



Responses of two contrasting saline-alkaline grassland communities to nitrogen addition during early secondary succession

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Keywords

Competitive exclusion; Functional group; N deposition; Plant diversity; Salinity-alkalinity; Successional dynamics

Nomenclature

Fu & Li (1995) for plants; Wu (1980) for plant vegetation

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Abstract

Question: (1) How do secondary successional dynamics differ in high and low saline-alkaline grassland communities in semi-arid lands; and (2) does nitrogen (N) enrichment promote plant secondary succession towards a perennial community under saline-alkaline stress?

Location: Saline-alkaline grassland in northeast China.

Methods: We selected two early successional communities differing in soil saline-alkaline stress and floristic composition: one 'high-stress community' co-dominated by the annual grass *Chloris virgata* and the annual forbs *Artemisia scoparia* and *Kochia sieversiana*, and one 'low-stress community' dominated by the annual forb *K. sieversiana*. A complete randomized design experiment with five N addition levels was conducted in triplicate for four consecutive years (2008–2011). The vegetation variables (above-ground biomass of individual species, functional group, community level, community species richness) and soil characteristics (pH, electrical conductivity as a proxy for salinity) were investigated every year. Detrended correspondence analysis (DCA) was used to evaluate the successional trajectories.

Results: DCA revealed clear differences in species composition and successional trajectories between the two communities. There were few changes in the species composition of the high-stress community, with annual species dominating all the plots over the 4 yr. However, most of the low-stress communities developed from the annual-dominated stage to the perennial-dominated stage. N addition had no effect on the relative biomass of the perennial functional group in the two communities. Thus, N addition did not obviously promote plant succession towards a perennial community, even under low saline-alkaline stress. Species richness did not respond to N addition in the high-stress community, whereas species richness declined across the N addition gradient in the low-stress community.

Conclusion: Our results provided direct empirical evidence that high saline-alkaline stress limits plant community succession, and low saline-alkaline stress communities exhibit a succession direction from the annual-dominated stage to the perennial-dominated stage. Interestingly, replicates within the same treatment under low-stress conditions did not develop towards a similar community composition, indicating the importance of environment heterogeneity at a small spatial scale. N addition did not promote the expected perennial plant succession; however, N enrichment reduced plant diversity and enhanced above-ground biomass, suggesting that artificial N addition (e.g. N deposition) may have an important effect in our system.

Introduction

Secondary succession is a pervasive ecological process in terrestrial ecosystems that is characterized by a change in plant community composition over time after disturbance. Soil nutrient availability (especially nitrogen, N) is an important driver of plant community diversity, structure and dynamics in terrestrial ecosystems (Tilman 1987; Paschke et al. 2000; Baer et al. 2004; Storm & Suss 2008; Faust et al. 2012). Soil N availability is particularly important in arid lands, where N is only behind water as the most common factor limiting plant primary productivity (Schlesinger et al. 1996). One popular hypothesis is that nutrient enrichment can accelerate the rate of plant succession because processes such as competition–exclusion are expected to be faster when resources are well supplied, thereby accelerating species turnover (Bazzaz 1996; Pickett et al. 2009). However, observational or experimental studies on the responses of the rate of succession to nutrient enrichment lead to inconsistent results. The effects of nutrient enrichment on the rate of succession largely vary among different communities, with an increase in sandy communities (Sparrius et al. 2013), decrease in a semi-arid sagebrush community (McLendon & Redente 1992) and no effect on an old-field community (Huberty et al. 1998).

The wide variety of results regarding nutrient enrichment effects on successional dynamics found in previous studies (McLendon & Redente 1992; Huberty et al. 1998; Sparrius et al. 2013) could be partly attributed to environmental stress. It has been suggested that under stressful conditions (e.g. salinity-alkalinity, cold, drought, etc.), nutrient competition may play a minor role in the successional development of plants because processes such as nutrient uptake could be limited (Walker & del Moral 2003). In particular, the soil phenomena of salinization and alkalization caused by both natural and human-induced processes are major environmental hazards (Metternicht & Zinck 2003). One billion hectares of land, covering approximately 8% of the land surface is suffering from salinity/alkalinity (Ghassemi et al. 1995). This issue of salinity/alkalinity could be especially important in arid lands because both the huge coverage of these ecosystems at a global scale (ca. 41%) and the predictions of aridity increasing by the end of this century will likely enhance soil salinity (Schlesinger et al. 2011). However, we lack evidence and mechanistic understanding of how soil saline-alkaline stress influences successional dynamics; in particular, more information is required on how soil saline-alkaline stress modifies responses of the plant community to nutrient enrichment (especially N).

