Effects of continuous nitrogen addition on the quality of *Leymus chinensis* and soil properties in saline-sodic soil of Northeast China

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Abstract

Leymus chinensis (Trin.) Tzvel. (Poaceae) is the dominant plant species in the Songnen grassland of China. Nitrogen addition is an important measure to prevent degradation of grassland due to soil salinization. To clarify the effects of long-term nitrogen addition on the quality of *L. chinensis* and soil properties, a field experiment was continuous conducted for 10 years in moderately saline-sodic grassland from 2009 to 2018, and the annual nitrogen addition rate was 0,30,60,90,120,150,180 and 210 kg N ha⁻¹, respectively. The results showed that with nitrogen addition rates increasing, the contents of protein and fat in shoot increased 20.5% to 80.9% and 6.9% to 69.3% compared with those of the control without nitrogen addition, respectively. When nitrogen addition rates reached 90 kg ha⁻¹ to 210 kg ha⁻¹, The ratio of K⁺/Na⁺ and Ca²⁺/Na⁺ in *L. chinensis* shoot increased 1.26 to 3.37 times and 1.56 to 2.98 times compared with those of the control, respectively. Nitrogen addition decreased significantly soil pH, electrical conductivity and exchange sodium percentage, and increased significantly the contents of soil organic matter, total nitrogen, available nitrogen, the activities of sucrase and urease and ecosystem multifunctionality(P<0.05). The redundancy analysis showed that there was a significant positive correlation between the improvement of *L. chinensis* quality and soil desalination and soil fertility increasing. Therefore, the suitable nitrogen addition rate of 90-120 kg N ha⁻¹ was an effective and necessary measure to maintain the healthy growth of *L. chinensis* and inhibit land degradation in moderately saline-sodic grassland of Northern China.

Abbreviations : *L. chinensis*, *Leymus chinensis* ; DASLES, Da'an Sodic Land Experiment Station; CAS, Chinese Academy of Sciences; MSSSL, moderately saline-sodic grassland; SSSL, severely saline-sodic grassland; N, nitrogen; EC, electrical conductivity; ESP, exchangeable sodium percentage; SOM, soil organic matter; TN, total nitrogen, AN, available nitrogen; C/N, the ratio of carbon to nitrogen; RDA, Redundancy analysis; EMF, ecosystem multifunctionality.

1. Introduction

Soil salinization is a worldwide environmental degradation problem. According to the statistics of FAO/UNESCO, the total area of salt affected soils is up to 100 million hectares, accounting for about 6% of the total land area in the world (Wong *et al.* , 2009; Hajiboland, 2013; Singh, 2015). With the global climate changing and increasing of the population, the area of salt affected soil is also increasing rapidly (Setia *et al.* , 2013). Due to soil salinization aggravating, many plants are facing unprecedented challenges. Taking Songnen Plain in as an example, soil salinization had resulted in a sharp decrease of grassland area by 64% from 1954 to 2000, most of which became saline-sodic wasteland (Wang *et al.* , 2009).

Leymus chinensis (Trin.) Tzvel.(Poaceae) is a perennial rhizome grass and the natural dominant species

in the western grassland of Songnen Plain in (Zhu, 2004). It has strong drought resistance, saline-alkali tolerance and low temperature resistance (Wang *et al.* , 1994; Bai *et al.* , 2004; Jin *et al.* , 2008). However, with the increasing of human interference in this area, overgrazing, reclaiming farmland, collecting soil and other production activities further aggravated soil salinization and sodicization, which to result to the continuous decline of soil fertility and further degradation of *L. chinensis* grasslands (Gao *et al.* , 2015; Qin *et al.* , 2016).

