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Continuous Nitrogen Application Differentially Affects Growth, Yield, and Nitrogen Use Efficiency of *Leymus chinensis* in Two Saline–Sodic Soils of Northeastern China

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ABSTRACT

Leymus chinensis **(Trin.) Tzvel. (Poaceae) is a dominant plant in the western Songnen Plain of northeastern China. Soil salinization, N deficiency, and current management practices have resulted in grassland degradation in the region. The objective of this study was to investigate the effect of N application on *L. chinensis*. A 3-yr field experiment was conducted in two nearby fields at the Da`an Sodic Land Experiment Station (DASLES) from 2009 to 2011. Both fields were classified as moderate saline–sodic grassland (MSSL) and severe saline–sodic grassland (SSSL), respectively. Nitrogen application rates were 0 (no fertilizer as control), 30, 60, 90, 120, 150, 180, and 210 kg N ha^{-1}. Nitrogen application improved herbage yield from 2.0 t ha^{-1} without N to more than 10.7 t ha^{-1} with 180 kg ha^{-1} N in MSSL, and from 1.0 t ha^{-1} without N to 4.9 t ha^{-1} with 180 kg ha^{-1} N in SSSL, herbage yields increased 5.4 and 4.9 times in MSSL and SSSL, respectively. Nitrogen use efficiency (NUE) was generally higher in MSSL than that in SSSL, the highest values of average NUE were the treatments with 120 kg N ha^{-1} in MSSL and 150 kg N ha^{-1} in SSSL based on the results of regression analysis. Our overall results support N applications of 90 to 120 kg N ha^{-1} in MSSL and 120 to 150 kg N ha^{-1} in SSSL. Nitrogen application can improve *L. chinensis* yield, and prevent grassland degradation in the region.**

Soil salinity is one of the main environmental problems affecting extensive areas of land in both developed and developing countries. Due to soil salinization and/or alkalization, periodic droughts, overgrazing, and human interference, one-third of the available grassland area has been degraded, and 2 million ha was added annually to that total, the production decreased by about 70% (the aboveground biomass decreased from 2.2 to 3.0 t ha^{-1} to 0.70 to 0.90 t ha^{-1}), from the 1950s to the 1990s (Kawanabe et al., 1998; Jia et al., 2006), and the direct economic loss from grassland degradation was estimated as 9 billion dollar per year in China (Akiyama and Kawamura, 2007). Grassland protection and restoration are urgent issues for both the central and provincial governments (Xu and Zhou, 2005; Yang et al., 1995).

Leymus chinensis (Trin.) Tzvel. (Poaceae) is a native, perennial rhizomatous grass with high palatability and forage value that is considered one of the most promising grass species for grassland rehabilitation and reconstruction in arid regions of northern China (Liu and Han, 2008). *Leymus chinensis* tolerates drought (Bai et al., 2004) and saline–sodic stresses, being able to survive in highly sodic soil with pH of 8.5 to 11.5 (Jin et al., 2008). Adding *L. chinensis* hay into diets of dairy cows can increase milk yield and provide good milk quality (Yan et al., 2011). Therefore, *L. chinensis* is recognized as an economically and ecologically important fodder crop.

The physiological response of *L. chinensis* to drought or saline–sodic stress is well documented (Gu et al., 2008; Cui et al., 2008; Jin et al., 2008). Huang et al. (2008, 2009) indicated that high water use efficiency and high K^+/Na^+ ratio in the shoot are two of the most important physiological mechanisms for salinity tolerance of *L. chinensis.* Saline–sodic stress reduces plant growth through both ionic toxicity and osmotic stress, and subsequently through nutrient deficiencies or imbalances (Hu and Schmidhalter, 2005; Upadhyay and Panda, 2005; Munns and Tester, 2008). In particular, N deficiency has become a major limiting factor for improving plant productivity and promoting vegetation restoration in grassland of northern China (Chen and Wang, 2000). Nitrogen nutrient deficiency is most severe in the saline–sodic grassland of Songnen Plain. A recent survey shows that the available N content is only 10 to 30 mg kg^{-1} in some saline–sodic soils that have been seriously degraded, representing less than a third

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Abbreviations: AGR, absolute growth rates; CAS, Chinese Academy of Sciences; DASLES, Da`an Sodic Land Experiment Station; LAI, leaf area index; MSSL, moderate saline–sodic grassland; NUE, nitrogen use efficiency; SSSL, severe saline–sodic grassland; RGR, relative growth rates.

of the soil available N in natural *L. chinensis* communities (Huang et al., 2010).

Nitrogen is one of the most important nutrients for plant growth, and the application of N fertilizer is usually essential to increase plant yields. Some studies shown that the application of N fertilizer has a positive effect on *L. chinensis* growth. In natural arid and semiarid grasslands, N application can increase the plant height, aboveground biomass and population density, carbohydrates, and chlorophyll content of *L. chinensis* to a certain extent (Pan et al., 2004, 2005; Wan et al., 2008). Nitrogen application often results in a significant increase in N concentration, a decrease in the C/N ratio of plant tissues, and enhances the photosynthetic rate and the ability of *L. chinensis* to adapt to drought stress (Chen et al., 2005; Xu et al., 2007). In saline and/or sodic environments, salinity can decrease NO_3^- uptake in both nonhalophytes (Peuke et al., 1996) and halophytes (Rubinigg et al., 2003). Nitrogen application may increase plant biomass production (Liu et al., 2004), and reduce ionic toxicity (Camara-Zapata et al., 2004). A factorial experiment indicated that effective N uptake of the shoots might contribute to high salt tolerance of *Suaeda physophora* under NaCl salinity (Yuan et al., 2010). Nitrogen application may help correct nutritional imbalances in plants exposed to salinity (Gómez et al., 1996); however, this appears to be species dependant. Saline soils do not necessarily require application of N to levels greater than required under non-saline conditions. Under controlled greenhouse conditions Semiz et al. (2014) observed that pepper plants (*Capsicum annuum* L.) required less applied N under saline as compared to non-saline conditions. To date, little experimentation has been conducted on plant growth of *L. chinensis* response in relation to N application in saline–sodic soils, especially under field conditions. Results from these studies should contribute to improved techniques for grassland restoration and reconstruction.