Because of its rapid economic growth (e.g. increase in food and energy demand; Cui et al. 2013), China has been

experiencing intense air pollution, especially anthropogenic emissions of reactive N species, resulting in enhanced N deposition rates (Liu et al. 2013). In the present study, we focus on the Songnen saline-alkaline grasslands in the central part of the northeast plains of China. This area is one of the three largest soda saline-alkaline lands in the world. Since the late 1970s, the saline-alkaline stress in the soil has been aggravated due to overgrazing or changes in land use (e.g. from grasslands to croplands), promoting regressive succession in the plant communities (e.g. from native perennial *Leymus chinensis* communities to secondary halophyte communities, such as *Chloris virgata*). In this respect, from a restoration standpoint, perennial grasses such as *L. chinensis* should be considered as the target climax community in our studied region. From the beginning of this century, many degraded grasslands in this area have been fenced to exclude grazing disturbance; consequently, secondary succession has been allowed to proceed. These contexts of vegetation and soil provide us an excellent opportunity to investigate how saline-alkaline stress influences secondary successional dynamics in natural grasslands.

Hence, to obtain more insight into the successional dynamics of plant communities in response to N enrichment under different soil saline-alkaline stress, we conducted N addition experiments on two early successional saline-alkaline plant communities in the Songnen grasslands of northeast China. The specific questions addressed were: (1) how do secondary successional dynamics differ in high and low saline-alkaline grassland communities; and (2) does N enrichment promote plant secondary succession towards a perennial community under saline-alkaline stress? We hypothesized that secondary successional dynamics might be faster in the low saline-alkaline grassland community compared to the high saline-alkaline community because of the lower environmental stress. In addition, we suggested that N enrichment might accelerate plant succession because processes such as competition–exclusion might be promoted when resources are well supplied (Bazzaz 1996; Pickett et al. 2009). This behaviour could be especially true for a low-stress plant community, where succession might be faster than under high stress environmental conditions.

Methods

Study site

This study was carried out at the Semi-Arid Climate–Environment Observatory of the Chinese Academy of Sciences at Tongyu, Jilin Province (44°25' N, 122°52' E), which is located in the Songnen saline-alkaline grasslands in the

central part of the northeast plains of China. Mean annual precipitation from 1971 to 2000 was 388.2 mm, with 80% falling during the growing season (May–Sept). Mean annual temperature was 5.7 °C. Soils in this area are mainly composed of sandy soil and saline-alkaline soil (Liu et al. 2008).

In 2006, an area of 47.7 ha overgrazing-induced, degraded saline-alkaline grassland was fenced and established for permanent observation at the observatory station. Because grazing disturbance was excluded, secondary succession started soon after fencing. Due to differences in microtopography, soil salinization is heterogeneous in this area (Yang et al. 2009). In 2008, we selected two sites within the fenced grassland that were located 100 m from each other at different topographic positions, being separated by ca. 20 cm in height. In this respect, in our region, the topographically elevated areas exhibit higher salinity levels in the topsoil than in the lowlands (Yang et al. 2009), creating a gradient of salinity stress. The highland community under high soil saline-alkaline stress (hereafter, high-stress community) was co-dominated by the annual grass *C. virgata* and the annual forbs *Artemisia scoparia* and *K. sieversiana* (Table 1). The lowland community under low soil saline-alkaline stress (hereafter, low-stress community) was dominated by the annual forb *K. sieversiana* (Table 1).

Experimental design

In each site, 15 (7 m × 7 m) plots were laid out in a complete randomized design. All plots were separated

by buffers of at least 1 m to avoid edge effects. The vegetation within each plot was homogeneous at the beginning of the experiment. The different plots in the two communities received annual commercial urea (46% N) at 0, 50, 100, 150 or 200 kg·ha⁻¹·yr⁻¹, which is equivalent to pure N additions of 0, 23, 46, 69 and 92 kg·ha⁻¹·yr⁻¹, respectively. These amounts were roughly in the range of the atmospheric N deposition rate of between 27 kg·ha⁻¹·yr⁻¹ (rate in last 10 yr) and 105 kg·ha⁻¹·yr⁻¹ (saturation rate of the community response) in this temperate grassland ecosystem (Zhang et al. 2008; Bai et al. 2010). Each treatment was completed in triplicate. N fertilizer was applied annually in early June from 2008 onwards.

Vegetation sampling and soil measurements

At peak biomass time (ca. mid-August) from 2008 to 2011, vegetation surveys and soil core sampling were conducted in three 1 m × 1 m quadrats randomly located in the central 5 m × 5 m area of each 7 m × 7 m plot. The location of the harvested quadrats varied each year to avoid spatial overlap among the years. The above-ground biomass in these quadrats was clipped to the soil surface, sorted into species, dried at 65 °C for 48 hr, and then weighed to the nearest 0.01 g. Species richness was defined as the number of species per quadrat. To analyse plant community dynamics, we classified all plant species into five functional groups based on the life form: annual forbs (AF) and annual grasses (AG), perennial forbs (PF), perennial grasses (PG) and shrubs and semi-shrubs (SS).

Table 1. Vegetation and soil characteristics of the two saline-alkaline grassland communities at initiation of the study.

