

## Responses of C:N:P stoichiometry of plants from a Hulunbuir grassland to salt stress, drought and nitrogen addition

Respuestas de la estequiometría C:N:P en un pastizal de Hulunbuir expuesto a estrés salino, sequía y adición de N

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**Abstract.** Chemical elements, such as carbon (C), nitrogen (N) and phosphorus (P), are major limiting nutrients in arid and semi-arid grasslands and their stoichiometry (C:N:P) is a very important ratio to determine. In addition, it is critical to understand how plant stoichiometry responds to multiple environmental factors at the species level. In this study, we conducted a greenhouse experiment to investigate the effects of salt stress (4 g NaCl/kg soil), drought (35% of the soil water holding capacity) and N addition (10 g N/m<sup>2</sup>), as well as their interactions, on C, N and P concentrations and C:N:P ratios in two dominant species (*Allium polyrhizum* and *Stipa krylovii*) from the Hulunbuir grassland of Inner Mongolia. *Allium polyrhizum* had higher N and P concentrations, and lower C:N and C:P ratios than *Stipa krylovii*. Salt stress significantly decreased plant C concentration, while drought had no significant effects on all the study variables of the two plant species. Nitrogen addition significantly increased plant N concentrations, and decreased C:N and C:P ratios. There were significant interactions between salt stress and species identity in affecting plant C and N concentrations as well as C:N and N:P ratios. This study could further enrich the knowledge of plant stoichiometry in response to multiple environmental stresses.

**Keywords:** Stoichiometry; Carbon; Nutrient; Salinization; Aridity; Fertilization.

**Resumen.** Los elementos químicos como el carbono (C), nitrógeno (N) y fósforo (P) son nutrientes limitantes importantes en los pastizales áridos y semiáridos, y su estequiometría (C:N:P) es muy importante en determinar esta relación. Además, es crítico entender como la estequiometría responde a factores ambientales múltiples a nivel de especies. En este estudio, efectuamos un experimento de invernáculo para investigar los efectos del estrés salino (4 g NaCl/kg de suelo), la sequía (35% de la capacidad de campo), y la adición de N (10 g N/m<sup>2</sup>), así como sus interacciones, en las concentraciones de C, N, y P y relaciones C:N:P en dos especies dominantes (*Allium polyrhizum* y *Stipa krylovii*) de los pastizales naturales de Hulunbuir en la parte central de Mongolia en China. *Allium polyrhizum* mostró concentraciones de N y P más altas, y menores relaciones C:N y C:P que *S. krylovii*. El estrés salino significativamente disminuyó la concentración de C de la planta, mientras que la sequía no tuvo los efectos significativos en todas las variables de estudio de las dos especies vegetales. La adición de N incrementó significativamente las concentraciones de N de la planta, y redujo las relaciones C:N y C:P. Hubo interacciones significativas entre el estrés salino y la identidad de las especies en afectar las concentraciones de C y N de la planta y las relaciones C:N y N:P. Este estudio podría enriquecer el conocimiento de la estequiometría de las plantas en respuesta a estreses ambientales múltiples.

**Palabras clave:** Estequiometría; Carbono; Nutriente; Salinización, Aridez, Fertilización.

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## INTRODUCTION

Ecological stoichiometry is a new discipline which yields new insights into the understanding of multiple chemical elements' balance in ecological interactions (Elser et al., 2000; Elser et al., 2010). The importance of chemical elements, such as carbon (C), nitrogen (N) and phosphorus (P), which are the basic components of molecules, cells, individuals, communities and even the biosphere, is overwhelming in ecology as well as their stoichiometry (Sterner & Elser, 2002). Both N and P are essential nutrients for plant metabolism, growth and reproduction in terrestrial ecosystems (Elser et al., 2004). For instance, plant N is closely connected with photosynthesis, primary productivity and litter decomposition (LeBauer & Treseder, 2008); plant P is a vital component of genetic material, cellular structures and energy storage (Elser et al., 2007; Elser et al., 2010).

