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Effects of continuous nitrogen application on seed yield, yield components and nitrogen-use efficiency of *Leymus chinensis* in two different saline-sodic soils of Northeast China

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Abstract. The effect of nitrogen (N) application on seed yields and yield components in *Leymus chinensis* (Trin.) Tzvel., a perennial rhizomatous grass, was measured in a field experiment with two saline-sodic soils at Da'an Sodic Land Experiment Station during 2010–11. Two grassland field sites were classified as moderately saline–sodic (MSSL) and severely saline–sodic (SSSL). Application rates of N at each site were 0, 30, 60, 90, 120, 150, 180 and 210 kg ha^{-1}. Application of N significantly improved seed yield mainly through increased spike number (R^2 = 0.96, $P \leq 0.001$). Compared with nil N, seed yield increased 7.4–10.9 times with N application of 150 kg ha^{-1} at MSSL, and 5.3–7.5 times with N application of 120 kg ha^{-1} at SSSL. However, absolute increases at SSSL were relatively small. Some significant differences ($P \leq 0.01$) in seed yield occurred between 2010 and 2011 with different N application rates in the same soil, and between MSSL and SSSL in the same year. Increasing N application rate significantly decreased N physiological efficiency (NPE) but increased N apparent-recovery fraction (NRF) and N partial-factor productivity (NPP) at both sites. Seed yield and NPP indicated that the optimal N application rates to increase yield were 150 kg ha^{-1} at MSSL and 120 kg ha^{-1} at SSSL. High soil pH was the major factor adversely impacting seed yield, and pH and soil salinity were major factors negative affecting NPE, NRF and NPP as well as decreasing the positive effect of N application. Nitrogen application is a practical and effective method to increase seed yield of *L. chinensis* in saline-sodic grasslands of Northeast China, particularly when soil pH and salinity are not limiting.

Additional keywords: Chinese ryegrass, false wheatgrass, soil electrical conductivity.

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Introduction

Soil salinity has an important impacts on plant establishment and growth (Maas and Hoffman 1977; Grieve *et al.* 2012; Han *et al.* 2015). Numerous studies have demonstrated that salinity can strongly hinder plant seed germination (Huang *et al.* 2008*a*; Zhang *et al.* 2010), seedling growth (Song *et al.* 2009) and productivity (Caines and Shennan 1999) through ion toxicity, osmotic stress and nutrient deficiencies or imbalances (Munns and Tester 2008). Some studies have also shown that nutrient addition can effectively increase plant yield under saline condition (Liu *et al.* 2005; Gimeno *et al.* 2009; Semiz *et al.* 2014). However, the effects of soil salinity on nutrient efficiency, and the impacts of the interaction of salinity and nutrients on plant growth, are still not well understood.

Leymus chinensis (Trin.) Tzvel. (family Poaceae), is a perennial rhizomatous grass that is widely distributed in the eastern region of the Eurasian steppe, and occurs in China in the Songnen Plain and in the eastern Inner Mongolian Plateau (Kuo 1987; Xiao *et al.* 1995). It is rich in vitamins, high-quality proteins, minerals and carbohydrates, and is highly palatable to animals. It tolerates drought (Bai *et al.* 2004) and saline-alkaline stresses, being able to survive in highly sodic soils and at soil pH of 8.5–11.5 (Jin *et al.* 2008). It is considered one of the most promising grass species for grassland rehabilitation and reconstruction in arid regions of northern China (Liu and Han 2008).

In recent decades, grasslands of *L. chinensis* in the western Songnen Plain have undergone significant degradation due to soil salinisation, droughts and human interference. The addition of nitrogen (N) increased plant height and population density of *L. chinensis* (Pan *et al.* 2004, 2005), significantly increased the N concentration and decreased the carbon (C) : N ratio of plant tissues, and enhanced the photosynthetic rate and water-use efficiency of this species (Chen *et al.* 2005; Ren *et al.* 2014). High water-use efficiency and high shoot potassium (K^+) : sodium (Na^+) ratio are two of the most important physiological mechanisms for salinity tolerance of *L. chinensis* (Huang *et al.* 2008*b*, 2009). Nitrogen application was found to improve *L. chinensis* hay yield as well as accelerating the restoration of vegetation in saline-sodic degraded grassland (Huang *et al.* 2010, 2015).

Several experiments have shown that seeding is the most economical and effective way to improve or re-establish *L. chinensis* populations on degraded grasslands (Montalvo *et al.* 2002; Liu *et al.* 2015). However, the limited availability of seed has severely constrained the practical application of seeding. *Leymus chinensis* has low heading percentage (average of 19.2%), low seed-setting percentage (average of 24.1%) and low seed yield (30–100 kg ha^{-1}) under natural conditions (Wang *et al.* 2010, 2013). Many researchers have focused on the problem of its low sexual reproductivity. Several investigations have found that climate (Yang *et al.* 2000), nutrient uptake (Wang 1998) and vegetative growth (Wang and Ripley 2000), as well as human and animal interference (Yang and Zhu 1989), can adversely influence seed production of *L. chinensis.* Nitrogen addition can markedly improve aboveground productivity of the plant (Zhu 2004; Huang *et al.* 2015), but it is unknown whether addition of N can increase seed yield of *L. chinensis*. In some studies, addition of N significantly reduced the flowering probability, individual seed mass and seed number (Bai *et al.* 2009). However, other studies have shown that addition of N at intermediate but not at higher levels in autumn can moderately increase seed yield and yield components of *L. chinensis* (Chen *et al.* 2013), or that addition of N at all application rates can dramatically increase yield (Wang *et al.* 2013). Thus, further investigation of the effects of N addition on seed yield of *L. chinensis* and an understanding of the N-utilisation strategy of this plant are very important for restoration and reconstruction of high-quality forage populations in the saline-sodic degraded grasslands of Songnen Plain in Northeast China.

Previous field studies have reported that continuous applications of N significantly affected the hay yield and N-utilisation efficiency (NUE) of *L. chinensis*, and that these effects were very different in two saline-sodic soils (Huang *et al.* 2015). Therefore, the purposes of the present study were (*i*) to determine seed yields under different N application levels in two saline-sodic soils, (*ii*) to examine the effects of spring N application on seed yield and NUE of *L. chinensis*, and (*iii*) to clarify the relationship between NUE and seed yield of *L. chinensis* in two different saline-sodic soils of Songnen Plain, Northeast China.