	High-Stress Community	Low-Stress Community	Significance
Vegetation Characteristics			
Dominant Species	<i>Kochia sieversiana</i> (59.24%), <i>Chloris virgata</i> (21.39%), <i>Artemisia scoparia</i> (13.11%)	<i>Kochia sieversiana</i> (82.27%)	–
Richness (no. m ⁻²)	4.89 ± 0.56	10.00 ± 1.02	*
Above-ground Biomass (g·m ⁻²)	278.41 ± 12.92	407.68 ± 36.03	*
Annual forbs (%)	72.41 ± 4.35	91.26 ± 4.78	*
Annual Grasses (%)	24.89 ± 5.83	3.47 ± 3.07	*
Perennial Forbs (%)	2.47 ± 1.49	4.40 ± 1.60	NS
Perennial Grasses (%)	0.11 ± 0.11	0.72 ± 0.24	NS
Shrubs and Semi-Shrubs (%)	–	0.16 ± 0.04	–
Soil Characteristics			
pH	9.50 ± 0.09	8.15 ± 0.03	**
Electrical Conductivity (µS·cm ⁻¹)	314.33 ± 32.53	158.45 ± 6.74	**
Organic Matter (g·kg ⁻¹)	14.82 ± 1.47	5.16 ± 0.96	**
Available N (mg·kg ⁻¹)	4.27 ± 0.72	7.94 ± 0.84	NS
Available P (mg·kg ⁻¹)	3.31 ± 0.23	6.14 ± 0.98	*
Available K (mg·kg ⁻¹)	103.86 ± 12.07	150.88 ± 12.08	NS

Significant differences between communities are reported from a t-test as NS, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$. Available soil N was determined in mid-August 2009; other plant and soil data were estimated based on sampling in mid-August 2008. Values (mean ± SE, $n = 3$) were calculated in control plots in each community.

After the beginning of the experiment and following the vegetation survey (ca. mid-Aug), three soil cores (3 cm in diameter, 15 cm in depth) were sampled in each plant sampling quadrat of each plot, and a total of nine cores were combined to analyse soil background characteristics in our studied sites. After removing stones and roots, all soil samples were transferred to the laboratory and stored in a refrigerator at 4 °C. A portion of the samples was air-dried, ground and then sieved through a 0.15-mm mesh to measure soil pH, electrical conductivity (EC) and the content of soil available N, P and K. Soil pH was determined using a PHS-3C pH meter (Lida Instrument Factory, Shanghai, China). EC was determined using a DDS-307 electrical conductivity instrument (Precision & Scientific Instruments, Shanghai, China). Available N was analysed using a Kjeltac 2300 analyzer unit (Foss Tecator AB, Hoganas, Sweden). Available P was extracted with 0.5 mol·L⁻¹ NaHCO₃ solution (pH 8.5). Available K was determined using flame spectrometry.

Data analyses

The mean values of vegetation variables (above-ground biomass, species richness and relative biomass of individual species and functional groups) of the three random quadrats in each plot were calculated to represent that plot. Relative biomass for individual species and functional groups was used to minimize variation due to inter-annual climate fluctuations.

Plant community composition (relative biomass values) was analysed with detrended correspondence analysis (DCA) using PC-ORD 5 (MjM Software, Gleneden Beach, OR, US). Rare species (those found in <10% of survey quadrats in each community type over all 4 yr) were removed before ordination, and the remaining rare species were down-weighted. The DCA ordination analyses were performed in two ways: first, we analysed the two communities together to compare overall differences in community composition and succession trajectories, and second, we analysed the two communities separately to evaluate N treatment effects on succession of each community.

The initial differences in vegetation and soil characteristics between the two communities were analysed using a *t*-test compared to control plots ($n = 3$) in 2008. The *t*-test was also used to compare differences in species richness of the two communities across all plots over the 4 yr ($n = 15 \times 4 = 60$ replicates for each community). Repeated measures ANOVA was used to test the effects of N addition on the community, functional groups, species and soil variables within each community. Time (year) was used as a repeated measures factor. Greenhouse-Geisser adjustment was used when the sphericity assumption was not verified. One-way ANOVA was used to test N

treatment effects within each year. Duncan's multiple-range *post-hoc* tests were used to test for differences between each treatment level when results of the one-way ANOVA exhibited a significant effect ($P < 0.05$).

To explore potential controlling factors of species richness in contrasting saline-alkaline communities, we conducted linear regression analysis between species richness with community above-ground biomass or soil saline-alkaline parameters (i.e. pH and EC). For these analyses, we used mean values for each studied variable (biomass, species richness, pH, EC) across the 4 yr ($n = 15$). The normality and homogeneity of variances were verified for all data using Kolmogorov-Smirnov and Levene tests, respectively. The *t*-test, ANOVA and linear regression analyses were performed with IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, US).

Results

Successional dynamics

The DCA ordination diagram showed a clear separation of plots of the two communities, indicating a strong difference in species composition of these communities (Appendix S1). Plots of the high-stress community were chaotic visually, whereas the low-stress community exhibited obvious successional trajectories (Appendix S1).