The experiment was conducted in two different saline–sodic soils from 2009 to 2011 at DASLES. The objectives of this study were: (i) to examine the effects of N application on plant growth and yield of *L. chinensis*; (ii) to determine the optimum of N application on *L. chinensis* through comprehensive comparison of NUE under different N concentration application; and (iii) to evaluate the role of N application in saline–sodic grassland restoration.

MATERIALS AND METHODS

Experimental Site

The study was conducted in an experimental field for established *L. chinensis* grass at the DASLES, Chinese Academy of Sciences (CAS) (45°35′58″–45°36′28″ N, 123°50′27″–123°51′31″ E), which is located in Da`an city of Jilin Province of China. The DASLES is a typical saline–sodic degraded grassland ecosystem located in the hinterland of Songnen Plain, and has an average elevation of 150 to 200 m above sea level. The area is semiarid with a temperate continental climate, dry in spring and fall, hot and moist in summer. In winter, the climate is dominated by the Mongolian anticyclone, which produces a westerly flow of cold, dry air with little snowfall. Annual mean precipitation is 414 mm that mainly falls during the period from April to September. The annual mean air temperature for the area is 4.3°C, varying from –20°C in January to 26°C in July (Huang et al., 2010). The maximum and minimum temperatures and total rainfall recorded during the *L. chinensis* growing seasons from 2009 to 2011 were collected from the DASLES weather station (Fig. 1).

The experimental field is dominated by *L. chinensis* and is a typical pure *L. chinensis* community. The vegetation showed a trend of decreased productivity (degradation) attributed to soil salinization and alkalization. The soil type is saline–sodic meadow soil (according to the soil classification by World Reference Base for Soil Resources [WRB]), its main soluble cations are Na^+ and K^+, and main soluble anions are HCO_3^- with lesser amounts of CO_3^{2-} with a soil pH usually above 8.5 (Deng et al., 2006). Two *L. chinensis* grasslands having different soil pH were selected. One was moderate saline–sodic grassland (MSSL) with soil pH below 9.0, clearly similar to that of the natural *L. chinensis* grassland; and the other was severe saline–sodic grassland (SSSL) with soil pH higher than 9.5, significantly higher than that of the common *L. chinensis* grassland. The basic chemical characteristics of the two grassland soils were shown in Table 1.

Experimental Design

We employed a randomized complete block design with three replicates in the field experiment. There were 24 (3 by 3 m) plots in each soil, and there was a 1-m gap between the adjacent plots with separation maintained with cement boards. Eight N application rates were designed as follows: 0 (the control with no applied fertilizer), 30, 60, 90, 120, 150, 180, and 210 kg N ha^{-1}.

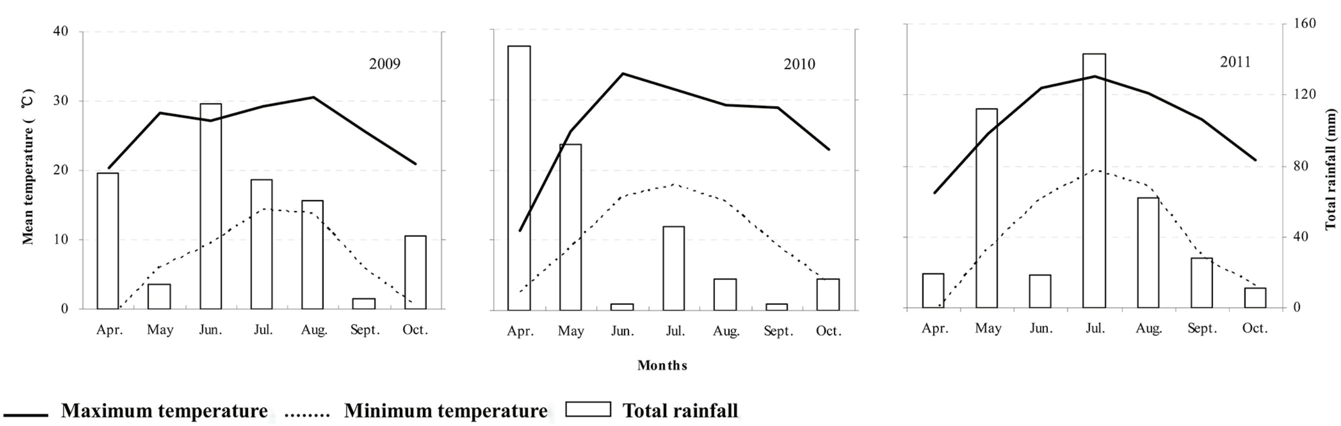

Fig. 1. Mean maximum and minimum temperature (°C), and total monthly rainfall (mm) during the plant growing seasons from 2009 to 2011.

Table 1. Initial soil properties of the 0- to 20-cm soil layer of the experimental site.

Soil type	Soil texture	pH† soil/water = 1:5	EC soil/water = 1:5	Salt content	CEC	ESP	Organic matter‡	Total N	Available N§	Available P	Available K
			mS cm^{-1}	g kg^{-1}	cmol kg^{-1}	%	—— g kg^{-1} ——		—————— mg kg^{-1} ——————		
MSSL¶	SCL	8.94	0.57	3.97	14.1	37.9	13.26	0.49	23.6	9.82	137
SSSL	SCL	9.80**	0.88**	6.13**	18.3**	50.3**	11.57ns#	0.39ns	21.2ns	9.80ns	141ns

** Significant at $P < 0.01$.

† pH and electrical conductivity (EC) were determined in soil/water = 1:5, cation exchange capacity (CEC) were determined using 1 mol L^{-1} NH_4OAC exchange methods, salt contents were calculated by the amount of main cations and anions, ESP was calculated by soil exchangeable sodium and CEC.