Basically, C:N and C:P could represent the ability of a plant species to assimilate C when absorbing N and P synchronously, and N:P ratio might reflect the dynamic balance that exists between soil nutrients and plant nutrient demands (Güsewell, 2004). Plant C:N:P stoichiometry reflect how well a plant can adjust itself to the local growth conditions (Elser et al., 2010). Many experiments have focused on the relationships between nutrient stoichiometry in plant communities and broad-scale geographical variations or climatic factors (Reich et al., 2004). However, it is significant to know how the plant C:N:P ratio varies with multiple environmental stresses at the species level in a typical ecosystem (Rong et al., 2015). To the best of our knowledge, few studies have focused on the effects of salinity, N addition and drought on C:N:P of grassland plant species.

Salinization is a major environmental problem all over the world with negative consequences on plant growth and productivity, especially in arid and semi-arid regions (Tilman et al., 2002). It has been reported that saline soils which occupied almost 8% of the earth surface are developing globally over time (Hajiboland, 2013). Soil salt imposes physiological constraints on plants, including osmotic and oxidative stresses, disturbance of photosynthesis, nutrition metabolic disorders and nutrient imbalances, and then effects on plant growth and nutrient contents (Flowers & Colmer, 2008; van Dijk et al., 2015; Sun et al., 2017). These constraints will alter plant stoichiometry (Rong et al., 2015; Sun et al., 2017). Compared to the comprehensive studies on the influence of salinity on plant ecophysiology, few studies have explored the effects of salt stress on plant stoichiometry (Sun et al., 2017). Some studies showed that plant N concentrations and N:P ratios increased, while P concentrations declined, with increasing soil salinity (Patel et al., 2010; Rong et al., 2015). In contrast, other studies observed that plant N concentrations decreased (Ramoliya et al., 2006), and changes of P concentrations depended on the plant species (Loupasaki et al., 2002; Sun et al., 2017).

Drought may increase salinization under the climate change scenario (Aragüés et al., 2015), as salt concentrations of the upper soil layers may increase due to higher losses via

evapotranspiration (Ebert et al., 2002). Droughts have increased over most terrestrial ecosystems since 1950 (Dai, 2013), and it has been promoted by the recent global warming (Trenberth et al., 2014). Drylands, such as arid and semi-arid grasslands, covering about 41% of the earth land surface, and hosting more than 38% of the global population, have always experienced drought (Delgado-Baquerizo et al., 2013). For example, China's grasslands cover over 40% of the country's total land area and these arid and semi-arid grasslands have suffered from increased drought during more than 30 years (Jiao et al., 2016). Moreover, climate warming that occurs in arid and semi-arid areas will make droughts even worse in the future (Zhou et al., 2015).

Soil water availability is the dominant limiting factor for plant growth in arid and semi-arid ecosystems (Lü et al., 2012), and drought would lead to a low soil water content. Proportional different effects of drought on the content of C with respect to the contents of N or P in plant biomass and litter could affect photosynthetic capacity, water use efficiency, and nutrient cycling (Sardans et al., 2008b). The effects of drought stress on C:N:P ratios remain unclear in terrestrial ecosystems (Jiao et al., 2016). Sardans et al. (2008b) found that drought significantly enhanced N:P ratios in wood and roots of *Q. ilex*, and concluded that drought has a greater impact on P than N content. The positive impact of drought on plant N:P ratios was also observed in manipulation experiments (Yuan & Chen, 2015).

The responses of ecosystem processes to salinization and drought might be modulated by other global change factors, such as N deposition or addition (Frechilla et al., 2001; Cui et al., 2010). Nitrogen deposition always leads to higher soil N availability, and changes plant nutrient concentrations and stoichiometry (Galloway & Cowling, 2002). As a strategy of grassland management, N addition has been widely used to improve forage quality and increase the productivity of degraded grasslands. It is widely accepted that N fertilization will alter ecosystem structure and function (Cui et al., 2010). Meanwhile, N fertilization will potentially affect the stoichiometric composition of the grassland community (Lü et al., 2018). Plant nutrient concentrations and stoichiometry will be altered by N enrichment, but empirical studies have obtained inconsistent results. It has been found that N addition would be expected to reduce plant C:N ratios and increase N:P ratios (Lü et al., 2012). Whereas, other studies observed that N fertilization had no effects on N:P ratios (Novotny et al., 2007). Lü et al. (2012) also found that the responses of stoichiometric ratios to N input varied greatly among plant species. Considering these uncertainties, general patterns of plant stoichiometry responding to increased N availability globally are in need of more experiments (Cui et al., 2010).