To examine the N effects on species composition and successional trajectories of each community, we performed DCA for the two communities separately. For the high-stress community, the first two axes explained 76.3% of variance in the vegetation data (Fig. 1a). The first axis was related to N addition, and the second weakly reflected the time series. The correlation between community composition and N addition was 0.562 for axis 1, and correlation between community composition and time series was 0.297 for axis 2. For the low-stress community, the first two axes explained 61.9% of the variance in vegetation data (Fig. 1b). The first axis was related to time and the second weakly reflected the N gradient. The correlation between community composition and time series was -0.882 for axis 1, and the correlation between community composition and N addition was -0.162 for axis 2. This result indicated that the changes in community composition of the low-stress community were mainly attributed to a succession process over time. Regarding functional composition, overall, the low-stress community developed from the annual plant community towards a perennial community. However, regarding species composition, successional trajectories of the low-stress community exhibited low predictability (Fig. 1b). Within each treatment, the three replicated plots showed divergent successional trajectories. In other words, even though the same treatment was applied, the three replicated plots did not

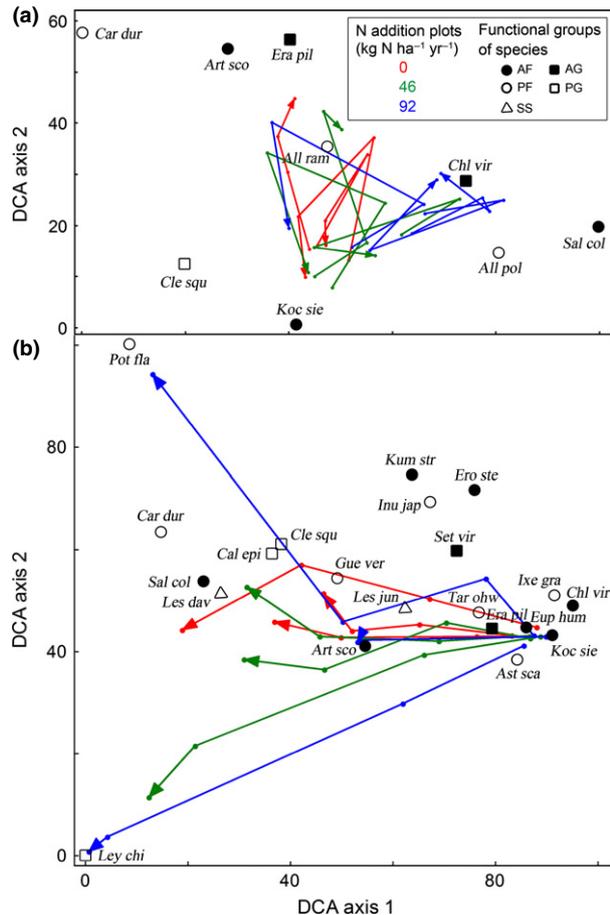


Fig. 1. DCA ordination of plots and species for (a) the high-stress community and (b) the low-stress community. Each of the three replicates was analysed separately. For clarity, 0, 46 and 92 kg·N·ha⁻¹·yr⁻¹ plots are presented. Each plot symbol represents one plot in a given year, and time points of each plot are connected by trajectories. Plot symbols close to arrows represent plots in the last year (2011). The species in different functional groups are shown. Functional group codes: AF, annual forb; AG, annual grass; PF, perennial forb; PG, perennial grass; SS, shrub and semi-shrub. Species codes: *All pol*, *Allium polyrrhizum*; *All ram*, *Allium ramosum*; *Art sco*, *Artemisia scoparia*; *Ast sca*, *Astragalus scaberrimus*; *Cal epi*, *Calamagrostis epigeios*; *Car dur*, *Carex duriuscula*; *Chl vir*, *Chloris virgata*; *Cle squ*, *Cleistogenes squarrosa*; *Era pil*, *Eragrostis pilosa*; *Ero ste*, *Erodium stephanianum*; *Eup hum*, *Euphorbia humifusa*; *Gue ver*, *Gueldenstaedtia verna*; *Inu jap*, *Inula japonica*; *Ixe gra*, *Ixeridium gramineum*; *Koc sie*, *Kochia sieversiana*; *Kum str*, *Kummerowia striata*; *Les dav*, *Lespedeza davurica*; *Les jun*, *Lespedeza juncea*; *Ley chi*, *Leymus chinensis*; *Pot fla*, *Potentilla flagellaris*; *Sal col*, *Salsola collina*; *Set vir*, *Setaria viridis*; *Tar ohw*, *Taraxacum ohwianum*.

develop towards a similar community composition. Among treatments, successional trajectories of N addition plots (46 and 92 kg·N·ha⁻¹·yr⁻¹) and control plots (0 kg·N·ha⁻¹·yr⁻¹) did not exhibit obvious separation, indicating that the expected accelerating effect of N enrichment on succession were contingent. Interestingly, the

degree of separation among the three replicates gradually increased along with the increasing N level (Fig. 1b).