‡ Organic matter content was determined with potassium dichromate method, and total N content was determined with Kjeldahl method.

§ Available-N, -P, and -K contents were determined using an alkaline hydrolysis diffusion method, 0.5 mol L^{-1} $NaHCO_3$ and 1 mol L^{-1} NH_4OAC extraction methods, respectively.

¶ MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland; SCL: sandy clay loam,

ns: not significant.

The site was fenced and had never been fertilized before the experiment was conducted. From 2009 to 2011, N fertilizer was applied at the proscribed rates for the treatments in the middle of May-once a year, after *L. chinensis* resumed growth.

Nitrogen fertilizer was applied as urea, of which, the N content was 46.2%. The fertilizer was applied on the surface of *L. chinensis* grassland in the early evening when it was cloudy. The water amount of 10 mm was slowly and uniformly sprayed to each plot after fertilizing so that urea granules could be dissolved quickly and fertilizer loss reduced due to volatilization. The field was managed as a single unit, maintaining the natural growth of *L. chinensis* and avoiding human interference.

Sampling and Measurement Methods

The dynamics of *L. chinensis* growth is exhibited by a single peak production curve in Songnen grassland of Northeast China. The maximum biomass production is usually from late July to early August every year (Zhu, 2004). Thus, annually we selected late July for field measurements. The experiment measurements were made at random sites with a sampling size of 0.5 by 0.5 m, with three replications within every plot. The LAI was measured using a LAI-2000 Plant Canopy Analyzer. The plant height was measured with a ruler for at least 10 plant individuals in each sampling quadrat. All the samples in a quadrat were cut in ground level. The population density was calculated as the number of plants per area. The harvested samples were weighed for fresh weight, then oven-dried at 65°C for 48 h, and next weighed again for dry weight (precision: 0.01 g). Plant biomass was expressed as aboveground dry weight per area, according to *L. chinensis* is usually cut for hay production during this period. Soil samples at 0- to 20-cm depth were collected at the end of July annually. Three soil cores (diameter of 40 mm) were taken at random from each plot and composited. Samples were mixed, air-dried, passed through a 2-mm sieve and then measured for the basic chemical characteristics of soils, pH, and electrical conductivity (EC) were determined in soil: water = 1:5, cation exchange capacity (CEC) were determined using 1mol L^{-1} NH_4OAC exchange methods, salt contents were calculated by the amount of main cations and anions, exchange sodium percentage (ESP) was calculated by soil exchangeable sodium and CEC, organic matter content was determined with K dichromate method; total N content was determined with Kjeldahl method; and available N, P, and K contents were determined using an alkaline hydrolysis diffusion method, 0.5 mol L^{-1} $NaHCO_3$ and 1mol L^{-1} NH_4OAC extraction methods, respectively (Bao, 2000).

We estimated the absolute growth rate (AGR, which was defined as the aboveground biomass increase per day), relative growth rate (RGR, which was defined as the per day aboveground biomass increase compared), and agronomic nitrogen use efficiency (NUE, which refers specifically to N agronomic efficiency, defined as the aboveground biomass increase divided by the amount of N applied) as follows:

$$\mathrm{AGR}\ (\mathrm{g\ m^{-2}\ d^{-1}}) = \mathrm{d}W/\mathrm{d}t$$

$$\mathrm{RGR}\ (\mathrm{g\ m^{-2}\ d^{-1}}) = \mathrm{d}\ (W_\mathrm{F} - W_\mathrm{C})/\mathrm{dt}$$

$$\mathrm{NUE}\ (\mathrm{kg\ kg^{-1}}) = (W_\mathrm{F} - W_\mathrm{C})/M$$

where W is dry weight of *L. chinensis* aboveground biomass per m^2, t is growth date (days); W_F, W_C, and M are dry weights of the aboveground biomass with N application treatment, without N application treatment and N application amount per m^2, respectively.

Statistical Analysis

The data were expressed as mean ± 1 SE. The treatments were run for a single factor ANOVA using the software program SPSS Version 16.0 (SPSS Inc., Chicago, IL). The significant analyses ($P < 0.05$) of the various response variables were separately undertaken under different N application rates, among different years and between two soils. The correlation analyses ($n = 27$, $P < 0.05$, $P < 0.01$ or $P < 0.001$) between N application rates and the various response variables, and the correlation analyses ($n = 27$, $P < 0.05$, $P < 0.01$ or $P < 0.001$) between one variable and other variables were also conducted, respectively. Regression analyses were conducted between N application rates and the various response variables, through the significant test ($P < 0.05$), the regression equations were established, and N application rates when the various response variables reached maximum were calculated. The optimum N application rates were determined according to the maximum of the various response variables corresponding N application rate in two different saline–sodic soils, respectively.

RESULTS

Plant Height

Plant height of *L. chinensis* increased significantly with increasing N application rates (Table 2). When N application rate was 210 kg N ha^{-1} in 2009 and 180 kg N ha^{-1} in 2010 and 2011 in both MSSL and SSSL, plant height of *L. chinensis* reached the maximum value. Compared with the control, plant

Table 2. Plant height and population density of *Leymus chinensis* under different N application rates in both soils from 2009 to 2011.