Most of the global change experiments have typically examined one or two plant nutrients responding to one or two global change drivers (Yuan et al., 2015). It has been reported that the negative effects of salinity on non-halophyte plants

could be exacerbated by drought in arid and semiarid regions (Wang & Li, 2013). In addition, there has been a renewed interest on the influences of N fertilization on plant nutrition under saline conditions. The results have suggested that plants (e.g., *Capsicum annuum* L.) under salt stress performed well when fertilized adequately (Villa-Castorena et al., 2003). The effects of salinity, drought and N addition on C, N and P concentrations as well as C:N, C:P and N:P ratios in grassland plants have not yet been well explored. However, the investigation of these variables is important because they can contribute to understand plant growth strategies and their ability to adapt to environmental changes and stresses. They might also be helpful for facilitating ecological conservation and environmental protection (Rong et al., 2015).

We conducted a pot experiment to determine the potential effects of salinity, drought and N fertilization and their interactions on grassland plants C:N:P stoichiometry. Treatments included salt stress, increased drought and N addition on two model plant species, *Allium polyrhizum* and *Stipa krylovii*. These are important plant species in the Hulunbuir grassland of Inner Mongolia. Based on our field observations of recent years, we found that there was a trade-off between *Allium polyrhizum* and *Stipa krylovii* regarding their richness among different years. We hypothesized that (1) salt stress and drought would decrease plant C, N and P concentrations, influencing C:N:P ratios; (2) N addition would result in higher plant N, unchanged C and P concentrations, and thus

higher N:P and lower C:N ratios (Bai et al., 2010; Wang et al., 2014). Based on these two hypotheses, interactions among salinity, drought and N addition in affecting plant nutrient concentrations and stoichiometric ratios were expected.

## MATERIALS AND METHODS

**Plant and soil material.** We collected the mature seeds of *Allium polyrhizum* and *Stipa krylovii* from the Hulunbuir grassland of Inner Mongolia at the end of September, 2015. The seeds of *Allium polyrhizum* and *Stipa krylovii* were sowed in two large plastic garden pots, respectively, in the early spring of 2016. Then the seedlings were transplanted to 80 experimental plastic pots (29 cm × 31 cm; 40 pots for *Allium polyrhizum* and 40 pots for *Stipa krylovii*). Pots were filled with soil collected at the Hulunbuir grassland. Each pot held one plant species consisting of six individuals.

The soil type was chestnut soil (Kastanozem soil group). The soil water holding capacity and soil pH were 24.5% and 6.66, respectively. After the soil was taken back to the greenhouse in the campus of Dalian Minzu University, we fully mixed it and removed the plant propagules.

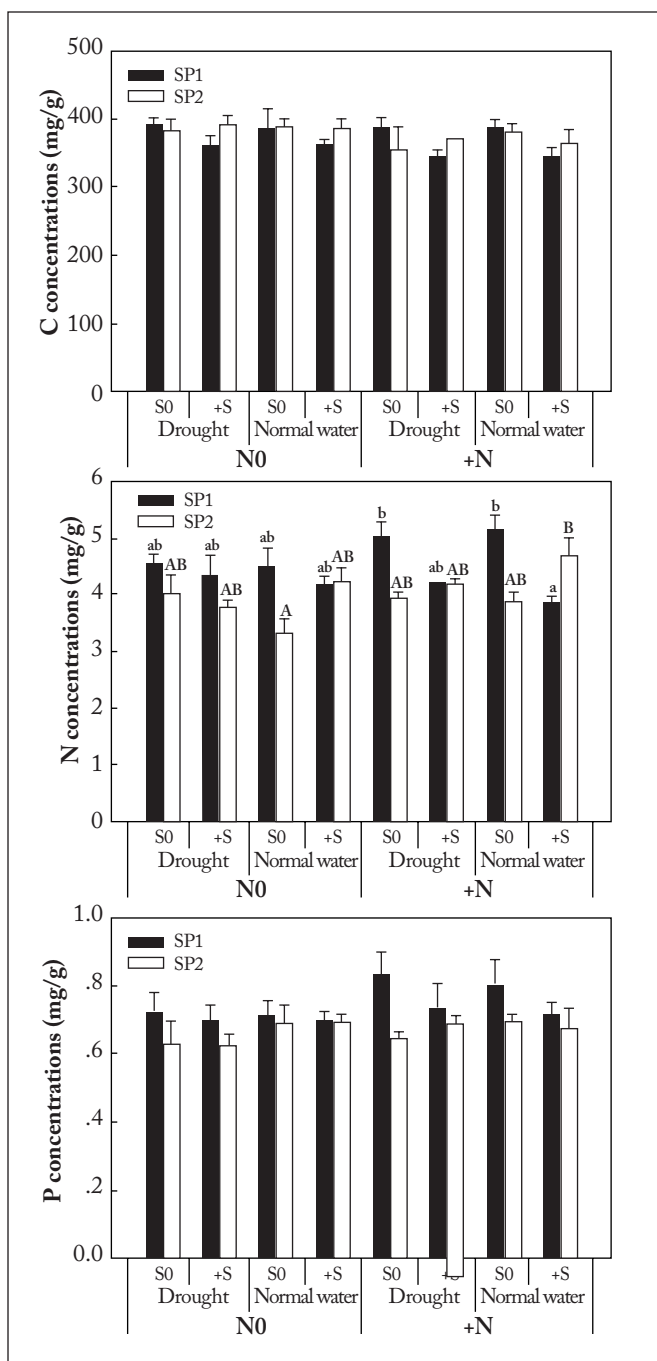
**Experimental design.** This experiment was conducted in the greenhouse of Dalian Minzu University in the year 2016. Salt was applied to the soil at two levels: no salt added and 4 g NaCl/kg soil (Chao et al., 2007), and N addition ( $\text{NH}_4\text{NO}_3$ )