Functional groups

Relative biomass of the functional groups of the two communities exhibited a significant effect of the year (Table 2, Appendix S2). In particular, in the low-stress community, relative biomass of perennials gradually increased from 2008 to 2011 (Fig. 2b). In addition, functional composition of the high-stress community was relatively stable, with annual species (i.e. annual forbs, AF; annual grasses, AG) dominating the plots across the entire experimental period (Fig. 2a, Appendix S2). The functional composition of the low-stress community changed dramatically, especially in 2010 and 2011 (Appendix S2). Relative biomass of annual species declined whereas perennial forbs (PF) and perennial grasses (PG) became the dominant functional groups over time (Table 2, Appendix S2).

Overall, there were no significant effects of N addition on relative biomass of the functional groups of the two communities across the 4 yr (Table 2). However, a trend appeared in which AG dominance increased with N gradient, while AF declined in the high-stress community (Appendix S2). Especially in 2008, the relative biomass of AF significantly declined with the N gradient ($P = 0.036$), and relative biomass of AG significantly increased with the N gradient ($P = 0.043$). N addition did not significantly affect the relative biomass of any functional groups of the low-stress community in any year (all $P > 0.05$).

Individual species

For most of the individual species of the high-stress community and half of the individual species of the low-stress community, there was a significant annual effect on relative biomass (Appendix S3, S4). In the high-stress community, this annual effect on relative biomass of the dominant species *K. sieversiana* and *C. virgata* was not due to directional temporal trends but was rather due to irregular inter-annual fluctuations (Appendix S3). Relative abundance of the subdominant species *A. scoparia* increased over time (Appendix S3). In the low-stress community, the initial dominant species *K. sieversiana* declined substantially from 2009 and disappeared in our survey during the last 2 yr (Appendix S4). The dominance of *L. chinensis* across all plots increased over time (Appendix S4).

Significant effects of N addition on the individual species were only detectable for the relative biomass of *Salsola collina* in the high-stress community (Appendix S3), and for *K. sieversiana*, *Kummerowia striata* and *Gueldenstaedtia verna* in the low-stress community (Appendix S4). In the case of *S. collina* in the high-stress community, however, we did

Table 2. Results (*P*-value) of repeated measures ANOVA for community above-ground biomass, species richness and relative above-ground biomass of the functional groups (annuals, perennials) in the high-stress and low-stress communities. Significant values (*P* < 0.05) are shown in bold.

	Above-Ground Biomass	Species Richness	Relative Biomass	
			Annuals	Perennials
High-Stress Community				
N Addition (N)	0.017	0.321	0.388	0.388
Year (Y)	<0.001	<0.001	0.048	0.047
Y × N	0.127	0.345	0.844	0.850
Low-Stress Community				
N Addition (N)	0.002	0.001	0.853	0.846
Year (Y)	0.004	<0.001	<0.001	<0.001
Y × N	0.380	0.278	0.954	0.956

not observe significant effects of N addition on relative biomass in any year (Appendix S3). The relative biomass of *K. sieversiana* of the low-stress community increased 11.1–15.4% and 85.6–90.3% compared to the control (0 kg-N·ha⁻¹·yr⁻¹) in 2008 and 2009, respectively (Appendix S4). N addition led to 92.1–95.9% and 57.2–95.9% decreases in relative biomass of *G. verna* of the low-stress community in 2010 and 2011, respectively (Appendix S4). Contrary to our expectations, we did not detect significant positive effects of N addition on the dominance of *L. chinensis* (Appendix S4).

Above-ground biomass

Addition of N had significant effects on the above-ground biomass in both communities (Table 2). Specifically, in year 4, the above-ground biomass increased with N addition in both communities (Fig. 2). The treatments with 46, 69 and 92 kg-N·ha⁻¹·yr⁻¹ increased above-ground biomass by 53.1%, 79.0% and 72.6%, respectively, compared to 0 kg-N·ha⁻¹·yr⁻¹ in the high-stress community (Fig. 2). In the low-stress community, treatments with 69 and 92 kg-N·ha⁻¹·yr⁻¹ increased above-ground biomass by 212.3% and 134.6%, respectively, compared to 0 kg-N·ha⁻¹·yr⁻¹. In addition, above-ground biomass of the low-stress community also significantly increased with N addition in year 1 (Fig. 2b). Overall, N addition resulted in a higher increase in above-ground biomass of the low-stress community compared to the increase in the high-stress community.

Species richness

On average, species richness of the high-stress community ($4.56 \pm 0.15 \text{ m}^{-2}$) was lower than that of the low-stress community ($7.82 \pm 0.34 \text{ m}^{-2}$) across all plots through the entire experimental period (Fig. 3; *t*-test: *P* < 0.001, *n* = 15 × 4 = 60).

Addition of N had no significant effect on plant species richness of the high-stress community over the 4 yr

(Table 2, Fig. 3a). In contrast, species richness of the low-stress community was sensitive to N addition (Table 2, Fig. 3b); from 2009 to 2011, species richness decreased significantly at the higher N levels (i.e. 69 and 92 kg-N·ha⁻¹·yr⁻¹). In 2009, species richness declined significantly in plots treated with 69 kg-N·ha⁻¹·yr⁻¹; in the following 2 yr, it declined in plots treated with 46 kg-N·ha⁻¹·yr⁻¹; by year 4, species richness in the low-stress community had declined by 9.4–52.6% across the N addition gradient compared with the control (0 kg-N·ha⁻¹·yr⁻¹).