Soil type	Treatment	Plant height			Plant population density		
		2009	2010	2011	2009	2010	2011
		cm			plants m^{-2}		
MSSL†	N_0	36.7 ± 2.1aA*‡	72.0 ± 0.9aC*	43.8 ± 1.4aB*	967 ± 79aAB*	1100 ± 90aB*	613 ± 35aA*
	N_{30}	39.4 ± 1.5aA*	85.5 ± 1.1bC*	52.5 ± 1.4bB*	1175 ± 63aA*	1558 ± 73aB*	989 ± 72abAns§
	N_{60}	48.2 ± 2.7bA*	87.7 ± 1.0bC*	63.6 ± 2.0cB*	1208 ± 129aA*	2775 ± 151bB*	1069 ± 217abcAns
	N_{90}	51.6 ± 1.8bcA*	93.2 ± 0.9cC*	75.5 ± 1.9dB*	1392 ± 147abA*	2817 ± 242bB*	1705 ± 173dAns
	N_{120}	54.8 ± 2.0cA*	93.5 ± 0.8cC*	80.3 ± 1.9deB*	1892 ± 51cA*	3058 ± 246bcB*	1464 ± 136cdAns
	N_{150}	63.3 ± 1.5dA*	92.5 ± 0.8cC*	80.0 ± 2.3deB*	1725 ± 123bcA*	3367 ± 213cB*	1541 ± 101cdAns
	N_{180}	77.1 ± 2.7eA*	97.0 ± 1.6dC*	86.1 ± 1.9fB*	2117 ± 137cA*	2908 ± 134bcB*	1405 ± 157bcdAns
	N_{210}	79.3 ± 2.4eA*	96.4 ± 0.8dB*	81.9 ± 1.7efA*	2833 ± 254dB*	2758 ± 98bB*	1589 ± 185dAns
SSSL	N_0	32.0 ± 1.7aA*	60.0 ± 1.2aC*	40.6 ± 1.4aB*	579 ± 73aAB*	825 ± 101aB*	403 ± 39aA*
	N_{30}	31.2 ± 1.1aA*	66.0 ± 1.1bC*	40.1 ± 1.2aB*	588 ± 47aA*	925 ± 52abB*	675 ± 113abABns
	N_{60}	31.5 ± 1.3aA*	70.7 ± 1.2cC*	44.9 ± 1.4bB*	772 ± 52bA*	1250 ± 80bcB*	920 ± 147bcABns
	N_{90}	34.4 ± 1.4abA*	76.5 ± 1.3dC*	54.7 ± 1.4cB*	837 ± 54bA*	1750 ± 80dC*	1231 ± 46deBns
	N_{120}	38.2 ± 1.9bcA*	76.7 ± 1.1dC*	54.1 ± 1.3cB*	1013 ± 18cA*	1050 ± 75abcA*	1337 ± 48deBns
	N_{150}	40.9 ± 2.6cA*	75.9 ± 1.2dC*	59.9 ± 1.6dB*	1547 ± 40dB*	1050 ± 101abcA*	1385 ± 94eABns
	N_{180}	41.6 ± 1.4cA*	83.3 ± 1.6eC*	61.0 ± 1.7dB*	1679 ± 71dB*	1342 ± 150cAB*	1064 ± 60cdAns
	N_{210}	42.3 ± 1.5cA*	80.6 ± 1.1eC*	60.4 ± 1.9dB*	1163 ± 41cA*	1350 ± 156cA*	1163 ± 138cdeAns

* Significance between MSSL and SSSL $P < 0.05$.

† MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland.

‡ Means ± 1 SE. Significance among different N application rates was marked with lowercase letters, significance among different years under the same N treatment was marked with uppercase letters.

§ ns: no significant.

height was increased by 116, 35, and 97% in MSSL, and by 32, 39, and 50% in SSSL in 2009, 2010, and 2011, respectively. However, as shown in Table 2, there was no difference ($P > 0.05$) between plant heights at 180 and 210 kg N ha^{-1} for MSSL and no difference ($P > 0.05$) among plant heights for SSSL with 150, 180, and 210 kg ha^{-1} N application. If we consider 2011 only (after 3 yr of N application), there were no significant differences in plant height above 120 kg ha^{-1} for SSSL and above 150 kg ha^{-1} for MSSL. Under the same N application rates, there were significant differences in plant height among the 3 yr in MSSL as compared to SSSL; and there were also significant differences between MSSL and SSSL in the same year (Table 2).

Plant Population Density

Increasing N application rates markedly increased plant population density of *L. chinensis* in both MSSL and SSSL (Table 2). Nitrogen application at most increased plant population density of *L. chinensis* by 1.93, 2.06, and 1.78 times in MSSL, and 1.90, 1.12, and 2.44 times in SSSL compared with the control in 2009, 2010, and 2011, respectively. Under the same N application, there were large changes in

Table 3. Yields of *Leymus chinensis* under different N application rates in both soils from 2009 to 2011.

Soil type	Treatment	Aboveground biomass			AGR† in 3 yr	RGR in 3 yr
		2009	2010	2011		
		dry wt. g m^{-2}			dry wt. g $m^{-2}d^{-1}$	
MSSL	N_0	208 ± 83aAns‡§	213 ± 16aA*	195 ± 15aA*	2.30 ± 0.06	–
	N_{30}	300 ± 50abA*	319 ± 29abA*	301 ± 17abA*	3.37 ± 0.07	1.13 ± 0.07
	N_{60}	483 ± 46bcA*	515 ± 55bcA*	462 ± 36bcA*	5.40 ± 0.17	3.17 ± 0.12
	N_{90}	558 ± 58bcdA*	717 ± 51cdA*	584 ± 73cA*	6.90 ± 0.56	4.60 ± 0.51
	N_{120}	683 ± 42cdA*	941 ± 151deA*	831 ± 24dA*	9.07 ± 0.81	6.83 ± 0.82
	N_{150}	767 ± 67dA*	876 ± 16deA*	965 ± 95deA*	9.63 ± 0.64	7.40 ± 0.69
	N_{180}	1108 ± 82eA*	1060 ± 105eA*	1044 ± 38deA*	11.90 ± 0.21	9.60 ± 0.20
	N_{210}	1442 ± 72fA*	1137 ± 148eA*	1151 ± 31eA*	13.80 ± 1.10	11.53 ± 1.09
SSSL	N_0	125 ± 26aAns	92 ± 13aA*	90 ± 8aA*	1.13 ± 0.13	–
	N_{30}	136 ± 12aA*	141 ± 8abA*	157 ± 24aA*	1.60 ± 0.06	0.43 ± 0.18
	N_{60}	171 ± 24aA*	196 ± 21bA*	201 ± 21abA*	2.10 ± 0.10	0.97 ± 0.23
	N_{90}	192 ± 20abA*	227 ± 25bA*	294 ± 18bcA*	2.63 ± 0.35	1.50 ± 0.46
	N_{120}	237 ± 10bA*	364 ± 25cA*	401 ± 69cdA*	3.70 ± 0.57	2.57 ± 0.70
	N_{150}	384 ± 23cA*	418 ± 13cdA*	452 ± 51dA*	4.63 ± 0.20	3.50 ± 0.32
	N_{180}	471 ± 20dA*	522 ± 10eA*	481 ± 47dA*	5.43 ± 0.19	4.30 ± 0.29
	N_{210}	443 ± 25cdA*	464 ± 69deA*	490 ± 32dA*	5.17 ± 0.15	4.00 ± 0.26