**Table 1.** Results (*F*-values) of four-way ANOVAs on the effects of species identity (SP), N addition (N), salt stress (S), drought stress (D) and their interactions on C, N and P concentrations and their stoichiometry values.

**Tabla 1.** Resultados (valores *F*) de ANOVA de 4 vías sobre los efectos de la identidad de la especie (SP), adición de N (N), estrés salino (S), estrés por sequía (D) y sus interacciones sobre las concentraciones de C, N y P y sus valores de estequiometría.

	d.f.	[C]	[N]	[P]	C:N	C:P	N:P
SP	1	1.849	<b>19.885***</b>	<b>8.945**</b>	<b>21.903***</b>	<b>11.319***</b>	0.052
N	1	1.896	<b>5.459*</b>	2.595	<b>9.02**</b>	<b>4.667*</b>	0.001
S	1	<b>7.312**</b>	1.011	1.046	2.002	0.603	0.006
D	1	0.096	0.045	0.291	0.111	0.933	0.472
SP×N	1	0.025	0.596	0.867	1.426	0.056	1.179
SP×S	1	<b>3.754^</b>	<b>24.591***</b>	1.39	<b>7.245**</b>	0	<b>4.524*</b>
SP×W	1	0.006	0.57	1.399	0.095	1.367	0.278
N×S	1	1.261	2.147	0.353	0.421	0.017	0
N×W	1	0.017	0.557	0.507	0.608	0.698	1.579
S×W	1	0.021	1.767	0.015	2.137	0.015	1.116
SP×N×S	1	0.009	<b>5.102*</b>	0.768	1.459	0.253	0.67
SP×N×W	1	0.073	0.658	0.05	0.939	0.103	0.653
SP×S×W	1	0.15	<b>6.924*</b>	0.149	<b>5.486*</b>	0.16	<b>4.832*</b>
N×S×W	1	0	1.196	0.088	1.715	0.06	0.304
SP×N×S×W	1	0.047	0.084	0.163	0.41	0.347	0.095

The *F*-values are presented, together with their level of significance. \**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001.



**Fig. 1.** The effects of salt stress, N addition and drought on C, N and P concentrations of *Allium polyrhizum* (SP1) and *Stipa krylovii* (SP2). Data are adjusted means + 1 SE ( $n=5$ ). Bars within species with dissimilar letters (lowercase letters for SP1, capital letters for SP2) represent significance difference ( $P<0.05$ ). Absence of letters above the bars indicate no significant differences ( $P>0.05$ ).