Materials and methods

Experimental site

A 2-year field experiment was carried out in 2010–11 at Da'an Sodic Land Experiment Station (DASLES, 45°35′58″–45°36′28″N, 123°50′27″–123°51′31″E, 150–200 m a.m.s.l.), part of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (CAS), in Da'an city, Jilin Province, China (Fig. 1). DASLES is a typical saline-sodic degraded grassland ecosystem in the hinterland of Songnen Plain. The climate is classified as semi-arid and temperate continental, with mean annual air temperature of 4.3°C, varying from –20°C (January) to 26°C (July), and mean annual rainfall of 414 mm. Potential evaporation is 1750 mm, 4.2 times the mean annual rainfall. Temperature and rainfall data were collected from the DASLES weather station during the experiment. Total rainfall was 336 mm in 2010 and 393 mm in 2011. Monthly distributions of rainfall and, to a lesser extent, temperature during the growing season were quite different between years, as shown in Fig. 2.

The experimental field soil was saline-sodic meadow (Huang *et al.* 2015). We selected two *L. chinensis* grasslands with different soil pH and salinity levels. One site was a moderately saline-sodic grassland (MSSL), with soil pH (1 : 5 soil–water extract) 8.94, soil electrical conductivity (EC_e) of saturation extract 5.7 dS m^{-1} (calculated from 1 : 5 soil extract), soil exchangeable sodium percentage (ESP) 37.9%, organic matter content 13.26 g kg^{-1}, and total N content 0.49 g kg^{-1}. The other site was classified as a severely saline-sodic grassland (SSSL), with soil pH 9.80, soil extract EC_e 8.8 dS m^{-1}, soil ESP 50.3%, organic matter content 11.57 g kg^{-1}, and total N content 0.39 g kg^{-1}. There were significant differences in soil salinity and sodicity, and no statistical differences in soil nutrient content between sites (Huang *et al.* 2015). Both sites were dominated by *L. chinensis* (>90%) and were typical pure *L. chinensis* communities. The vegetation showed evidence of degradation (decreased productivity) because of soil salinisation (Huang *et al.* 2015).

Experimental design

The experiment was a completely randomised block design, with three replicates at each site. Eight N levels applied as urea (N content 46.2%) were as follows: 0 (control), 30, 60, 90, 120, 150, 180 and 210 kg N ha^{-1}. Hence, there were 24 plots (3 m by 3 m each) at each site. There was a 1-m gap between adjacent plots with separation maintained by cement boards installed to a depth of 10 cm in the soil. The experiment sites had been fenced and they had never received fertiliser before the experiment was started.

Nitrogen fertiliser application to the experimental sites began in 2009, and N was applied once per year. *Leymus chinensis* annually resumes growth in early April at this location, and the N fertiliser was applied on the surface of the grassland in early evenings of cloudy days in mid-May. In order to dissolve urea granules quickly and minimise fertiliser loss due to volatilisation, water (10 mm) was slowly and uniformly sprayed on each plot after fertilisation. Field management was the same for all plots at both sites. We maintained natural growth of *L. chinensis* and avoided any other human interference.

Sampling and measurement

Heading of *L. chinensis* in Songnen grassland is generally initiated in early June and seeds are mature in late July; thus, late July was selected for sampling in this experiment. The sampling methods were reported previously (Huang *et al.* 2015). All of the spikes of *L. chinensis* were cut along the spike shank in a sampling area of 0.5 m by 0.5 m at three

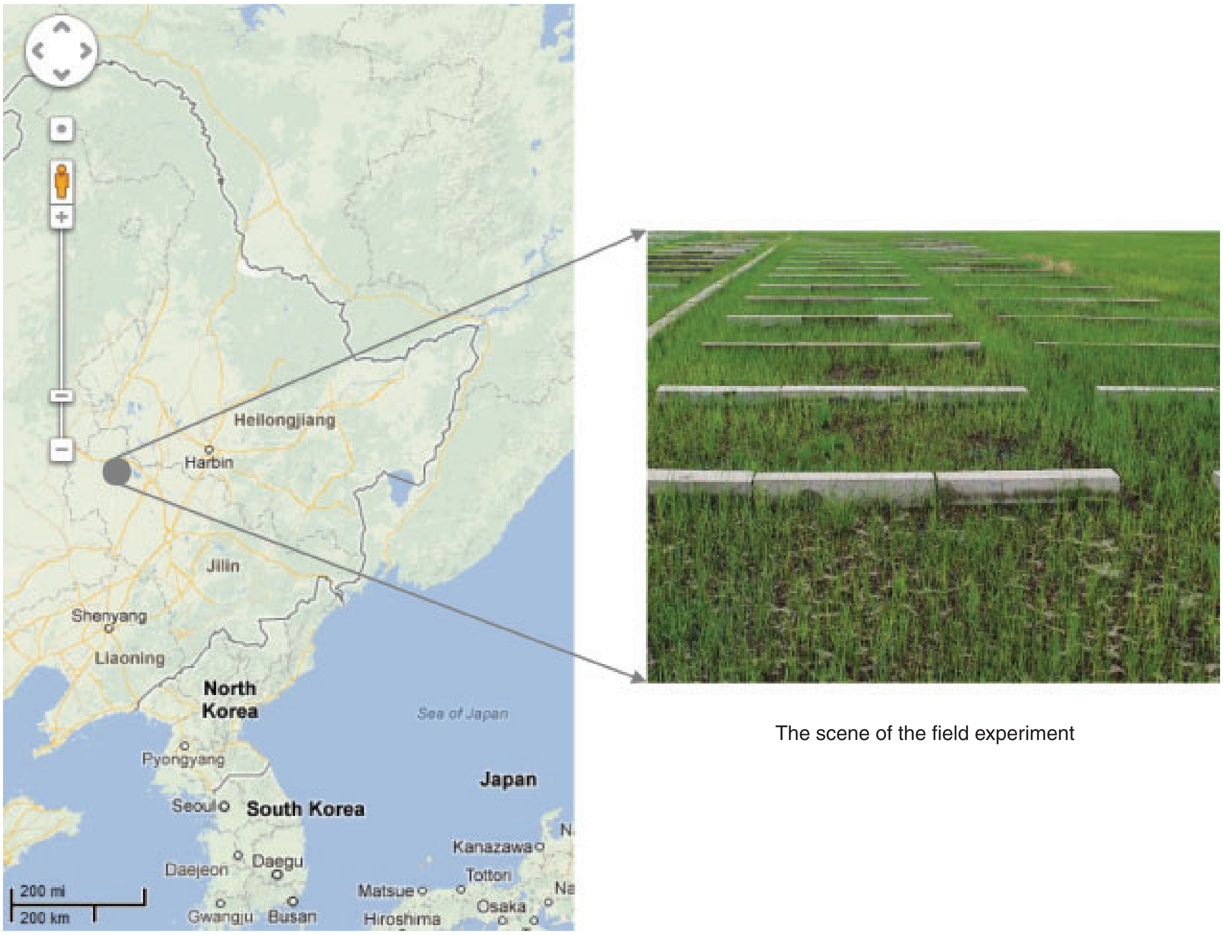

Fig. 1. Location of the study sites, Da'an city, Jilin, China (based on map data ©2016 Google Maps).

