], resulting in a larger proportion of axillary shoot buds among the different bud types, while ignoring whole rhizome-type buds. In this study, axillary shoot buds were compared to rhizome buds which was more conducive to determining the population growth strategy of *L. chinensis* in different seasons than using multiple rhizome bud categories as seen in other studies. Other related research has shown that rhizome buds (especially vertical apical rhizome buds) are precursors of aboveground shoots that form in the spring [7].

After irrigation and nitrogen application in autumn, the soil environment and plant traits acted more on the axillary shoot buds (Table 2, Figure 6a), and the composition of the bud bank was dominated by this bud type (Figure 3). The seasonal dynamics of the bud bank were closely related to the growth of rhizomes [7]. The aboveground tillers that form in spring begin to senesce during autumn, and the vegetation supplies more nutrients to the rapid input and output of the bud bank as a population reserve for wintering [35,36]. The axillary shoot buds originate from the spring tiller base and grow close to the parent shoots to obtain more nutrients, and compared to the rhizome buds, they have a higher survival rate and rapid output ability [13,14,17,24]. Irrigation improves the nitrogen uptake and utilization rate of plants, and preferentially promotes axillary shoot bud formation and output [23,25,37]. In the present work, a proportion of the rhizome buds formed in spring developed into rhizomes, which improved the rhizome length and number to ensure access to limited soil resources. Seasonal effects have a greater influence on the bud bank in spring than irrigation and nitrogen application regulation, whereas the bud bank composition is more sensitive to changes from irrigation and nitrogen applied during autumn.

4.2. Relationship between the Bud Bank and Its Adaptive Strategy and Shoot Population Density Increase

The aboveground tillers of most perennial, rhizomatous grasses mainly originate from underground bud banks [17,30,38,39]. Our results showed that there was a significant positive correlation between the total bud number in spring and autumn and the increase in the aboveground shoot population density (Figure 5). However, the relationship between the two types of buds and the population was significantly different in spring and autumn. In spring, both types of buds were positively correlated with population density increase. In autumn, *L. chinensis* invested more resources in the rapid input and output of axillary shoot buds, which in relative terms reduced the proportion of rhizome buds in the total bud population. There was a significant positive correlation between the number of axillary shoot buds and population density increase (Figure 5b). Axillary shoot buds are closely related to the population density increases in spring and autumn and contribute the most to the forage yield of the *L. chinensis* population. It is likely that rhizome buds grow primarily into rhizome shoots in the spring and therefore are relatively unaffected in the autumn, and perennial rhizome grasses prioritize rhizome production over budding [30]. This allows for expansion into new habitats, providing more environmental resources for the rapid expansion of autumn populations via new buds. In spring, rhizome buds form new vegetative shoots, driving the increase in shoot population density.

Clonal plants have two growth strategies: "guerrilla" type and "phalanx" type [19]. "Guerrilla"-type growth mainly sends rhizome buds and daughter shoots away from the parent shoots, with a loose population distribution. "Phalanx" type keeps axillary shoot buds close to the parent shoots, and the population is distributed in clusters [19,26]. Under natural conditions, spring is the key period for the formation of aboveground spring populations. This period is generally based on the "guerrilla"-type growth mode of expanding territory [5,19]. Nevertheless, the current study found that irrigation and nitrogen application regulation in spring increased the input and output of buds but did not change the "guerrilla"-type growth strategy. This plant population grows by producing a large number of rhizome buds that are conducive to clonal reproduction. The investment in rhizome buds enables the establishment of a huge underground rhizome system for the population to access a greater amount of environmental resources, ensuring the growth and development of the aboveground population during the growing season [9,40]. The density of the aboveground population formed in spring stops changing in autumn, which is a critical period for preparing for the formation of aboveground populations in the subsequent spring [35,36]. After the application of irrigation and nitrogen, the *L. chinensis* population tended to have a more stable "phalanx" growth strategy with increases in the number of axillary shoot buds. More resources were invested in the rapid growth of the new tillers to increase the autumn shoot population density in the short term. This is also likely due to the fact that shoots would have finished their growth, whereas at the earlier sample date, shoots would still be adding nodes onto their vegetative tillers. The axillary daughter shoots developed from the axillary shoot buds can better utilize local resources and provide for more successful interspecific competition [40–43]. However, the "guerrilla" growth mode in spring enables the formation of rhizome buds far from the parent shoot population, avoiding competition with the parent shoots for limiting resources, thereby increasing forage yield.