**Fig. 1.** Efectos del estrés salino, adición de N y sequía sobre las concentraciones de C, N y P de *Allium polyrhizum* (SP1) y *Stipa krylovii* (SP2). Los datos son el promedio + 1 EE de  $n=5$ . Dentro de cada especie, letras minúsculas (SP1) o mayúsculas (SP2) diferentes indican diferencias significativas ( $P<0,05$ ). La ausencia de letras sobre las barras indican diferencias no significativas ( $P>0,05$ ).

at two levels: 0 or 10 g N/m<sup>2</sup> (Han, Sistla, Zhang, Lü & Han 2014). Both the NaCl and NH<sub>4</sub>NO<sub>3</sub> were added to the soil in wet form. Drought was applied at two levels: 75% (normal water) and 35% (drought) of the soil water holding capacity (Huang et al., 2013). In order to maintain the 75% or 35% soil moisture, a weighing method was used during the whole experiment since the time that the N addition and salt stress treatments were applied.

All of the treatments and their possible combinations were started in June 2016. In total, there were eight treatments (2 salt levels × 2 water levels × 2 N levels) for each plant species, replicated five times (five blocks). Pots were randomly arranged in each block.

**Sampling and chemical analysis.** In each pot, we harvested all plants (leaves and stems) at the end of the growing season as one sample. The collected samples were transported to the laboratory, oven-dried at 65 °C for 72 h, and then ground with a ball mill. The plant samples were then kept dry and cool in plastic valve bags for chemical analysis.

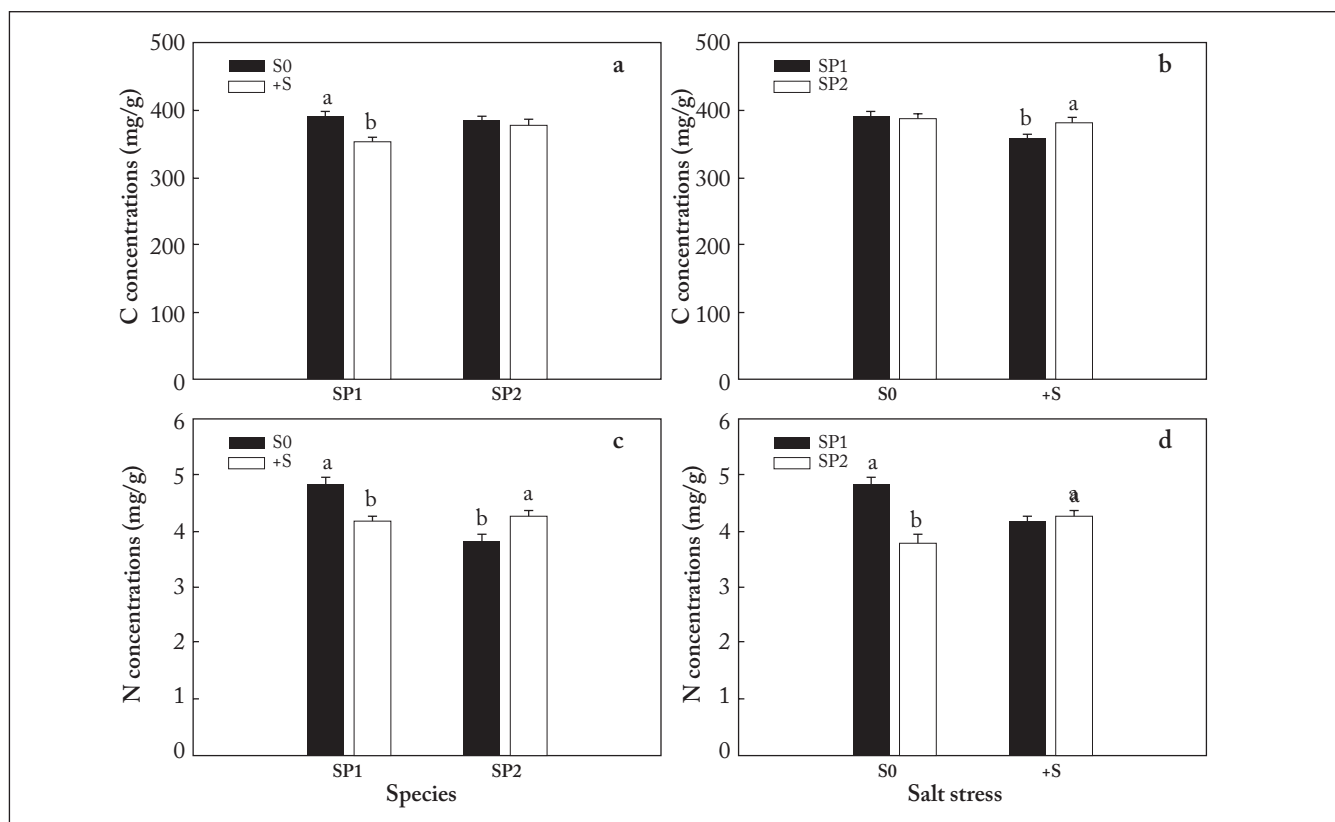
Carbon concentrations of the plant were determined with the wet oxidation method (potassium dichromate volumetric method) (Shaw 1959). Plant N concentrations were measured used the micro-Kjeldahl method (Iswaran & Marwah 1980). We used H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> to digest the plant materials; after reaction with molybdenum blue, P concentrations of the digest were analyzed colorimetrically at 880 nm. The stoichiometric ratios of C:N, C:P and N:P were reported as mass ratios.