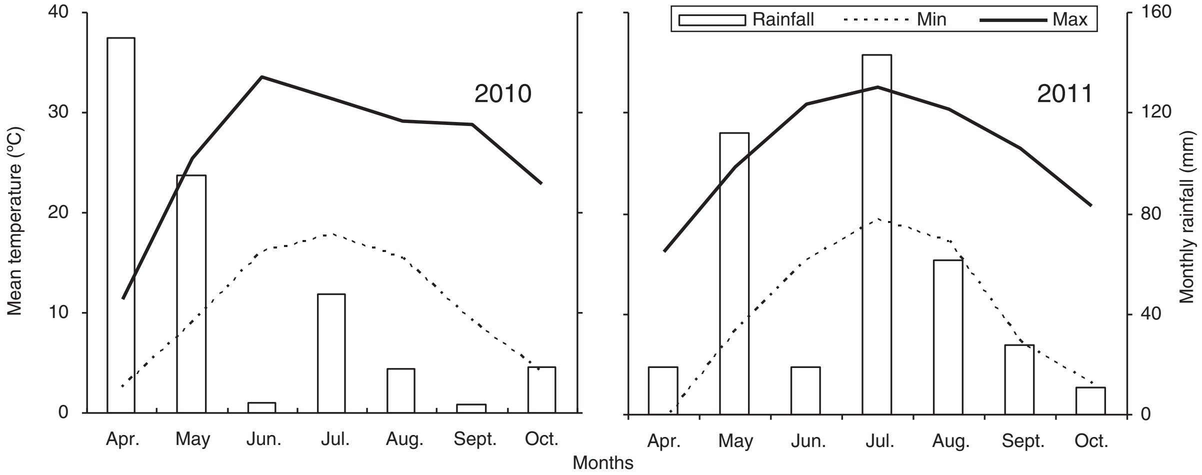

Fig. 2. Mean maximum and minimum temperature (°C) and monthly rainfall (mm) during the growing seasons of 2010 and 2011.

random sites in each plot in both 2010 and 2011. The lengths of all spikes were measured in each sampling area, then all spikes from each sampling site were placed into individual paper bags and air-dried for three weeks in a laboratory. We analysed the shoot concentration of total N (H_2SO_4–H_2O_2 digestion, semi-micro distillation method) to calculate N-absorption efficiency of *L. chinensis* (Bao 2000), and measured the population density and hay yield based on a biomass sample (Huang *et al.* 2015).

Heading percentages were calculated as the ratio of number of spikes producing seeds to plant number per unit area multiplied by 100. Thousand-seed weights (g) were calculated from subsamples, each with 100 seeds. Seed yield was determined as:

$$\begin{aligned}\text{Seed yield } (\text{g m}^{-2}) &= \text{no. of spikes } (0.25\ \text{m}^{-2})\\ &\times 4\ (\text{conversion coefficient})\\ &\times \text{no. of filled seeds per spike}\\ &\times 1000\text{-seed weight}\end{aligned}$$

Nitrogen-efficiency terminology followed Moll *et al.* (1982), Pierce and Rice (1988) and Delogu *et al.* (1998). The following three N-efficiency parameters were calculated for each treatment in the present study:

1. Nitrogen physiological efficiency (NPE, g g^{-1}), calculated as the ratio of (hay yield at N_x – hay yield at N_0) to (N uptake at N_x – N uptake at N_0), i.e. NPE (g g^{-1}) = $(W_{FD} - W_{CD})/(N_F - N_C)$ (López-Bellido and López-Bellido 2001).
2. Nitrogen apparent-recovery fraction (NRF, %), calculated as the ratio of (N uptake at N_x – N uptake at N_0) to applied N at N_x, i.e. NRF (%) = $(N_F - N_C)/M_F \times 100$ (López-Bellido and López-Bellido 2001).
3. Nitrogen partial-factor productivity (NPP, g g^{-1}), calculated as the ratio of seed yield at N_x to applied N at N_x, i.e. NPP (g g^{-1}) = W_G/M_F (Xu *et al.* 2009).

Here, N_x is N application where x is rate from 30 to 210 kg N ha^{-1} and N_0 is nil N treatment; W_{FD} and W_{CD} are the dry weight of the *L. chinensis* aboveground biomass per m^2 with and without N application, respectively; N_F and N_C are the total aboveground plant N uptake with and without N application, respectively; and M_F is the N application amount per m^2 and W_G is the seed yield at a particular N application rate.

Statistical analyses

Data are expressed as mean ± 1 standard error. Treatments were analysed in a two-way analysis of variance (N treatment and soil type) by year, using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Analyses of significance ($P < 0.05$) of the various response variables were undertaken for different N application levels and between the two soils by general linear model (*F*-test). Annual differences of the various response variables were compared individually by paired two-sample average analysis (*t*-test). Correlation analyses ($n = 27$, $P < 0.05$, $P < 0.01$ or $P < 0.001$) between N application rates and the various response variables, and between one variable and other variables, were also conducted. Stepwise regression analyses were used to examine the contribution of site (soil salinity and pH), year (climatic variation) and N application rates or yield components to seed yield of *L. chinensis*.