There was no significant relationship between species richness and above-ground biomass across the 4 yr in the high-stress community. In contrast, a significant negative linear relationship ($R^2 = 0.696$, *P* < 0.001, *n* = 15) between species richness and above-ground biomass was observed in the low-stress community across the 4 yr. Species richness in the high-stress community decreased linearly with the increase in soil pH ($R^2 = 0.358$, *P* = 0.018, *n* = 15) and EC ($R^2 = 0.319$, *P* = 0.028, *n* = 15). However, there were no significant relationships between species richness and soil pH or EC in the low-stress community (*P* > 0.05).

Discussion

Successional dynamics in high- and low-stress communities

In the present study, contrasting successional patterns were observed in the two saline-alkaline grassland communities over the entire experimental period. The high-stress community underwent very few changes in community composition, with annual species dominating the plots during all 4 yr. In contrast, relatively rapid changes in dominance of the different functional groups occurred in the low-stress community. For example, annual forbs initially dominated all the plots and then diminished as succession proceeded, but the dominance of perennial forbs and perennial grasses gradually increased during the course of succession and almost

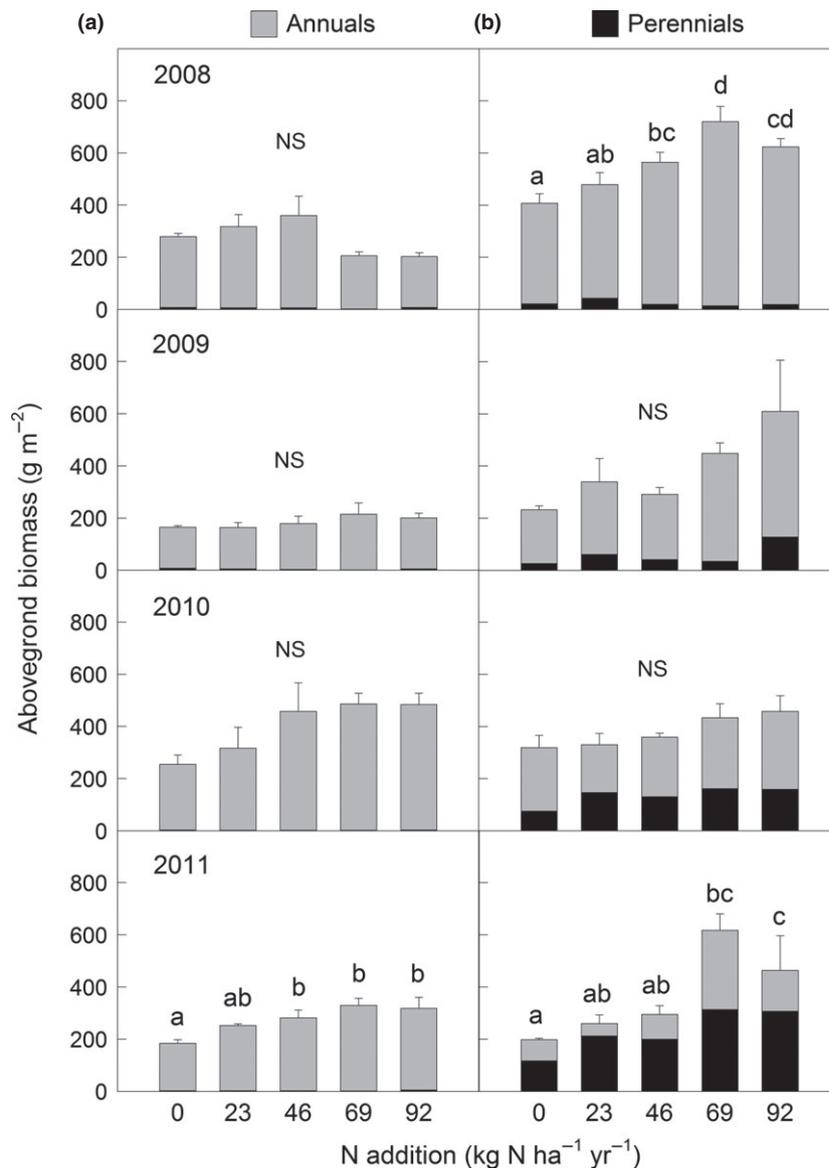


Fig. 2. Effects of N addition on above-ground biomass in (a) the high-stress community and (b) the low-stress community (mean \pm SE). Annuals include annual forbs and annual grasses, and perennials include perennial forbs, perennial grasses and shrubs and semi-shrubs. Different letters above bars indicate significant differences among treatments ($P < 0.05$), and NS indicates a non-significant difference ($P > 0.05$).

dominated all plots in year 4. This type of replacement of short-lived species by perennial species in the low-stress community is typical during the course of secondary succession (McLendon & Redente 1991; Huberty et al. 1998; Paschke et al. 2000; Albert et al. 2014). Here, we provide evidence that high saline-alkaline stress can limit plant community succession as compared to low saline-alkaline areas.