**Statistical analysis.** The normality of the data was tested using the Levene's test. The main and interactive effects of species identity, salt stress, drought and N addition on plant C, N and P concentrations, and their stoichiometric ratios, were determined by four-way analysis of variances (ANOVAs). Significant differences among treatments were analyzed using a Tukey HSD post hoc test following the four-way ANOVAs. When there were two-way interactions between species and treatments (such as salt stress) for certain variables, one-way ANOVAs were performed for each species. Statistical significance was determined at  $P=0.05$ . All statistical analyses were performed using SPSS V16.0 (SPSS, Chicago, IL, USA).

## RESULTS

**Plant C, N and P concentrations.** Nitrogen and P concentrations varied significantly between the two species (Table 1; Fig. 1). The species of *Allium polyrhizum* had higher N and P concentrations than *Stipa krylovii*. Across the two species, N addition significantly increased N concentration, while salt stress significantly decreased C concentration. However, drought had no significant effects on the concentrations of all elements.

Interactions among salt stress, drought and N addition in affecting plant nutrient contents were not observed in this



**Fig. 2.** The responses of C and N concentrations to the interaction of salt stress and species identity (SP1, *Allium polyrhizum*; SP2, *Stipa krylovii*). Data are adjusted means + 1 SE (n=5). Bars within species with dissimilar letters represent significance difference (P<0.05). Absence of letters above the bars indicate no significant differences (P>0.05).

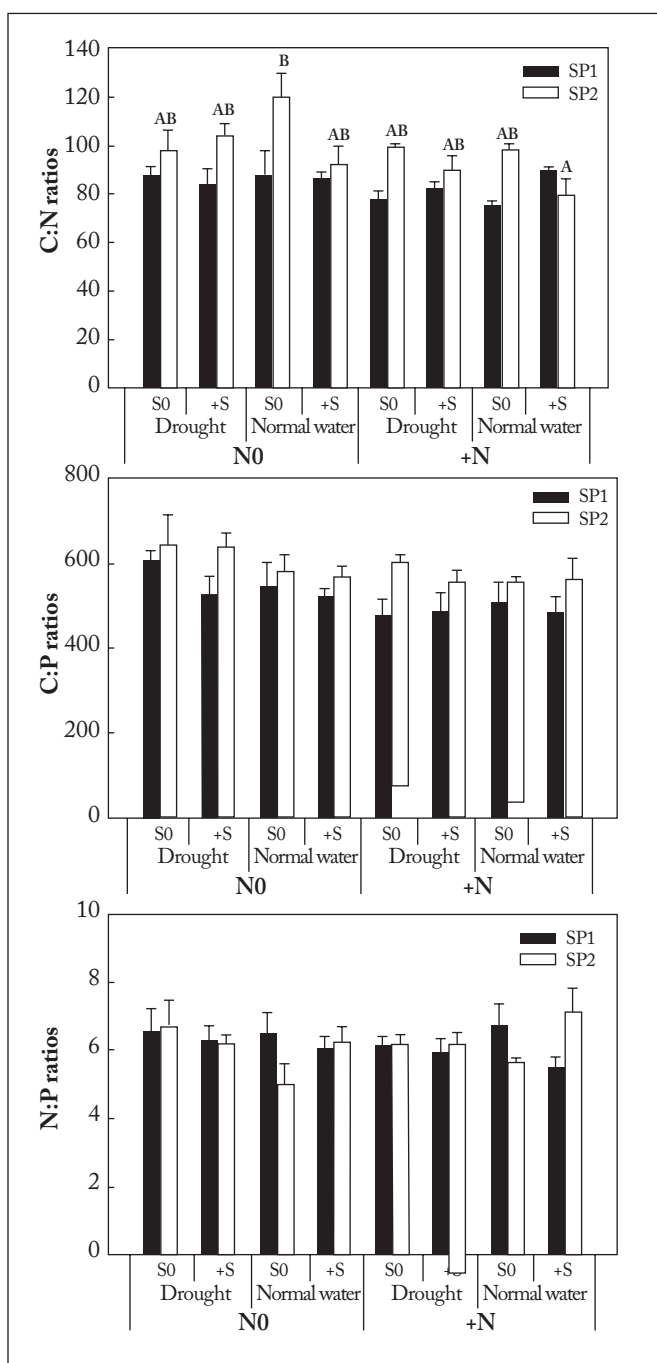
**Fig. 2.** Respuestas de las concentraciones de C y N a la interacción del estrés salino y la identidad de la especie (SP1, *Allium polyrhizum*; SP2, *Stipa krylovii*). Los datos son el promedio + 1 EE de n=5. Dentro de cada especie, letras diferentes indican diferencias significativas (P<0,05). La ausencia de letras sobre las barras indican diferencias no significativas (P>0,05).