Numerous studies have shown that nutrients addition was an important measure to improve soil fertility and maintain high productivity of grassland, especially nitrogen (N) fertilizer addition was widely used in pasture land (Han *et al.* , 2007; Clark & Tilman, 2008). Also, N fertilizer addition can be used as soil restoration method in infertile or salt-affected soils (Murtaza *et al.* , 2000). As one of the key nutrient elements, N addition can promote leaf chlorophyll (Song *et al.* , 2016) and crude protein contents of *L. chinensis* (Hu *et al.* , 2017), and increase the hay yield and quality of *L. chinensis* (Qi *et al.* , 2013; Song *et al.* , 2016). Meanwhile, high soil N mineralization (Zhang *et al.* , 2009) and nitrification rates (Qin *et al.* , 2016) were observed in the pasture which N addition. However, Zhang *et al.* (2008) found that excessive N addition led to inefficient utilization of N fertilizers, and increased nitrate leaching and high environmental risks (Qin *et al.* , 2016). According to different soil types and plant species, the reasonable amount of N addition determined the crop yield and ecosystem stability (Vitousek *et al.* , 2009). The effects of N addition on the characteristics of plant or soil were often demonstrated lively in terms of ecosystem multifunctionality (EMF). Therefore, the study of EMF driven by plant or soil characteristics had become a recently hot spot in various ecosystem researchs (Hector & Bagchi, 2007; Manning *et al.* , 2018; Jing *et al.* , 2015), while the research on the EMF affected by salts had received little attention.

Given the acknowledged importance of rational nutrients addition on ecologically fragile area, studying the effects of N addition on *L. chinensis* performance and ecosystem stability would provide a new insight on ecosystem productivity and saline-sodic land restoration. So we had established a field experiment about the effects of N addition on *L. chinensis* in two kinds saline-sodic soils since 2009, and the effects of continuous application of N on hay yield and seed yield of *L. chinensis* were also reported successively, and the previous studies demonstrated that reasonable N addition could improve the biomass and seed yield of *L. chinensis* (Huang *et al.* , 2015 & 2019). However, the effects of long-term continuous N addition on the quality of *L. chinensis* , the characteristics of soil salt and the stability of ecosystem need to be further studied. Thus, the purposes of this study were (i) to reveal the effect of long-term continuous N addition on the nutritional quality of *L. chinensis* , (ii) to clarify the effect of long-term N addition on the salt characteristics of saline-sodic soil, (iii) to analyze comprehensively the roles of N addition on the restoration of saline-sodic grassland and EMF in Northeast China.

2. Materials and methods

2.1 Experimental site

The field experiment of this study has been carried out for 10 years since Da'an Sodic Land Experiment Station (DASLES), Chinese Academy of Sciences (CAS) (45°35'58"~45deg36'28"N, 123deg50'27"~123deg51'31"E), which is located in Da'an city of Jilin Province of China. The DASLES is a typical saline degraded grassland ecosystem located in the Western of Songnen Plain, . The area is semi-arid with a temperate continental climate. The annual mean temperature is 4~, the annual mean precipitation is 350~ in the area. While evaporation is about , which is 4~5 times of the precipitation.

The original field experiment was conducted in two different kinds of saline-sodic grassland: moderately saline-sodic grassland (MSSL), with soil pH 8.94 and ECe 5.7 dS m⁻¹, and severely saline-sodic grassland (SSSL), with soil pH 9.80 and ECe 8.8 dS m⁻¹. This study was only carried out in MSSL, for other basic chemical characteristics of grassland soils, please refer to our previous studies (Huang *et al.* , 2015 & 2019).

2.2 Experimental design

The experiment was a completely randomized block design, with 3 replicates in MSSL. Eight N addition levels were 0 (control), 30, 60, 90, 120, 150, 180 and 210 kg N ha⁻¹, respectively. The area of each plot was 9 m² (3 by 3 m), and there were 24 plots in total. 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 fertilizer before the experiment was started.

Nitrogen was added as urea (the content of pure N 46.2%), when the *L. chinensis* resumed growth in the middle of May once a year, N fertilizer was applied to the surface of the *L. chinensis* grassland in the early evening since 2009. After N addition, 10 mm water was slowly and evenly sprayed to each plot to make urea granule quickly dissolved and reduce the loss of fertilizer caused by volatilization. The same field management was applied to each plot, and maintained *L. chinensis* natural growth without human interference.