Different degrees of limitations to plant establishment and growth could be the main reason for the contrasting successional patterns between high- and low-stress communities. High-stress habitats present significant barriers

to plant establishment, especially for later successional species, because early successional species are often more tolerant to stress than later successional species (Grime 2001; Walker & del Moral 2003). In the high-stress community, only a few species, especially *C. virgata* and *K. sieversiana*, can survive, grow and reproduce because of their salt- and alkali-tolerant physiological traits (Yang et al. 2007, 2008). However, due to the persistent high saline-alkaline stress over the 4 yr, later successional species such as *L. chinensis*, *C. epigeios* and *A. glabrum* were not able to become established in the high-stress community. Overall, the high-stress community retained the characteristics of the early

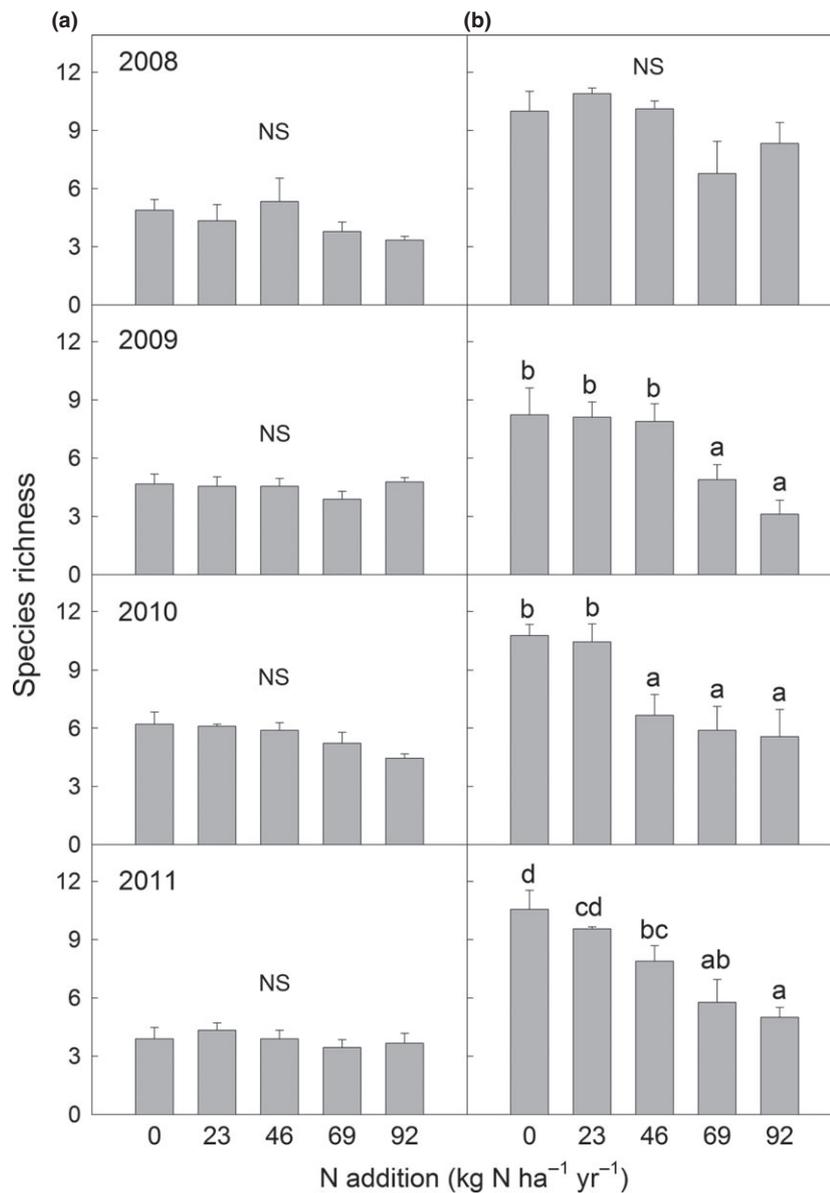


Fig. 3. Effects of N addition on species richness in (a) the high-stress community and (b) the low-stress community (mean \pm SE). Different letters above bars indicate significant differences ($P < 0.05$) among treatments, and NS indicates a non-significant difference ($P > 0.05$).

successional stage and only exhibited inter-annual fluctuations (i.e. non-directional irregular changes), whereas the low-stress community developed from the early successional stage to a later successional stage. Our results suggest that plant communities may follow different strategies in response to different levels of stress. For example, under high-stress conditions (i.e. salinity), plants may follow a stress-tolerant strategy. However, under low-stress conditions, competition–exclusion may be the most important process controlling community dynamics in the late successional stage (Walker & Chapin 1987; Callaway & Walker 1997).