* Significance between MSSL and SSSL $P < 0.05$.

† AGR: absolute growth rates; RGR: relative growth rates; MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland.

‡ Means ± 1 SE. Significance among different N application rates was marked with lowercase letters, significance among different years under the same N treatment was marked with uppercase letters.

§ ns: no significant.

plant population density among the 3 yr in both MSSL and SSSL treatments. There were also large differences in plant population densities between MSSL and SSSL plots in the same year (Table 2). In 2011 there were no significant increases in plant density above N application of 90 kg ha^{-1} in SSSL and MSSL.

Leaf Area Index

The leaf area index (LAI) values were lower than 1.0 in the controls (0 applied N) of SSSL in the 3 yr, and they showed a decreasing trend with time from 2009 to 2011. The LAI values rose sharply after N was applied, and they increased at all N levels with time from 2009 to 2011 (Fig. 2a). The increase in LAI was large for the same N application treatment after 3 yr, indicating that N application significantly increased LAI compared to the control, and N application had a significant cumulative effect. Using the N_{180} treatment as an example, LAI values increased 2.36, 5.86, and 8.23 times compared to the control in 2009, 2010, and 2011, respectively.

The LAI values of the MSSL control treatment showed a decreasing trend with time (as did SSSL) during the 3 yr. The LAI values of the N application treatments were relatively constant with time under the same N application (Fig. 2b). The LAI values increased with N application up to 210 kg ha^{-1} during the first year. During subsequent years increase in N level above 150 kg ha^{-1} did not significantly increase LAI in 2010 and 2011. Although N application significantly increased LAI as N rate went up, there was no significant difference between the averaged LAI at the N level of 120 kg ha^{-1} and the LAI at 210 kg ha^{-1} in the 3 yr. In 2011 there were no increases in LAI above application of 120 kg ha^{-1} for SSSL and above 90 kg ha^{-1} for MSSL.

Aboveground Biomass

The biomass of *L. chinensis* increased significantly by N application from 2009 to 2011, as shown in Table 3. The control treatment always resulted in the lowest biomass either in MSSL or in SSSL in the 3 yr (Table 3). In MSSL, N application rate of 210 kg ha^{-1} always produced the highest biomass, but biomass values tended to decrease from 2009 to 2011. There were no statistical differences in yield above N 150 kg ha^{-1} in year 2010 and 2011.

In the SSSL treatments, N application rates of 180 kg ha^{-1} in 2009, 2010, and 210 kg ha^{-1} in 2011 produced the highest biomass, and biomass values tended to increase with time and cumulative effects. In 2011, there were no significant differences in yield above N applications of 120 kg ha^{-1}. Also, no significant differences were found among the 3 yr either in MSSL or in SSSL under the same N application. The biomass in MSSL was significantly larger than that in SSSL under the same N application in the same year; approximately double or greater at all N levels and years.

In MSSL, the AGR and RGR of *L. chinensis* increased linearly with the increase in N application ($R^2 > 0.99$). However, an increasing trend in biomass from 2009 to 2011 was only found with the N application rates of 90, 150, and 180 kg ha^{-1}, while the other treatments showed a decreasing trend with time (Table 3). In SSSL, AGR and RGR were the largest when N application rate was 180 kg ha^{-1}, and further increases in N resulted in decreased biomass. However, all N application treatments showed a growth trend in biomass from 2009 to 2011 ($R^2 > 0.95$).

Nitrogen Use Efficiency

Nitrogen use efficiency was significantly influenced by N application rates, years, and sodicity (Table 4). In MSSL, NUE increased significantly and linearly with increasing N application in the first year. The NUE first increased and then decreased with increasing N application above 120 kg ha^{-1} in 2010 and 2011. No notable differences for NUE values were observed under the same N application rates among the 3 yr. The result of continuous N application showed that the mean value of NUE was largest when the N application rate was 120 kg ha^{-1}.