Results

Seed yield and yield components

Seed yield

Seed yield of *L. chinensis* significantly ($P < 0.05$) increased with increasing N application rate (Table 1). Seed yield reached a maximum with N application rate of 150 kg ha^{-1} at MSSL and 120 kg ha^{-1} at SSSL; seed yield thus increased from the control value of 14.7 to 123 g m^{-2} in 2010 and from 3.6 to 42.8 g m^{-2} in 2011 at MSSL, and from 4.7 to 29.6 g m^{-2} in 2010 and from 2.3 to 19.6 g m^{-2} in 2011 at SSSL (Table 1). For both soils, there were highly significant ($P < 0.01$) differences in seed yield of *L. chinensis* between 2010 and 2011, and the average seed yields in 2010 were 3.4 and 2.2 times those in 2011 at MSSL and SSSL, respectively (Table 2). Within the same year there were also highly significant ($P < 0.01$) differences in seed yield between sites MSSL and SSSL. The average seed yield at MSSL was much higher than at SSSL in both 2010 (76.7 vs 17.6 g m^{-2}) and 2011 (22.7 vs 7.9 g m^{-2}), as shown in Table 2. Stepwise

Table 1. Seed yields (g m^{-2}) of *Leymus chinensis* under different nitrogen application rates at both sites in 2010 and 2011, and two-way ANOVA (nitrogen treatment and soil type) by year

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland. Values are means ± 1 standard error. Within columns, means followed by the same letter are not significantly different ($P > 0.05$). *F*-values: for block, $F_{0.05}$ (2,30) = 3.32 and $F_{0.01}$ (2,30) = 5.39; for the factor soil, $F_{0.05}$ (1,30) = 4.17 and $F_{0.01}$ (1,30) = 7.56; for the factor nitrogen, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (2,30) = 3.30; for the interaction, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (7,30) = 3.30. **$P \leq 0.01$; n.s., not significant ($P > 0.05$)

Treatment (kg N ha^{-1})	2010		2011	
	MSSL	SSSL	MSSL	SSSL
0	14.7 ± 2.00d	4.7 ± 1.14	3.6 ± 0.44c	2.3 ± 0.50b
30	29.3 ± 1.53d	7.9 ± 0.37	5.8 ± 1.80c	3.3 ± 0.60b
60	90.2 ± 11.66bc	15.3 ± 0.89	12.8 ± 2.35c	6.0 ± 1.07b
90	76.7 ± 13.32c	19.3 ± 1.08	25.1 ± 3.97b	7.2 ± 0.35b
120	96.5 ± 14.13abc	29.6 ± 1.77	30.5 ± 2.15b	19.6 ± 3.11a
150	123.1 ± 22.82a	28.2 ± 3.52	42.8 ± 2.01a	11.2 ± 0.25ab
180	107.8 ± 7.08ab	23.0 ± 4.78	31.9 ± 3.88b	8.3 ± 1.62b
210	75.1 ± 16.64c	13.0 ± 1.86	28.9 ± 0.66b	5.0 ± 0.67b

Source	*F*-value	
Block	0.333n.s.	0.911n.s.
Soil	146.754**	81.710**
Nitrogen	10.972**	14.957**
Soil × nitrogen	4.577**	5.771**

Table 2. Annual mean for seed yield and yield components of *Leymus chinensis* under different nitrogen application rates at the same site, and significance analysis (*t*-test) for 2010 and 2011

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland. Values are means ± 1 standard error. $t_{0.05}$ (7) = 2.3646, $t_{0.01}$ (7) = 3.4995. $^*P \leq 0.05$; $^{**}P \leq 0.01$; n.s., not significant ($P > 0.05$)

Site/soil type	Year	Seed yield (g m^{-2})	Length of spikes (cm)	No. of spikes (per m^2)	Heading percentage (%)	No. of filled seeds per spike	1000-seed weight (g)
MSSL	2010	76.7 ± 13.21	13.4 ± 0.69	751 ± 107.8	28.4 ± 1.57	41 ± 3.0	2.39 ± 0.037
	2011	22.7 ± 4.90	13.3 ± 0.60	211 ± 43.6	15.4 ± 2.49	41 ± 1.6	2.60 ± 0.049
	t-value	5.8963**	0.2007n.s.	7.1646**	8.8046**	0.0490n.s.	4.6761**
SSSL	2010	17.6 ± 3.20	12.5 ± 0.31	234 ± 41.1	21.2 ± 3.99	33 ± 1.6	2.25 ± 0.070
	2011	7.9 ± 1.95	12.2 ± 0.48	104 ± 30.8	10.8 ± 1.91	43 ± 2.6	1.92 ± 0.079
	t-value	5.6560**	0.7919n.s.	5.2918**	3.1902*	3.2581*	3.8421**

Table 3. Spike characteristics of *Leymus chinensis* under different nitrogen application rates at both sites in 2010 and 2011, and two-way ANOVA (nitrogen treatment and soil type) by year

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland. Values are means ± 1 standard error. Within columns and sites, means followed by the same letter are not significantly different ($P > 0.05$). *F*-values: for block, $F_{0.05}$ (2,30) = 3.32 and $F_{0.01}$ (2,30) = 5.39; for the factor soil, $F_{0.05}$ (1,30) = 4.17 and $F_{0.01}$ (1,30) = 7.56; for the factor nitrogen, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (2,30) = 3.30; for the interaction, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (7,30) = 3.30. $^*P \leq 0.05$; $^{**}P \leq 0.01$; n.s., not significant ($P > 0.05$)