The different growth strategies of *L. chinensis* in spring and autumn are the embodiment of its ecological adaptability. Adjustment of bud composition is an evolutionarily favorable behavior that assists population maintenance and renewal in the face of environmental change. Furthermore, this experiment found that in both spring and autumn the shoot population density increased following irrigation, nitrogen application or simultaneous applications of irrigation and nitrogen. Such management techniques should provide a new way to improve the yield of grasslands dominated by perennials.

5. Conclusions

We conclude as follows: (1) the bud bank size and its components were significantly influenced by irrigation and nitrogen application timings in *L. chinensis*; (2) spring irrigation and nitrogen application had no influence on bud bank composition, but autumn treatments increased the proportion of axillary shoot buds and ultimately increased the shoot population density; (3) available nitrogen and available phosphorus, rhizome number and rhizome length had high correlations with and contributions towards the two types of buds in spring, but more resources were invested into the production of axillary shoot buds; and (4) in spring, the bud bank was composed mainly affected the axillary shoot buds; and (4) in spring, the bud bank was composed mainly of rhizome buds and the population seemed to adopt a "guerrilla"-type growth strategy for exploiting resources, whereas in autumn, the population seemed to adopt a combination of "guerrilla"-type and "phalanx"-type growth strategies for population maintenance and renewal. Thus, a robust water and nitrogen supply can provide a stable growth environment for the formation of the *L. chinensis* bud bank, thereby enabling population increase.

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Season	Controlling Factors		Treatment						
		Parameters	СК	Ν	Ι	N*I			
Spring		TN (mg·kg ⁻¹)	235.76 ± 10.80	76 ± 10.80 249.90 ± 61.19 $277.49 \pm 70.$		234.14 ± 22.97			
1 0		TP (mg·kg ⁻¹)	300.70 ± 68.36	192.41 ± 39.60	255.77 ± 19.37	121.68 ± 10.33			
	SE	AN (mg·kg ⁻¹)	$2.95\pm0.10~\text{C}$	$5.33\pm0.55~\text{AB}$	$4.80\pm0.25~\mathrm{B}$	$6.74\pm0.65~\mathrm{A}$			
		AP (mg·kg ⁻¹)	$11.35\pm2.13~\mathrm{C}$	$16.21\pm0.44~\text{AB}$	$15.12\pm0.69~\text{AB}$	$17.98\pm0.64~\mathrm{A}$			
		SM (%)	$13.37\pm0.25~\mathrm{B}$	$13.94\pm0.19~\text{AB}$	$14.75\pm0.20~\mathrm{A}$	$14.15\pm0.40~\text{AB}$			
	PT	PI (No \cdot m ⁻²)	$704.75 \pm 147.68 \text{C}$	$1382.50 \pm 73.66 \text{ AB}$	$1153.00 \pm 53.70 \text{ B}$	$1608.25 \pm 137.81 \; \mathrm{A}$			
Autumn		TN (mg⋅kg ⁻¹)	233.19 ± 10.09	311.91 ± 45.03	245.13 ± 51.68	259.35 ± 29.34			
		TP (mg·kg ^{-1})	$306.76\pm17.46~\mathrm{A}$	$189.29\pm11.95~\mathrm{B}$	$196.80\pm41.86~\mathrm{B}$	$199.27\pm9.79~\mathrm{B}$			
	SE	AN (mg⋅kg ⁻¹)	$3.15\pm0.16C$	$5.15\pm0.44~\mathrm{B}$	$5.50\pm0.24~\mathrm{B}$	$7.44\pm0.34~\mathrm{A}$			
		AP (mg·kg ^{-1})	$12.55\pm1.60~\mathrm{C}$	$17.31\pm0.32~\mathrm{A}$	$15.38\pm0.68~\text{AB}$	$19.11\pm0.45~\mathrm{A}$			
		SM (%)	$13.38\pm0.25\mathrm{C}$	$14.01\pm0.13~\text{BC}$	$14.95\pm0.29~\text{AB}$	$15.08\pm0.16~\mathrm{A}$			
	PT	PI (No \cdot m ⁻²)	$1492.00 \pm 195.59 \text{ C}$	$2188.00 \pm 72.57 \; \text{AB}$	$2039.50 \pm 86.20 \text{ B}$	$2639.50 \pm 242.48 \; \text{A}$			

Appendix A

Table A1. Different treatment effects on parameters in spring and autumn.

Data are presented as average values \pm SE. The differences were considered significant when p < 0.05 and indicated by different letters. SE, soil environment; PT, plant trait; TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; AP, available phosphorus; SM, soil moisture content; PI, population density increase; CK, control; N, nitrogen application; I, irrigation; NI, irrigation and nitrogen application.



Figure A1. Precipitation and temperature during the growing season in 2018 and 2019.

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