2.3 Sampling and measurement methods

According to the cutting habits of local *L. chinensis* as forage, it is usually harvested in early September every year. Thus, the plant and soil samples of the *L. chinensis* were collected in early September in 2018. The sampling was carried out at random sites with a sampling size of 0.5 by 0.5 m, with three replications within each plot. The plant samples were cut the shoot at ground level in a sampling size zone, put them into the oven and killed green at 105 for 2 h, oven dried at 65 for 12 h, and then grated them less than 1 mm. The soil samples were randomly taken three soil cores (diameter of 40 mm) at 0 to 20 cm depth within each plot, then mixed well and air dried, passed through a 2-mm sieve and then measured for the concentrations of soil salinity, nutrients and soil enzyme activities.

The protein content of *L. chinensis* was determined by continuous flow analyzer (SKALAR SAN++), the fat content was determined by soxhlet extraction method, the coarse fiber content was determined by acid-base washing method, the ash content was determined by using burning method of high temperature at 550±20, and the concentrations of K⁺, Na⁺, Ca²⁺ and Mg²⁺ were determined by ICPS-7500 plasma emission spectrometer after the plant samples were combined digestion by nitric acid and perchloric acid (Bao, 2000),

Soil salinity and nutrients were measured by using conventional methods, and the specific methods were the same as those in our previous study (Huang et al., 2015). The related soil enzyme activities were determined by using the method of Guan *et al.* (1986), soil samples were cultured in a constant temperature incubator at 37 °C for 24 h, and then soil urease activity was determined by sodium hypochlorite phenol colorimetry, soil catalase activity was determined by potassium permanganate titration, and soil sucrase activity was determined by 3, 5-Dinitrosalicylic acid colorimetry.

2.4 Statistical analysis

The data were expressed as mean ± 1 SE. The treatments were run for a one-way ANOVA using the software of R version 3.5.2. Redundancy analysis (RDA) was implemented with the vegan package in the software R. The EMF was as a uniteless index that usually combines soil characters and plant performance indexes, such as soil fertility, N cycling and plant productivity. Here, we examined 17 indexes including soil pH, EC, ESP, available nitrogen (AN), total nitrogen (TN), soil organic matter (SOM), catalase activity, urease activity, sucrose activity, and plant coarse protein, fat, coarse fiber, ash, K⁺, Na⁺, Ca²⁺, Mg²⁺ concentrations under different N addition rates. The averaging-based EMF measure was applied on Z-score transformed data to calculate the EMF value into a single index. The EMF of different treatments was compared through one-way ANOVA analysis followed by the Duncan's test. The relationship between EMF and N addition rates was best fitted by quadratic equation model, with the highest determinate coefficient. Other figures in this article were manufactured by Origin Pro 8.0 (Microcal, Malvern, England).

3. Results

3.1 The quality of *L. chinensis*

3.1.1 Protein and fat

The crude protein content of *L. chinensis* increased significantly from 2.73% to 4.94% with N addition increasing ($P < 0.05$). In particular, when N addition rate was from 0 kg ha⁻¹ to 90 kg ha⁻¹, the crude protein content of *L. chinensis* increased in a straight line (Fig. 1A). Compared with the control, the protein content increased 20.5%, 38.5% and 66.3%, respectively. However, the protein content increasing was no significance when N addition was more than 90 kg ha⁻¹ ($P \geq 0.05$). In all treatments, the crude protein content of *L. chinensis* was the highest when N addition rate reached 150 kg ha⁻¹, and increased by 80.9% more than that of the control.

Similarly, the fat content of *L. chinensis* also increased markedly with increasing of N addition rates (Fig.1B). The fat contents of N addition treatments increased 6.9%-69.3% compared with that of the control, and the difference was very significant ($P < 0.05$). While N addition rate reached 90-210 kg ha⁻¹, there was no significant differences between the fat contents of each treatment ($P \geq 0.05$).

3.1.2 Coarse fiber and ash

The coarse fibers content of *L. chinensis* showed an overall downward trend with N addition rates increasing (Fig.1C). Compared with the control, the coarse fibers content reduced by 1.0 % to 16.3 % with N addition increasing, but there was no significant differences between most test treatments ($P \geq 0.05$).