Site/soil type	Treatment (kg N ha^{-1})	Length of spike (cm) 2010	Length of spike (cm) 2011	No of spikes (per m^2) 2010	No of spikes (per m^2) 2011	Heading percentage (%) 2010	Heading percentage (%) 2011
MSSL	0	10.8 ± 0.33d	10.9 ± 0.10c	229 ± 11.0d	31 ± 1.3e	21.2 ± 2.59c	5.1 ± 0.46d
	30	12.5 ± 0.41cd	10.9 ± 0.24c	354 ± 46.4d	66 ± 3.4de	22.6 ± 2.26bc	6.7 ± 0.32cd
	60	11.9 ± 0.30cd	12.4 ± 0.20bc	817 ± 90.2bc	123 ± 10.0d	29.8 ± 4.25abc	12.0 ± 1.28cd
	90	12.8 ± 0.52cd	13.9 ± 0.23ab	850 ± 94.6bc	221 ± 50.8c	30.1 ± 1.48abc	13.6 ± 1.85bc
	120	12.8 ± 0.44cd	13.7 ± 0.24ab	1029 ± 112.3ab	293 ± 4.4ab	33.6 ± 2.41a	20.3 ± 1.76ab
	150	14.1 ± 0.56bc	15.1 ± 0.21a	1075 ± 138.3a	340 ± 12.7	32.0 ± 3.56ab	22.2 ± 1.44a
	180	15.5 ± 0.74ab	14.6 ± 0.37ab	908 ± 83.9abc	270 ± 52.5bc	31.1 ± 1.56abc	22.8 ± 1.44a
	210	16.7 ± 0.51a	15.0 ± 0.20a	744 ± 70.4 c	346 ± 24.1	27.1 ± 3.12abc	20.2 ± 1.97ab
SSSL	0	11.2 ± 0.34	9.8 ± 0.16c	71 ± 10.4d	34 ± 3.5b	9.1 ± 2.28b	8.7 ± 1.78bc
	30	12.0 ± 0.58	10.8 ± 0.11bc	105 ± 7.9cd	41 ± 2.6b	11.4 ± 1.02b	11.0 ± 1.76bc
	60	13.0 ± 0.58	12.6 ± 0.15ab	163 ± 5.8bcd	56 ± 3.2b	13.2 ± 0.99 b	8.8 ± 1.62bc
	90	13.2 ± 0.38	13.8 ± 0.30a	272 ± 43.4abcd	62 ± 2.8b	15.5 ± 2.34 b	7.3 ± 1.61c
	120	12.7 ± 0.32	11.8 ± 0.17abc	339 ± 20.1ab	270 ± 23.2a	32.7 ± 3.52 a	21.9 ± 1.71a
	150	13.4 ± 0.33	13.1 ± 0.21ab	403 ± 61.0a	209 ± 23.1a	38.4 ± 4.22a	15.6 ± 1.14ab
	180	13.4 ± 0.38	12.7 ± 0.46ab	305 ± 36.5abc	100 ± 19.7b	31.5 ± 1.77a	7.3 ± 1.44c
	210	11.4 ± 0.40	13.2 ± 0.39ab	214 ± 56.3abcd	62 ± 1.7b	17.5 ± 2.83b	5.9 ± 0.42c
Source				*F*-value			
Block		0.086n.s.	0.143n.s.	0.132n.s.	0.991n.s.	0.263n.s.	0.103n.s.
Soil		4.703*	8.089**	219.245**	94.779**	20.778**	14.855**
Nitrogen		3.819**	7.366**	17.687**	37.474**	11.113**	8.745**
Soil × nitrogen		3.171*	0.749	4.161**	9.720**	3.333**	5.176**

regression analysis showed that site (soil pH and EC) was the major factor affecting seed yield of *L. chinensis* (R^2 = 0.31, $P \leq 0.001$) followed by year and then N (overall R^2 = 0.65).

Spike length

Spike length of *L. chinensis* significantly ($P < 0.05$) increased with increasing N application rate at both sites (Table 3). At MSSL, when N application rate increased from 30 to 210 kg ha^{-1}, spike length increased from the control value of 1.1 to 5.9 cm in 2010 and from 0 to 4.1 cm in 2011. At SSSL, spike length correspondingly increased from 0.2 to 2.2 cm in 2010 and from 1.0 to 4.0 cm in 2011. Soil type and N application level significantly ($P < 0.05$) affected spike length of *L. chinensis*. In the same soil (at either MSSL or SSSL), there were no significant differences in spike length changes between 2010 and 2011 (Table 2). Stepwise regression analysis showed that N application rate was the main factor affecting spike length of *L. chinensis* (R^2 = 0.52, $P \leq 0.001$).

Spike number

Spike number of *L. chinensis* increased with increasing N application rate (Table 3), similar to the trend in spike length. There were significant differences in spike number between 2010 and 2011 at both soils ($P \leq 0.01$). We also found differences between soils in the same year ($P \leq 0.01$). Compared with the control, spike number increased 0.5–3.7 times with increasing N in 2010 and 1.1–10.2 times in 2011 at MSSL, and 0.5–4.7 times in 2010 and 0.2–6.9 times in 2011 at SSSL (Table 3). Stepwise regression analysis showed that the spike number of *L. chinensis* was most influenced by year, followed by site (pH and EC) and N application rate (overall $R^2 = 0.69$, $P \leq 0.001$).

Heading percentage

In both soils, there were significant ($P < 0.05$) annual differences in heading percentage of *L. chinensis*. Heading percentages in 2010 were much higher than in 2011 for both soils (Table 2). There were also significant ($P < 0.01$) differences in average heading percentages between MSSL and SSSL. As N rate increased, heading percentage increased by 6.7–58.5% in 2010 and 31.4–347.1% in 2011 compared with the control at MSSL, and by 25.2–321.9% in 2010 at SSSL. However, heading percentages under some N application treatments were even lower than under the control at SSSL in 2011 (Table 3).

Filled seed number and 1000-seed weight

At MSSL, the number of filled seeds per spike significantly ($P < 0.05$) increased with increasing N only in 2010. At SSSL, the number of filled seeds per spike significantly ($P < 0.05$) increased when N application rate was 60 kg ha^{-1} in 2010 and 90 kg ha^{-1} in 2011 compared with the control. Significant ($P < 0.05$) differences in 1000-seed weight of *L. chinensis* occurred under different N applications only at SSSL in 2010 (Table 4). At MSSL, the mean of number of filled seeds was the same in 2010 and 2011 (Table 2), but the average 1000-seed weight was significantly ($P < 0.01$) greater in 2011 than in 2010. By contrast, at SSSL, the average number of filled seeds per spike was less in 2010 than in 2011, and the average 1000-seed weight was also significantly ($P < 0.01$) greater in 2010 than in 2011. In 2011, the average 1000-seed weight was significantly ($P < 0.01$) greater at MSSL than at SSSL.

Correlation analyses between seed yield and yield component factors

Correlation analysis between seed yield and yield component factors of *L. chinensis* indicated that seed yield, spike number and heading percentage were highly correlated ($P < 0.001$) in both soils (Table 5). Stepwise regression analysis showed that application of N increased seed yield of *L. chinensis*, mainly by increasing spike number (or heading percentage) in saline-sodic grassland ($R^2 = 0.96$, $P \leq 0.001$). At MSSL, there were significant ($P < 0.01$) negative correlations between 1000-seed weight and seed yield, spike number and heading percentage. There were also significant ($P < 0.05$) correlations between the number of filled seeds and seed yield and spike length. However, no significant correlations were found among seed-yield component factors (except for spike number or heading percentage) of *L. chinensis* at SSSL.