Similarly, in SSSL, NUE first increased with increasing N application and then decreased above N of 180 kg ha^{-1} in 2009, 2010, and above 120 kg ha^{-1} in 2011. The NUE values in 2010 and 2011 were significantly higher than that in 2009 when N application was <150 kg ha^{-1} and there were no significant differences for NUE in the 3 yr when N application was >150 kg ha^{-1}. The mean value of NUE was the largest when the N application rate was 150 to 180 kg ha^{-1}. Nevertheless, under the same N application rate, the NUE in MSSL was significantly greater than that in SSSL. The values of

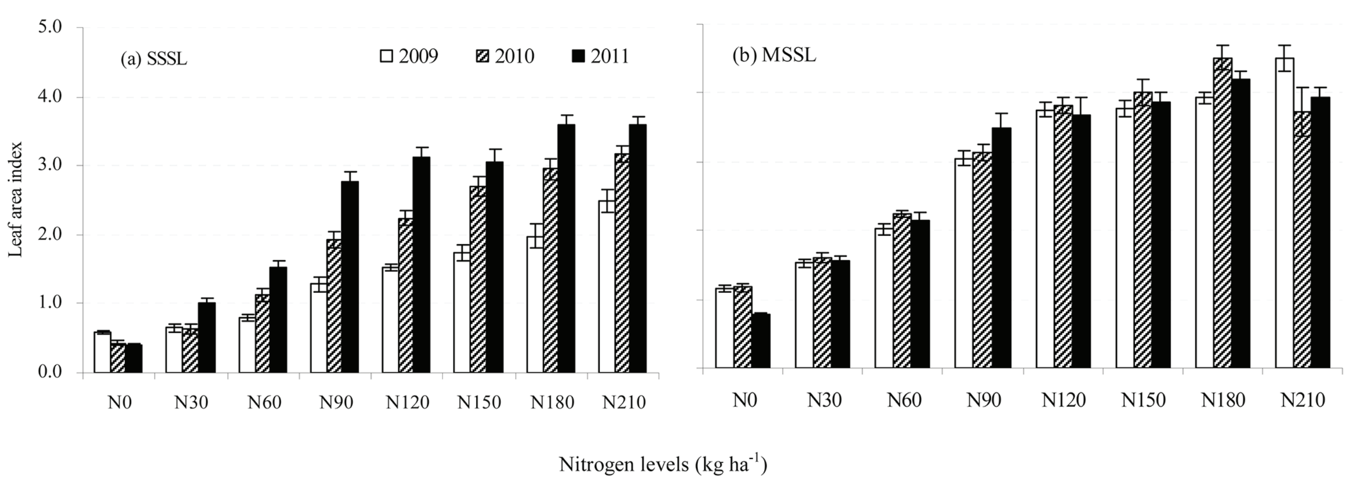

Fig. 2. Leaf area index of *Leymus chinensis* under different N application rates.

Table 4. Nitrogen use efficiency (NUE) of *Leymus chinensis* under different N application rates in both soils from 2009 to 2011.

Soil type	Treatment	NUE 2009	NUE 2010	NUE 2011	Average NUE in 3 yr	Trends of NUE from 2009 to 2011
		dry wt. kg N kg^{-1}				
MSSL†	N_0					
	N_{30}	30.5 ± 3.3aA*‡§	35.6 ± 4.6aA*	35.3 ± 5.6aA*	33.8 ± 1.6a*	+
	N_{60}	45.8 ± 7.7abcA*	50.4 ± 5.9aA*	44.5 ± 6.0aA*	46.9 ± 1.8b*	+
	N_{90}	38.9 ± 6.5abA*	56.0 ± 4.8aA*	43.3 ± 8.1aA*	46.1 ± 5.1ab*	+
	N_{120}	39.6 ± 3.5abA*	60.7 ± 6.9aA*	53.0 ± 2.0aA*	51.1 ± 6.2b*	+
	N_{150}	37.2 ± 4.4abA*	44.2 ± 0.9aA*	51.4 ± 7.0aA*	44.3 ± 4.1ab*	+
	N_{180}	50.0 ± 4.6bcA*	47.1 ± 5.9aA*	47.2 ± 2.1aA*	48.1 ± 1.0b*	–
	N_{210}	58.7 ± 8.2cA*	44.0 ± 7.4aA*	45.5 ± 6.2aA*	49.4 ± 4.7b*	–
SSSL	N_0					
	N_{30}	3.6 ± 0.8aA*	16.4 ± 2.8abB*	22.4 ± 2.6aB*	14.1 ± 5.6a*	+
	N_{60}	7.6 ± 2.3abA*	17.3 ± 3.5abB*	18.4 ± 2.7aB*	14.4 ± 3.4a*	+
	N_{90}	7.4 ± 2.2abA*	14.9 ± 2.8bB*	22.6 ± 2.2aB*	15.0 ± 4.4a*	+
	N_{120}	9.3 ± 0.8abA*	22.6 ± 2.1abB*	25.9 ± 5.2aB*	19.3 ± 5.1a*	+
	N_{150}	17.2 ± 1.5cA*	21.7 ± 0.9abA*	24.2 ± 3.6aA*	21.0 ± 2.0a*	+
	N_{180}	19.2 ± 1.1cA*	23.8 ± 0.5aA*	21.7 ± 2.6aA*	21.6 ± 1.3a*	+
	N_{210}	15.1 ± 1.2bcA*	17.7 ± 3.3abA*	19.1 ± 1.8aA*	17.3 ± 1.2a*	+

* Significance between MSSL and SSSL $P < 0.05$.

† MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland.

‡ Means ± 1 SE. Significance among different N application rates was marked with lowercase letters, significance among different years under the same N treatment was marked with uppercase letters.

§ +/– means that the trend of increasing or decreasing about NUE.

NUE in 2010 and 2011 indicate that NUE decreased when N application rate was more than 120 kg ha^{-1} in MSSL.

The results of the correlation analysis among different indicators of *L. chinensis* also indicated that N application significantly affected growth, yield, and NUE. Herbage yield and N application rates were highly correlated ($P < 0.001$), as shown in Table 5. Nitrogen application was crucial to increasing herbage yield in both MSSL and SSSL. Nitrogen use efficiency was significantly correlated with herbage yield ($P < 0.01$), while no significant correlation was found between NUE and N application rates. The lack of correlation is attributed to the initial increase in NUE and subsequent decrease with increasing N applications.

Nitrogen Status of Soil

Increasing N application resulted in a trend of increasing total N above N_{60} (Table 6). In a similar manner there was a general trend of increasing available N with increasing N application. Available N was still below typical levels under non-saline conditions (Huang et al., 2010).