Of special interest, while perennial species such as *L. chinensis*, *C. epigeios*, *A. glabrum* and *P. flagellaris* were not common at the beginning of the study, only 4 yr later, these species dominated most of the low-stress community. All these species are clonal, and may have originated from surviving rhizomes or a seed bank. These results suggest that legacy effects (e.g. intact plants, seed bank and rhizomes) may be important for plant succession after disturbance (Franklin 1990). Clonal species have previously been shown to increase with site successional age (Albert et al. 2014). Such clonal species have the ability to spread rapidly, expand their territory through physiological inte-

gration among ramets, track resources in the habitat (i.e. foraging), have short-range dispersal using competitively superior ramets and longevity of genets enhance spopulation persistence (Svensson et al. 2013).

Effects of N addition on plant community dynamics

According to the theoretical predictions, N enrichment should increase the rate of succession through stimulating competitive exclusion, thereby increasing species turnover (Bazzaz 1996; Pickett et al. 2009). However, we found that N addition did not promote the expected late successional stage in our study region (i.e. perennial grass species), which was contrary to our expectations and that of some other studies (Storm & Suss 2008; Xu et al. 2012). It is well known that arid lands such as those in this study lack nutrients. Phosphorus deficiency (Table 1) may limit incorporation of N into our ecosystem and restrain any positive effect of N on plant succession in semi-arid lands (McLendon & Redente 1991). Only one of the three highest N addition replicates in the low-stress community reached a climax community situation (i.e. perennial grass species such as *L. chinensis*; Fig. 1b), suggesting that the effects of N enrichment on plant succession might depend on micro-environmental conditions (e.g. availability of other nutrients) linked to the huge spatial heterogeneity.

Although N addition did not have obvious effects on the different functional groups, it altered both plant above-ground biomass and species richness. For the low-stress community, N addition decreased species richness and increased biomass. Regarding species loss caused by N enrichment-induced competitive exclusion, two common mechanisms have been proposed: the random loss hypothesis and the functional-based hypothesis (Suding et al. 2005). The random loss hypothesis states that rare species are the most likely to be excluded due to their small population size or low initial abundance (Goldberg & Miller 1990; Stevens & Carson 1999). In contrast, the functional-based hypothesis emphasizes the importance of plant functional traits that determine competitive ability (Craine & Dybzinski 2013). Thus, the increase in biomass of fast-growing species such as annual forbs (low-stress community; Appendix S4) and grasses (high-stress community; Appendix S3) under high N conditions might be favoured by their particular functional traits. The dominant species (e.g. *L. chinensis* in the low-stress community and *C. virgata* in the high-stress community) may have out-competed other species in our plots, thus decreasing species richness, which is likely linked to random loss mechanisms. The overall increase in biomass in both low- and high-stress communities supports the idea that our ecosystem is highly limited by N. However, other environmental conditions (e.g. saline-alkaline stress, drought, etc.) might also

modify responses of these N-deficient ecosystems to N addition (Walker & del Moral 2003). In our study, plant succession of the high-stress community exhibited a similar random pattern for all N treatments (Appendix S1, Fig. 1a), indicating the limitation imposed by high saline-alkaline stress in N-deficient ecosystems. N addition promotes a shift in the successional pattern of low-stress communities, from a convergent pattern within plots with 0 kg-N-ha⁻¹·yr⁻¹ to a divergent pattern within plots given 92 kg-N-ha⁻¹·yr⁻¹ (Fig. 1b). This observed divergent pattern at high levels of N addition may have favoured different communities for several reasons. For instance, in one of the plots with 92 kg-N-ha⁻¹·yr⁻¹ in 2011, the decline in species richness could be attributed to enhanced light limitation caused by the high stature of *A. scoparia* (Hautier et al. 2009; Borer et al. 2014). The development of such dominance hierarchies of undesired species (e.g. *A. scoparia*) could thus arrest the course of succession. Whereas in the other two plots with 92 kg-N-ha⁻¹·yr⁻¹, clonal plants (*L. chinensis* and *P. flagellaris*) may have dominated over other species due to their superior nutrient capture ability (Svensson et al. 2013).

Conclusions

Our results provide direct empirical evidence that relative to low saline-alkaline stress in soil, high saline-alkaline stress limits plant community succession. After the cessation of intensive grazing, the low-stress community exhibited an obvious succession direction from a stage dominated by annuals to one dominated by perennials. N addition did not promote the rate of plant succession, but N enrichment reduced plant diversity and enhanced above-ground biomass, suggesting that N additions may have important effects in our N-deficient system. Moreover, the successional pattern of low-stress communities from convergent to divergent across the increased N gradient indicates the importance of environment heterogeneity at a small spatial scale. As a result, considering spatial and temporal scales is essential in future studies on the effects of artificial N addition (e.g. N deposition).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. DCA ordination of plots of the two communities over time (2008–2011).

Appendix S2. Effects of N addition on relative above-ground biomass of plant functional groups in both the high-stress and the low-stress communities during the four experimental years.

Appendix S3. Effects of N addition on relative above-ground biomass of the main species in the high-stress community during the four experimental years.

Appendix S4. Effects of N addition on relative above-ground biomass of the main species in the low-stress community during the four experimental years.

Appendix S5. Effects of N addition on soil pH and electrical conductivity in response to N addition in both the high-stress and the low-stress communities during the four experimental years.