It was different from the protein, fat and coarse fibers, there was almost no significant difference in the ash content of *L. chinensis* under different N addition treatments (Fig.1D). In MSSL, the ash content of *L. chinensis* was kept 4%-5%, and N addition had little effect on ash content of *L. chinensis*.

3.1.3 Main mineral elements

With different N addition rates increasing, the total content of mineral elements in *L. chinensis* shoot showed an increasing trend (Fig.2). Specifically, the contents of K⁺ and Ca²⁺ in shoot increased significantly with N addition rate increasing ($P < 0.05$), on the contrary, the content of Na⁺ in shoot decreased significantly with the increase of N addition ($P < 0.05$), and the content of Mg²⁺ in shoot hardly changed. The total contents of mineral elements in shoot in the treatments with 150 kg N ha⁻¹ and 180 kg N ha⁻¹ increased 27.6% and 34.2% compared with that in the control treatment, respectively. While the content of K⁺ in shoot in the treatments with 150 kg N ha⁻¹ and 180 kg N ha⁻¹ increased 71.9% and 99.3% compared with that in the control treatment, the content of Ca²⁺ in shoot in the treatments with 90 kg N ha⁻¹ to 210 kg N ha⁻¹ increased 45.0% to 80.7% compared with that in the control treatment, and the difference were all significant ($P < 0.05$). Instead, the content of Na⁺ in shoot in the treatment with 120 kg N ha⁻¹ to 180 kg N ha⁻¹ decreased significantly 32.3%, 30.9% and 40.8% compared with that in the control treatment ($P < 0.05$), respectively.

With the increase of N addition level, the ratio of K⁺/Na⁺ and the ratio of Ca²⁺/Na⁺ in shoot of *L. chinensis* also increased constantly (Fig.3). When N addition level reached 90 kg N ha⁻¹ to 210 kg N ha⁻¹, the ratio of K⁺/Na⁺ in shoot increased 1.26 to 3.37 times compared with that in the control treatment, and the ratio of Ca²⁺/Na⁺ in shoot increased 1.56 to 2.98 times compared with that in the control treatment, and the differences all reached significant levels ($P < 0.05$). However, when N addition level was 210 kg N ha⁻¹, the both ratios decreased, but still higher than those in the control treatment. Comprehensive mineral elements content, K⁺/Na⁺ and Ca²⁺/Na⁺ in shoot, when N addition rates were 120 kg ha⁻¹ to 180 kg ha⁻¹ the mineral quality of *L. chinensis* reached the best state, and there was a significant difference compared with those in the control treatment.

3.2 Soil characteristics

3.2.1 pH, EC and ESP

Soil pH, EC and ESP of N addition treatments decreased significantly compared with those of non-N addition treatment when N addition rate was more than 90 kg ha⁻¹ ($P < 0.05$), and three indexes showed a downward trend with the increase of N addition rates (Fig.4). Compared with the treatment without N addition, soil pH of the N addition treatments with 90-210 kg ha⁻¹ decreased by 0.71-1.19 units, soil EC decreased by

34.4%-67.2%, and soil ESP decreased by 55.7%-65.3%, respectively. In MSSL of *L. chinensis*, N addition can reduce effectively salinity and alkalinity of 0-20 cm soil.

3.2.2 SOM, TN, AN and C/N

In MSSL of *L. chinensis*, N addition also increased the contents of SOM, TN and AN, and reduced the ratio of carbon to nitrogen (C/N). With N addition rate increasing, the content of SOM increased in a straight line, and it reached a significant level when N addition rate was 60 kg ha⁻¹ compared with that of the control treatment ($P < 0.05$). When N addition rates were 150-210 kg ha⁻¹, the contents of SOM increased 2.16-2.53 times compared with that of the control treatment (Fig.5A). Similarly, soil TN and AN contents were also improved significantly with N addition rates increasing ($P < 0.05$). Soil TN content was the highest when N addition rate reached 150 kg ha⁻¹, and increased by 269.3% compared with that of the treatment without N addition (Fig.5B). Soil AN content was the highest when N addition rate reached 180 kg ha⁻¹, and increased by 255.1% compared with that of the treatment without N addition (Fig.5C). Nitrogen addition significantly decreased the soil C/N ($P < 0.05$). The C/N ratio of the N addition treatments with 90-210 kg ha⁻¹ was stable at about 15 (14.6-16.1), and those were lower by 38.3% - 44.3% than that of the control treatment without N addition (Fig.5D).