The total N content for the MSSL plots showed a large increase relative to the initial condition, as shown in Table 6. Final soil N increased with increasing N application. The N_0 treatment had 0.5 and the N_{90} treatment a final N content of 1.19 g kg^{-1}, while the N_{180} treatment had a total N content of 1.79 g kg^{-1}. While increasing N application increased soil N, there was a diminishing increase in soil N with increasing N application. The available N contents also increased with increasing N application and were much greater than the initial values (Table 6). Above N_{90} the final values in the MSSL plots were within the range of natural non-saline communities of *L. chinensis*.

DISCUSSION

Nitrogen Application Restrains Grassland Degradation

Many studies have reported that N deficiency in grasslands is one of the dominant factors that hinder improvements in grassland productivity (Ries and Shugart, 2008; O'Halloran et al., 2010). The negative impacts of N deficiency on aboveground production has increased extensively in terrestrial ecosystems (LeBauer and Treseder, 2008), and especially in marginal lands such as salt-affected soils (Wang et al., 2010). Some researchers have evaluated the effects of N application on tomato (*Lycopersicon esculentum* Mill.) (Kutuk et al., 2004) and cotton (*Gossypium hirsutum* L.) (Chen et al., 2010; Dong et al., 2012) under salinity stress, and found that N application improved yield (Chen et al., 2010). The data from 3 yr of field trials indicated an almost linear relationship between N application rates and yields of *L. chinensis* in both MSSL and SSSL conditions (Tables 3 and 5).

Nitrogen application may improve not only yield of *L. chinensis*, but also restrain grassland degradation. The most typical and prominent feature of grassland degradation is the decline of aboveground biomass. Our results demonstrated that aboveground biomass decreased with time under no N application on saline–sodic soil. Nitrogen application improved aboveground biomass of *L. chinensis* year by year (Table 3). Miller (2001) estimated that about 34% of all grasslands in China were moderately to severely degraded due to environmental and human interference factors. There are 2.37×10^6 ha of saline–sodic grasslands in western Songnen Plain. Soil severe salinization is expanding at the rate of 1.4% annually in the western Songnen Plain (Liu et al., 2002). Grassland protection and restoration are urgent needs for the local governments. Nitrogen application is an effective method to quickly increase yield of *L. chinensis* and effectively suppresses grassland degradation under naturally saline–sodic

Table 5. Simple correlation coefficients between investigated traits.

Item	Nitrogen application rate	x_1	x_2	x_3	x_4
MSSL†					
Total aboveground biomass (herbage yield)-x_1	0.9829***				
Plant height-x_2	0.5967**	0.6416**			
Population density-x_3	0.6270**	0.6501**	0.7708***		
Leaf area index (LAI)-x_4	0.9318***	0.9256***	0.6506**	0.6352**	
Nitrogen use efficiency(NUE)-x_5	0.5335*	0.6583**	0.6181**	0.6226**	0.5751**
SSSL					
Total aboveground biomass (herbage yield)-x_1	0.9437***				
Plant height-x_2	0.6830**	0.6771**			
Population density-x_3	0.3616	0.4440	0.4814*		
Leaf area index (LAI)-x_4	0.8687***	0.9169***	0.6139**	0.5055*	
Nitrogen use efficiency(NUE)-x_5	0.4505	0.6871**	0.4301	0.5030*	0.6684**

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

† MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland.

Table 6. The changes in soil N content in different treatments after 3 yr of N application.

Soil type	N rates	Available N	Total N
		mg kg^{-1}	g kg^{-1}
MSSL†	N_0	37.3 ± 4.6ab‡	0.495 ± 0.033a
	N_{30}	29.2 ± 2.5a	0.610 ± 0.065ab
	N_{60}	43.8 ± 5.4b	0.755 ± 0.033b
	N_{90}	59.5 ± 4.6c	1.188 ± 0.084c
	N_{120}	68.3 ± 3.0cd	1.402 ± 0.052d
	N_{150}	76.4 ± 6.0d	1.565 ± 0.078e
	N_{180}	99.2 ± 3.6e	1.790 ± 0.030f
	N_{210}	93.9 ± 3.3e	1.735 ± 0.035f
SSSL	N_0	16.3 ± 1.7ab	0.313 ± 0.021ab
	N_{30}	15.2 ± 1.1a	0.278 ± 0.022a
	N_{60}	18.1 ± 1.2abc	0.315 ± 0.019ab
	N_{90}	25.7 ± 1.4de	0.338 ± 0.030ab
	N_{120}	23.3 ± 1.9cde	0.363 ± 0.025b
	N_{150}	21.6 ± 1.5bcd	0.635 ± 0.019c
	N_{180}	32.1 ± 2.3e	0.600 ± 0.018c
	N_{210}	24.5 ± 2.6de	0.597 ± 0.031c

† MSSL: moderate saline–sodic grassland; SSSL: severe saline–sodic grassland.

‡ Means ± 1 SE. Significance among different N application rates was marked with lowercase letters, $P < 0.05$.

field conditions. Therefore, understanding and exerting the positive role of N application is of great economic importance to *L. chinensis* production and salinization degradation grassland restoration in western Songnen Plain of northeastern China.

Nitrogen Application Promotes the Growth of *Leymus chinensis*

Some physiological effects of N application or climate change on plant growth of *L. chinensis* have been recognized in semiarid grassland conditions (Pan et al., 2004, 2005; Wan et al., 2008; Chen et al., 2005; Liu et al., 2012). However, the interaction effect of climate change and N application is still not fully understood, especially under naturally saline–sodic field conditions. Generally, the grassland production may vary considerably among years and also within a growing season due to climatic factors, even in grasslands with a relatively uniform rainfall distribution throughout the year (Herrmann et al., 2005). A smaller precipitation with uneven distribution is a typical characteristic of the climate in western Songnen Plain (Fig. 1), the growth of *L. chinensis* was greatly affected by interannual variability of the precipitation. Our data demonstrated differences in plant height, population density, and LAI of *L. chinensis* among the 3 yr under the same N application treatment in both salinity conditions.