Table 4. Filled seed number and 1000-seed weight of *Leymus chinensis* under different nitrogen application rates at both sites in 2010 and 2011, and two-way ANOVA (nitrogen treatment and soil type) by year

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland. Values are means ± 1 standard error. Within columns and sites, means followed by the same letter are not significantly different ($P > 0.05$). *F*-values: for block, $F_{0.05}$ (2,30) = 3.32 and $F_{0.01}$ (2,30) = 5.39; for the factor soil, $F_{0.05}$ (1,30) = 4.17 and $F_{0.01}$ (1,30) = 7.56; for the factor nitrogen, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (2,30) = 3.30; for the interaction, $F_{0.05}$ (7,30) = 2.33 and $F_{0.01}$ (7,30) = 3.30. *$P \leq 0.05$; **$P \leq 0.01$; n.s., not significant ($P > 0.05$)

Site/soil type	Treatment (kg N ha^{-1})	No. of filled seeds per spike		1000-grain weight (g)	
		2010	2011	2010	2011
MSSL	0	25 ± 2.7d	43 ± 3.6	2.56 ± 0.054	2.71 ± 0.054
	30	34 ± 2.8c	33 ± 3.0	2.50 ± 0.100	2.52 ± 0.306
	60	46 ± 2.2ab	41 ± 6.0	2.38 ± 0.080	2.61 ± 0.087
	90	37 ± 0.8bc	40 ± 2.2	2.43 ± 0.181	2.77 ± 0.215
	120	43 ± 3.4abc	43 ± 1.4	2.22 ± 0.075	2.43 ± 0.039
	150	49 ± 3.4a	47 ± 1.6	2.33 ± 0.167	2.71 ± 0.138
	180	50 ± 2.3a	44 ± 4.6	2.37 ± 0.086	2.67 ± 0.221
	210	42 ± 4.9abc	35 ± 0.2	2.33 ± 0.075	2.39 ± 0.165
SSSL	0	27 ± 3.0b	35 ± 3.1bc	2.39 ± 0.093abc	1.88 ± 0.185
	30	30 ± 2.8	41 ± 4.8abc	2.52 ± 0.170a	2.01 ± 0.359
	60	41 ± 1.9a	48 ± 4.7ab	2.28 ± 0.040abcd	2.20 ± 0.238
	90	33 ± 1.0ab	53 ± 3.7a	2.11 ± 0.104cd	2.24 ± 0.279
	120	35 ± 2.3ab	38 ± 4.9bc	2.47 ± 0.111ab	1.93 ± 0.096
	150	35 ± 1.5ab	32 ± 1.1c	2.00 ± 0.077d	1.74 ± 0.174
	180	34 ± 2.9ab	45 ± 7.2abc	2.17 ± 0.136bcd	1.68 ± 0.184
	210	27 ± 5.2b	48 ± 2.4ab	2.03 ± 0.082d	1.65 ± 0.137
Source		*F*-value			
Block		1.186n.s.	1.352n.s.	2.377*	0.534n.s.
Soil		25.547**	0.768n.s.	7.211*	45.922**
Nitrogen		7.415**	0.996n.s.	2.797*	1.069n.s.
Soil × nitrogen		2.247n.s.	2.686*	1.789n.s.	0.638n.s.

Table 5. Correlation analysis of nitrogen application rate, seed yield, yield component factors and nitrogen physiological efficiency (NPE) of *Leymus chinensis* at two saline-sodic sites on the western Songnen Plain of China

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland. *$P < 0.05$ (0.482); **$P < 0.01$ (0.606); ***$P < 0.001$ (0.725)

	N application rate	Seed yield	Spike length	No. of spikes	Heading percentage	No. of filled seeds	1000-seed weight
				MSSL			
Seed yield	0.4830*						
Spike length	0.9342***	0.4224					
No. of spikes	0.4438	0.9816***	0.3681				
Heading percentage	0.5071*	0.8844***	0.4457	0.9142***			
No. of filled seeds	0.5060*	0.6046*	0.5135*	0.4786	0.3619		
1000-seed weight	–0.3585	–0.7230**	–0.2014	–0.7894***	–0.7136**	–0.1497	
NPE	–0.7178**	0.2007	–0.6330**	0.2573	0.2247	–0.1787	–0.2552
				SSSL			
Seed yield	0.4067						
Spike length	0.5317*	0.4638					
No. of spikes	0.4642	0.9697***	0.4160				
Heading percentage	0.3477	0.9252***	0.2798	0.9300***			
No. of filled seeds	0.0835***	–0.2826	0.3739	–0.4168	–0.3806		
1000-seed weight	–0.5047*	0.2290	0.0098	0.1097	0.1991	–0.2539	
NPE	–0.8743***	–0.2581	–0.2927	–0.3330	–0.1560	0.2940	0.4198

Nitrogen efficiency

Nitrogen physiological efficiency

The NPE was significantly ($P < 0.05$) influenced by N application rate whether at MSSL or SSSL (Tables 5 and 6). NPE decreased with increasing N application rate in both soils. There was a significant difference in NPE between 2010 and 2011 in each soil. NPE was much higher in 2010 than in 2011 under different N application treatments at MSSL; conversely, NPE was lower in 2010 than in 2011 at SSSL. There was no significant difference in average NPE between MSSL and SSSL in 2010, but the difference in NPE was significant for the same N applications in both soils in 2011. Stepwise regression analysis showed that N application rate and site (soil pH and EC) were the main factors affecting NPE of *L. chinensis* (overall $R^2 = 0.66$, $P \leq 0.001$). Correlation analysis also indicated lesser effects of NPE on seed yield (Table 5).

Nitrogen apparent-recovery fraction

The NRF is often used as an index for evaluating N-uptake efficiency. Unlike NPE, the NRF improved with increasing N application for both soils (Table 6). The NRF of *L. chinensis* was higher at MSSL than at SSSL under different N application rates. Average NRF was 2.3 times and 3.5 times at MSSL than at SSSL in 2010 and 2011, respectively. There were significant ($P < 0.05$) differences in NRF of *L. chinensis* between both soils. Stepwise regression analysis showed that site (soil EC and pH), N application rate and year together affected NRF (overall $R^2 = 0.85$, $P \leq 0.001$). The annual difference in NRF was significant at MSSL, and not significant at SSSL.

Nitrogen partial factor productivity

Like NPE and NRF, the NPP was significantly ($P < 0.05$) influenced by N application rate at both sites (Table 6). Mean NPP at MSSL was 8.45 g grain g^{-1} N in 2010 and 2.20 g grain g^{-1} N in 2011. Mean NPP at SSSL was 1.93 g grain g^{-1} N in 2010 and 0.86 g grain g^{-1} N in 2011. There were significant ($P < 0.05$) differences between years for each soil and between soils (Table 6). Stepwise regression analysis showed that the main factors affecting NPP of *L. chinensis* were site (soil pH and EC), year and N application rate, in turn (overall $R^2 = 0.68$, $P \leq 0.001$). In contrast to the results for seed yield in 2010 (Table 1), NPP reached the maximum when N application rates were 60 kg ha^{-1} at MSSL and 30 kg ha^{-1} at SSSL (Table 6).