3.2.3 Soil enzyme activity

Soil enzyme activity is a living index to reflect soil environmental changes and fertility conditions, and they are more sensitive to soil environment. In MSSL, with N addition rates increasing, soil urease and sucrase activities increased significantly ($P < 0.05$), and soil catalase activity decreased gradually (Table 1). The soil urease activities in the N addition treatments with 90-210 kg ha⁻¹ increased by 91.5%-110.6% compared with that in no N addition treatment, and the soil sucrase activities increased by 3.74-5.42 times, respectively. On the contrary, the soil catalase activities in the N addition treatments with 90-210 kg ha⁻¹ decreased by 3.2%-13.6% compared with that in no N addition treatment.

3.3 The relationship between *L.chinensis* quality and soil chemical characteristics

In order to clarify the effects of soil salinity and nutrients on the quality of *L. chinensis*, RDA was conducted between *L. chinensis* quality and soil chemical characteristics. The results of RDA revealed that the variance ratio of RDA1 (35.20%) reached a significant level in the biplot ($P < 0.01$), there were some negative correlations between soil salinity (pH, EC and ESP) and most quality indexes of *L. chinensis* (except for Na⁺ and coarse fiber content), and there were some positive correlations between SOM, Sucrase, Urease and most quality indexes (Fig.6). RDA also indicated that *L. chinensis* had higher Na⁺ and coarse fiber in no N addition or low N addition treatments (N 0-90), which was mainly related to higher salinity in soil, on the contrary, *L. chinensis* had higher contents of K⁺, Ca²⁺, Mg²⁺, protein and fat in high N addition treatments (N 120-210), which was mainly related to higher SOM contents, urease and sucrose activities. Thus, N addition increased the quality of *L. chinensis* mainly through improved soil nutrient contents, especially carbon and N contents in saline-sodic soils.

3.4 Ecosystem multifunctionality

Nitrogen addition significantly affected the EMF of *L. chinensis* grassland in saline-sodic soil (Fig.7). The analysis of one way ANOVA showed that there were significant differences on the EMF of *L. chinensis* grassland under different N addition rates ($F=35.33, P < 0.001$, Fig.7A). The EMF was negative when N addition rates were from 0 to 60 kg ha⁻¹, and the EMF was positive while N addition rates were from 90 to 210 kg ha⁻¹. This demonstrated that the EMF of *L. chinensis* grassland increased with N addition rates increasing in saline-sodic soil, but the EMF didn't increase when N addition rates reached enough levels (from 120 to 210 kg ha⁻¹ in the study). It was found that the relationship between the EMF and N addition level was quadratic curve model ($R^2=0.88116, P < 0.0001$, Fig.7B). In the study, the EMF was the maximum when N addition rates were 150 or 180 kg ha⁻¹, while the EMF would decrease if N addition rate reached at 210 kg ha⁻¹. Therefore, the EMF of *L. chinensis* grassland was affected by N addition rates to a large extent in saline-sodic soil of Northeast China.

4. Discussion

4.1 Effect of N addition on the quality of *L. chinensis*

Our previous studies have demonstrated that N addition can increase hay yield of *L. chinensis* and inhibit grassland degradation in different saline-sodic soils, and determined the optimum N addition rate was 90 to 120 kg N ha⁻¹ in MSSL according to the yield and N use efficiency (Huang *et al.* , 2015). Many other researchers have also reported that N addition improved the quality of various herbages (Chen & Carran, 1990; Dong *et al.* , 2008; Tan *et al.* , 2014; Hu *et al.* , 2017). However, the effect of N addition on the quality of *L. chinensis* was rarely studied in saline-sodic grassland. It is very worth studying how long-term continuous N application will affect the quality of *L. chinensis* in MSSL.