Under the same N application treatment either in MSSL or in SSSL, plant height and population density of *L. chinensis* in 2010 were significantly greater than those in 2009 and 2011 (Table 2), but there were no notable differences on the aboveground biomass among the 3 yr (Table 3). This demonstrated that *L. chinensis* growth might be related to conditions of more rainfall and higher temperatures in the early growth of *L. chinensis* in 2010 even though the total rainfall and mean temperature were similar for the 3 yr. The aboveground biomass might be more related to total monthly rainfall and mean temperature under the same N application treatment in both salinity conditions (Fig. 1).

The LAI is a variable defining the assimilation area capable of absorbing photosynthetically active radiation (PAR), on which photosynthesis depends, and indirectly increases the biomass. Lepiarczyk et al. (2005) and Kulig et al. (2010) found a significant and high correlation between the values of LAI and aboveground dry mass and grain yield of wheat. Salvagiotti and Miralles (2008) found that the LAI of plants depended on the level of N fertilization. In the present study, the correlation analysis confirmed the above correlations between the values of LAI and N application rates and aboveground biomasses of *L. chinensis*. High correlation coefficients were obtained between N application rates and aboveground biomass and LAI of *L. chinensis* in both soils (Table 5). Compared with plant height and population density, the values of LAI and aboveground biomass had a better response to N application. The values of LAI can also represent well the aboveground biomass changes of *L. chinensis* under different N applications. Moreover, LAI can easily be determined by nondestructive means or by remote sensing and thus used to monitor the growth condition of plants and forecast yield of *L. chinensis*.

The Role of Nitrogen Application in Saline–Sodic Grassland

It is well known that soil salinity and sodicity can affect plant growth, yield, and quality. Recently, some studies found that N fertilizer application could improve the yield of plants grown under saline conditions (Jabeen and Ahmad, 2009; Chen et al., 2010; Yuan et al., 2010). This might be because N played both nutritional and osmotic roles in saline–sodic conditions (Ding et al., 2010). However, N management in saline–sodic land is particularly difficult due to influence of drought or salinity stresses (Rinehardt et al., 2004; Dong et al., 2012). The role and feasibility of N application is attracting increasing attention. Our previous research results showed that *L. chinensis* production could be maximized if soil pH was at or below 8.5(Huang and Liang, 2009). The soils used in this study belong to typical sodic meadow with characteristics of elevated pH and ESP levels, and low EC and N contents (Table 1). The 3-yr field study indicated that the growth and yield of *L. chinensis* were significant difference in both soils. Under no N fertilizer, the SSSL values of aboveground biomass and AGR of *L. chinensis* in the 3-yr study was only a half of those in MSSL. When N application rate was 180 kg N ha^{-1}, yields of *L. chinensis* increased by 5.4 and 4.9 times in MSSL and SSSL, respectively, revealing that the role of N application in increasing herbage yield in MSSL is more evident than that in SSSL.

Generally, N fertilizer can increase dry matter production of rangelands up to two- to three-fold depending on the annual rainfall and moisture in the region (Elliott and Abbott, 2003). Guevara et al. (2000) regarded that in regions where annual rainfall is <300 to 400 mm, fertilization of rangelands is not profitable. In the 3 yr (2009–2011) of this study the total rainfall in *L. chinensis* growing season was 397 mm in 2009, 336 mm in 2010, and 393 mm in 2011 with a gap in rainfall and snow accumulation during the winter season. Combining snow and rainfall the annual mean precipitation is only 414 mm in this region, very close to the range of 300 to 400 mm cited by Guevara et al. (2000). Therefore, it is significant that we established the feasibility of N application in saline–sodic grassland in this region.

Nitrogen Use Efficiency and the Optimum Nitrogen Rates

The NUE is an important indicator that is often used to evaluate the feasibility of N application. In recent years, improving NUE in agriculture production has become an urgent requirement due to increasing N fertilizer costs and increasing off-site pollution resulting from N fertilization (Bronson, 2008; Rochester et al., 2009). Generally, the parameters for evaluating NUE include N agronomic efficiency, N physiological efficiency, and N uptake efficiency (Delogu et al., 1998; López-Bellido and López-Bellido, 2001). Among these parameters, N agronomic efficiency can directly reflect the relationship between output and input (Vanlauwe et al., 2011). In the present study, NUE clearly showed the contribution of N application to increasing yield of *L. chinensis*. At the same time, it was also consistent with the definition of Oenema et al. (2012) about NUE for grassland as the ratio of N output and N input (N output in herbage yield, N input in application rate). Our data also indicated that N application had a cumulative effect over the 3 yr of study. The NUE showed an increasing trend from 2009 to 2011. The NUE was higher in MSSL than that in SSSL and NUE value was largest when the N application rate was 120 kg N ha^{-1} and 180 kg N ha^{-1} in MSSL and SSSL, respectively. Further N application above these values will result in decreasing NUE (Table 4). The increase in available N in the MSSL soil condition indicates that over the long term annual N applications can be reduced to below those observed in this study. In contrast under the SSSL soil condition there are relatively small increases in total and available N, indicating substantial N losses and a need to continual high N applications.

In conclusion, N application can promote growth of *L. chinensis* and increase herbage yield, and accelerate vegetation restoration in saline–sodic degraded grassland. Our study provided a favorable and effective method for *L. chinensis* production and vegetation restoration in western Songnen Plain of northeastern China and other areas with similar ecologies. Comprehensive consideration of yield of *L. chinensis* and NUE, the optimum rates of N application should be 90 to 120 kg N ha^{-1} in MSSL and 120 to 150 kg N ha^{-1} in SSSL.

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