Discussion

Nitrogen is an essential nutrient for forage crop growth and has been shown previously to improve significantly biomass and productivity of *L. chinensis* (Pan *et al.* 2004, 2005), including in some marginal lands such as saline-sodic soils (Huang *et al.* 2010, 2015). Experiments evaluating the effects of N addition on seed yields of *L .chinensis* found that addition of N in autumn improved seed yields (Chen *et al.* 2013; Wang *et al.* 2010, 2013). Our investigation demonstrated that spring application of N could also significantly improve seed yields of *L. chinensis*, and that there were differences between two saline-sodic soils. Under the same N application levels, seed yield of *L. chinensis* was much higher at MSSL than at SSSL in the same year. Nitrogen application had a greater effect on increasing seed yield at MSSL than at SSSL, and this result was consistent with the effects of N application on hay yield in the same experiment (Huang *et al.* 2015).

There were significant ($P < 0.05$) differences in seed yield and yield components of *L. chinensis* under the various N application levels between 2010 and 2011 in each soil. These results were similar to the experimental results of Wang *et al.* (2013) in 2008 and 2009, but different from the results of Chen *et al.* (2013) in 2009 and 2010. It is likely that findings of differences between the first and second year in one experiment and no differences in another are related to climatic conditions (e.g. temperature,

Table 6. Nitrogen efficiencies of *Leymus chinensis* under different nitrogen application rates at both sites in 2010 and 2011

MSSL, Moderately saline-sodic grassland; SSSL, severely saline-sodic grassland; NPE, nitrogen physiological efficiency; NRF, N apparent-recovery fraction; NPP, N partial-factor productivity. Within columns and sites, individual treatment means followed by the same lower case letter are not significantly different. Within sites, treatment averages for each parameter followed by different upper case letters are significantly different ($P \leq 0.05$) between years 2010 and 2011. Within years, treatment averages for each parameter followed by different Greek letters are significantly different ($P \leq 0.05$) between sites MSSL and SSSL

Site/soil type	Treatment (kg N ha^{-1})	NPE (g g^{-1}) 2010	NPE (g g^{-1}) 2011	NRF (%) 2010	NRF (%) 2011	NPP (g g^{-1}) 2010	NPP (g g^{-1}) 2011
MSSL	30	210a	112a	17.6c	32.3d	9.78b	1.93
	60	213a	103ab	23.1c	43.8cd	15.04a	2.13
	90	143b	96ab	39.4b	44.6cd	8.52b	2.79
	120	126bc	85b	48.0ab	62.9bc	8.04b	2.54
	150	101bc	64c	43.0ab	79.1ab	8.20b	2.85
	180	97bc	55c	48.1ab	85.7a	5.99bc	1.77
	210	78c	57c	55.6a	79.8ab	3.58c	1.38
	Average	138A	82Bα	39.4Bα	61.2Aα	8.45Aα	2.20Bα
SSSL	30	163a	229a	9.9c	10.6bc	2.62a	1.11b
	60	132b	200ab	12.7bc	9.4c	2.54a	1.01b
	90	132b	175bc	11.4c	12.9bc	2.14a	0.80bc
	120	130b	154c	17.5bc	17.5abc	2.46a	1.64a
	150	126b	119d	17.4bc	20.3ab	1.88ab	0.75bcd
	180	112b	87de	21.4ab	24.9a	1.28bc	0.46cd
	210	66c	72e	27.7a	26.3a	0.62c	0.24d
	Average	123B	148Aβ	16.9β	17.4β	1.93Aβ	0.86Bβ

rainfall, etc.), because similar significant differences between years were observed in the controls (Chen *et al.* 2013; Wang *et al.* 2013).

In our study, although the total rainfall was similar for both years, the distribution was quite different. Total rainfall for April, May and June was 255 mm in 2010 and only 155 mm in 2011. In addition to lower spring rainfall, spring temperatures were higher in 2011 than 2010. Drier and hotter conditions in 2011 likely caused the decreased seed yield in the control treatment in 2011 compared with 2010 for both soils. However, there was a common trend for both soils between 2010 and 2011. Seed yield, spike number and heading percentage of *L. chinensis* were significantly higher for both soils in 2010 than in 2011; moreover, they all increased with an increase in N application. By contrast, spike length, number of filled seeds per spike and 1000-seed weight were not significantly different between 2010 and 2011, again in both soils.

Our results showed a strong response to application of N, the maximum seed yields of *L. chinensis* at MSSL increasing 7.4–10.9 times with N application rates up to 150 kg ha^{-1} relative to the control, and at SSSL 5.3–7.5 times with N application rate of 120 kg ha^{-1} relative to the control. These results are similar to those reported by Wang *et al.* (2013), but different from those of Chen *et al.* (2013), who found only modest increases in seed yield after application of N at 29–90 kg ha^{-1} and no significant increase in seed yield at an application rate of 19 kg ha^{-1} for either of the years of their study. Although it was not possible to explain definitively these contrasting results, we note that major differences in these studies were soil pH, initial N status and absolute seed yields.

In the present study, the average soil pH was 8.94 at MSSL and 9.80 at SSSL. The site used by Wang *et al.* (2013) had a pH of 9.0, whereas the site used by Chen *et al.* (2013) had a pH of 8.12. In both the present study and that of Wang *et al.* (2013), seed yields were much lower than those reported by Chen *et al.* (2013), for a site with much lower pH than at our sites, suggesting that pH is a very important variable affecting seed yield. In addition, our site SSSL had much lower seed yields than MSSL at the same N application rates. The result from stepwise regression analysis also showed that site (EC and pH) was the major factor adversely impacting seed yield ($R^2 = 0.31$, $P \leq 0.001$).

On the sole basis of the yield data from the two sites, we cannot directly evaluate the relative effects of EC and pH. However, based on the salt tolerance tables of Grieve *et al.* (2012), we can calculate the expected yield losses related to salinity and compare them to our measured yields. The grain or seed yield of listed grasses (Grieve *et al.* 2012) showed a decrease in the range of 5–10% per unit increase in EC$_e$ beyond the threshold value (EC$_e$ value at which yield first starts to decrease). Thus, the maximum difference in yield expected between the two sites as a result of salinity would be in the range of 15–30% lower at SSSL than at MSSL, based on the EC$_e$ difference of 3.1 dS m^{-1}. The measured yield decline for the control treatments was 68% in 2010 and 27% in 2011. The yield decline at SSSL relative to MSSL was 76% in 2010 and 74% in 2011 when the optimal N treatments are considered. From this analysis, we conclude that the major yield differences between the two sites or soils are attributed to the higher pH at SSSL. Soil salinity was thus secondary but would also reduce the positive impact of N application. The positive effects of N application on *L. chinensis* could not overcome the negative effects of soil pH and salinity in saline-sodic soils.