The results of this experiment indicated that the protein and fat contents of *L. chinensis* were significantly increased by N addition for 10 years in MSSL, and the coarse fiber and ash contents did not change significantly under different N addition levels (Fig.1). In saline or alkali condition, *Leymus chinensis* usually has high protein content (Shi *et al.* , 2008; Zhou *et al.* , 2009). Generally, N addition increased the protein and fat contents and decreased the coarse fiber content of *L. chinensis* , which has been confirmed by many studies (Dong *et al.* , 2014; Bai *et al.* , 2017; Hu *et al.* , 2017). Previous studies also demonstrated that N addition did not affect the ash content of more plants (Brown *et al.* , 1932; Dong *et al.* , 2014).

Furthermore, N addition had a positive effect on the optimization of ion-allocated proportion in *L. chinensis* plants, and the content of K⁺ and Ca²⁺ in *L. chinensis* plants increased, while the content of Na⁺ decreased with N addition rates increasing (Fig.2). The ratio of K⁺/Na⁺ and Ca²⁺/Na⁺ in *L. chinensis* shoot also increased with N addition rates increasing (Fig.3). The ratio of K⁺/Na⁺ and Ca²⁺/Na⁺ in shoot of plants was usually regarded as an important index of salt tolerance (Huang *et al.* , 2008). Under N addition, both of them increasing indicated that the toxicity of sodium ions to *L. chinensis* was reduced (Yin *et al.* , 1993) and salt tolerance of *L. chinensis* was enhanced (Li *et al.* , 2002). Therefore, the reasonable N addition not only improved the quality of *L. chinensis* , but also improved its salt tolerance and reduced the degradation of saline-sodic grassland.

4.2 Effect of N addition on soil characteristics

With the development of modern agriculture, nutrient addition (especially N addition) has become an essential cultivation measure to improve the productivity of most food crops and forages. However, the influences of long-term fertilization on cultivated soil have also aroused widespread concern in recent decades. For example, long-term application of N fertilizer resulted in the decline of SOM in black soil in Northeast China (Qiao *et al.* , 2007), and the aggravation of soil acidification in Southern China, soil pH dropped by 0.5 units in past 20 years (Guo *et al.* , 2010). So far, little research had been done about the effect of N addition on the characteristics of saline-sodic soil. Our results in saline-sodic paddy field showed that the N addition level had no significant effects on soil pH and EC in the short term (3 years), but the soil N contents were significantly increased, soil pH and EC also decreased significantly with cultivation time increasing (Huang *et al.* , 2016). In this study, soil pH, EC and ESP decreased significantly when N addition rate was more than ha⁻¹ after 10 years of continuous N addition (Fig.4). Similar results were also found in alfalfa pastures in North America and mountain heathlands in Spain (Chen *et al.* , 2001; Marcos *et al.* , 2003). These results further indicated that long-term continuous N addition had a positive effect on reducing grassland salinization.

Many literatures have demonstrated that N addition improved soil fertility, especially in some marginal lands (Wang & An, 2010; Liet *et al.* , 2017; Kumar *et al.* , 2019). Our previous study also showed that soil inorganic N and TN contents increased significantly in the 0-20 cm soil layer of saline-sodic paddy field with the increasing of cultivation years or N application rates (Huang *et al.* , 2016). The experimental results indicated that the contents of SOM, TA and AN under different N addition rates were significantly increased ($P < 0.05$) than those of no N treatment after 10 years of continuous N addition in *L. chinensis* grassland (Fig.5). However, compared with the experimental results of 7 years ago (Huanget *et al.* , 2015), the contents

of TA and AN in soils from different N treatments had little change. Under the condition of continuous N addition for many years in saline-sodic grassland, in addition to increasing the hay yield of *L. chinensis*, where did the rest of the N go? Why can't soil N content be further improved? These problems should be further studied in the future.

Interestingly, with N addition rates increasing, the content of SOM increased significantly, while C/N ratio decreased significantly in saline-sodic grassland of *L. chinensis* ($P < 0.05$, Fig.5A & 5D). The soil C/N ratio was maintained at about 15:1 when N addition rate was more than 90 kg N ha⁻¹, which was close to the conventional value of SOM. Some studies had pointed out that soil C/N ratio usually directly influences the nitrification and denitrification process of soil microorganisms, and then affects the conversion and discharge of soil N (Rizhiya, *et al.*, 2011; Kraft, *et al.*, 2014). In this experiment, soil C/N ratios were higher in the original saline-sodic grassland without N addition and the treatment with N addition rate less than 60 kg N ha⁻¹, so the growth of *L. chinensis* shoot was inhibited. Therefore, N addition can increase the aboveground yield of plants and improve the quality of plants/soils (Mou, 2015; Chang *et al.*, 2017).

Based on the analysis of soil enzyme activities related to the transformation of soil C, N and P, it was found that the activities of soil sucrase and urease increased significantly with N addition rates increasing ($P < 0.05$), while the activities of catalase decreased, and there was no significant difference among different N treatments (Table.1). Many previous studies have also demonstrated that urease activity increased significantly and promoted N fertilizer hydrolysis and mineralization under exogenous nN addition (Ge *et al.*, 2010), thus, more absorbable N was provided for plants (Aerts & Chapin, 1999). The similar changes of soil sucrase activity indicated that exogenous N promoted plant growth and increased soil organic carbon pool (Zhao *et al.*, 2008), while the response of soil catalase activity to exogenous N addition was almost insignificant (Song *et al.*, 2009; Wang *et al.*, 2015; Peng *et al.*, 2017).

4.3 The optimum of N addition and its effects on multifunctionality

Our previous study determined the optimal N addition rate was 90 to 120 kg N ha⁻¹ in MSSL according to the hay yield and N use efficiency (Huang *et al.*, 2015). The overall results of this study strongly support this conclusion again from the quality of *L. chinensis* and soil characteristics. Although *L. chinensis* may have the maximum seed yield when N addition rate was 150 kg N ha⁻¹ in MSSL (Huang *et al.*, 2019) and other higher quality indicators, such as protein contents and K⁺/Na⁺ ratio in this study, comprehensive analysis indicated that this recommendation was still the best. The relationship between *L. chinensis* quality and soil chemical characteristics also fully illustrated that soil desalination was consistent with the improvement of soil fertility and plant quality (Fig.6), and N addition was beneficial to achieve the goal.

In recent years, there has been a surge of interest in ecosystem multifunctionality which including 'ecosystem function multifunctionality' and 'ecosystem service multifunctionality' (Manning *et al.*, 2018). As a whole, the EMF has been widely studied in natural ecosystem (Pfisterer & Schmid, 2002). In this study, the effect of N addition on the EMF of *L. chinensis* showed a quadratic curve relationship, and there was a positive correlation between the EMF and N addition rates of more than 90 kg N ha⁻¹ (Fig.7). When N addition rate was 120 to 210 kg N ha⁻¹, there were no significant changes on the EMF among different N treatments, indicating that the EMF basically reached a steady state. Consequently, N addition of 90-120 kg N ha⁻¹ was also suitable in moderately saline-sodic grassland of *L. chinensis* from the perspective of the EMF.

5. Conclusions

It took nearly 10 years to conduct a field experiment of N addition on *L. chinensis* in MSSL. Nitrogen addition not only increased the contents of protein and fat, the ratio of K⁺/Na⁺ and Ca²⁺/Na⁺ of *L. chinensis* shoot, but also decreased the degree of soil salinization and increased soil fertility and the EMF. The suitable N addition rate of 90-120 kg N ha⁻¹ effectively inhibited the yield, quality and soil degradation of *L. chinensis* in MSSL. Thus, scientific data once again proved that N addition was an effective and necessary measure

to maintain the healthy growth of *L. chinensis* and inhibit land degradation in saline-sodic grassland of northern China.

Conflicts of interest

Authors do not have any conflicts of interest.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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