A relationship between low soil nutrient status and response to addition of N was also evident, and that this was likely pH-related. The relationship between N nutrient status and site (pH) was examined by a comparison of N status and response among the various datasets. In the present study, both soils had lower nutrient contents than the soil used by Chen *et al*. (2013), which we attribute to the elevated pH of our soils. The optimal N requirement in our study was thus likely to be higher than in the previous study. We observed an increase in seed yield up to an N application of 150 kg ha^{-1} at MSSL. However, site interacted with N response, because the much lower response to N at SSSL than MSSL appears related to the higher pH at SSSL. At MSSL (pH 8.94), application of 150 kg N ha^{-1} increased seed yield relative to the control by 108 g m^{-2} in 2010 (from 14.7 to 123 g m^{-2}) and by 39.2 g m^{-2} in 2011 (from 3.6 to 42.8 g m^{-2}). At SSSL, application of 150 kg N ha^{-1} increased yield by only 23.5 g m^{-2} in 2010 and 8.9 g m^{-2} in 2011. Higher N application rate did not increase seed yield; additionally, the adverse environmental and economic effects of excessive N application should not be ignored. Therefore, optimal N application rates should be comprehensively determined by considering the response to other stresses such as elevated pH that reduce the yield response to N.

Seed yield of *L. chinensis* was determined by spike number per unit area, number of filled seeds per spike and grain weight. The number of filled seeds per spike increased in 2011 compared with 2010 at SSSL, and 1000-grain weight increased in 2011 compared with 2010 at MSSL, but all seed yields decreased in 2011 compared with 2010 at both MSSL and SSSL. The correlation analysis indicated a significant ($P \leq 0.001$) positive correlation between seed yield and spike number/heading percentage of *L. chinensis* in two saline-sodic soils. Stepwise regression analysis also showed that increasing seed yield was mainly determined by increasing spike number ($R^2 = 0.96$, $P \leq 0.001$) with increasing N application, and that the increase in spike number was mainly affected by annual variations in temperature and rainfall and soil pH. With regard to various seed-yield component factors of *L. chinensis*, the relationships among number of filled seeds per spike, 1000-grain weight and seed yield were inconsistent between MSSL and SSSL. Several different studies report the effects of N application rates on 1000-seed weight. Wang *et al*. (2013) and Chen *et al*. (2013) reported that application of N significantly increased 1000-seed weights; Hocking and Stapper (2001) and Ma *et al*. (2015) found that application of N had no significant effect on 1000-seed weights; and Kutcher *et al*. (2005), Bai *et al*. (2009) and Ahmad *et al*. (2011) found that 1000-seed weight decreased with increasing N application rate. Our data show significant differences in 1000-seed weights between 2010 and 2011 ($P \leq 0.01$) and between the two saline-sodic soils ($P \leq 0.05$). Stepwise regression analyses for seed yield component factors indicated that spike number and 1000-seed weights were mainly affected by site (soil pH, $R^2 = 0.27$ and 0.48, $P \leq 0.01$), and that spike length, heading percentage and number of filled seeds per spike were mainly affected by N application rate (R^2 = 0.52, 0.16 and 0.24, $P \leq 0.05$). Interannual differences in seed yield and yield components of *L. chinensis* are likely related to the different climatic conditions in these two consecutive years, but further study is required to determine how the interannual differences were related to soil salinity, soil water status and pH during reproductive growth. Thus, N application had a complex effect on various seed-yield component factors of *L. chinensis*.

Nitrogen application is a practical and effective method to increase seed yield of *L. chinensis* in saline-sodic grasslands, but the response depends on initial soil N content, soil pH, soil salinity and soil water status during the growing season. Simultaneously, in order to minimise N-fertiliser costs and the risk of N leaching, NUE issues must be considered (Bronson 2008; Rochester *et al.* 2009). NUE is an important indicator used to evaluate the feasibility of N application. Generally, the parameters for evaluating NUE include N agronomic efficiency, N physiological efficiency, N uptake efficiency, etc. (Delogu *et al.* 1998; López-Bellido and López-Bellido 2001). Nitrogen agronomic efficiency of *L. chinensis* in two saline-sodic soils was discussed in our previous study (Huang *et al.* 2015). The present study showed that NPE, NRF and NPP were influenced significantly by N application rate, and by site (pH) for NPE, site and N for NPF, and site and year for NPP, based on the stepwise regression analysis. The lower N efficiency at SSSL was likely related to its elevated soil pH. Our analysis also demonstrated significant correlations between N application rate and NPE ($P \leq 0.01$) of *L. chinensis* whether at MSSL or SSSL. However, NPE of *L. chinensis* significantly decreased and NRF significantly increased with increasing N application rate, especially in soil with high pH and EC (e.g. SSSL). These results indicate that greater N uptake could not be converted entirely into a corresponding increase in hay yield of *L. chinensis*. When N application rate was >150 kg ha^{-1} at MSSL and 120 kg ha^{-1} at SSSL, the value of NPP decreased. Moreover, NPE, NRF and NPP of *L. chinensis* were significantly higher at MSSL than at SSSL; therefore, reducing N application rate below optimal levels established under non-stressed conditions would also be advisable in high-pH and high-EC soils.

In conclusion, significant differences in seed yield of *L. chinensis* existed between years and between two different saline-sodic soils in Northeast China. Soil pH was the major limiting factor affecting seed yield. Nitrogen application in spring significantly improved seed yield of *L. chinensis*, mainly by increasing spike number. High soil pH and salinity (expressed as EC) were major negative factors affecting NPE, NRF and NPP. Nitrogen application also significantly affected various N efficiencies of *L. chinensis*. We conclude that application rates of N for maximum seed yield in *L. chinensis* were 150 kg ha^{-1} at MSSL and 120 kg ha^{-1} at SSSL. Therefore, it is important to determine the optimal N application rate for increasing the seed yield of *L. chinensis* according to soil pH and EC, annual climatic characteristics and extent of yield increase relative to N input. Reducing N application rate is recommended if the soil has a high pH and EC.

Conflicts of interest

Authors do not have any conflicts of interest.

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