study. Whereas, there were interactions between species identity and salt stress to influence C and N concentrations based on the four-way ANOVA (Table 1). For the species of *Allium polyrhizum*, C concentration was significantly decreased by salt stress; however, salt stress had no significant effect on C concentration of *Stipa krylovii*. When there was no salt stress, C concentrations of the two species were not different from each other, while under salt stress, C concentrations varied significantly between *Allium polyrhizum* and *Stipa krylovii* (Fig. 2a, b). As for C concentrations, salt stress significantly decreased C concentration of *Allium polyrhizum* while increased N concentration of *Stipa krylovii*. Under ambient salt conditions, N concentration of *Allium polyrhizum* was higher than that of *Stipa krylovii*; when suffered from salt stress, N concentrations of the two species were not significantly different from each other (Fig. 2c, d).

**Plant C:N:P ratios.** The two species varied greatly in C:N and C:P ratios (Table 1; Fig. 3). The species of *Allium polyrhizum*

had lower C:N and C:P ratios than *Stipa krylovii*. Across the two species, N addition significantly decreased C:N and C:P ratios. None of the plant C:N:P ratios were significantly influenced by salt stress and drought.

Inconsistent with our expectation, interactions among salinity, drought and N addition in affecting plant stoichiometric ratios were not found in this study. But, salt stress significantly interacted with species identity to affect the ratios of C:N and N:P (Table 1). Salt stress led to a decrease in C:N ratio of *Stipa krylovii*, but had no significant influences on this variable of *Allium polyrhizum*. Under ambient salt conditions, C:N ratio of *Allium polyrhizum* was lower than that of *Stipa krylovii*, while under salt conditions, C:N ratios of the two species did not differ from each other (Fig. 4a, b). As for N:P ratios, the results of one-way ANOVAs suggested that there were no significant differences between two kinds of salt conditions for *Allium polyrhizum* or *Stipa krylovii*, and between the two kinds of plant species under ambient or salt conditions (Fig. 4c,d).



**Fig. 3.** The effects of salt stress, N addition and drought on C:N, C:P and N:P ratios of *Allium polyrhizum* (SP1) and *Stipa krylovii* (SP2). Data are adjusted means + 1 SE ( $n=5$ ). Bars within species with dissimilar letters represent significance difference ( $P<0.05$ ). Absence of letters above the bars indicate no significant differences ( $P>0.05$ ).

**Fig. 3.** Efectos del estrés salino, adición de N y sequía sobre las relaciones C:N, C:P y N:P de *Allium polyrhizum* (SP1) y *Stipa krylovii* (SP2). Los datos son el promedio + 1 EE de  $n=5$ . Dentro de cada especie, letras minúsculas (SP1) o mayúsculas (SP2) diferentes indican diferencias significativas ( $P<0,05$ ). La ausencia de letras sobre las barras indican diferencias no significativas ( $P>0,05$ ).

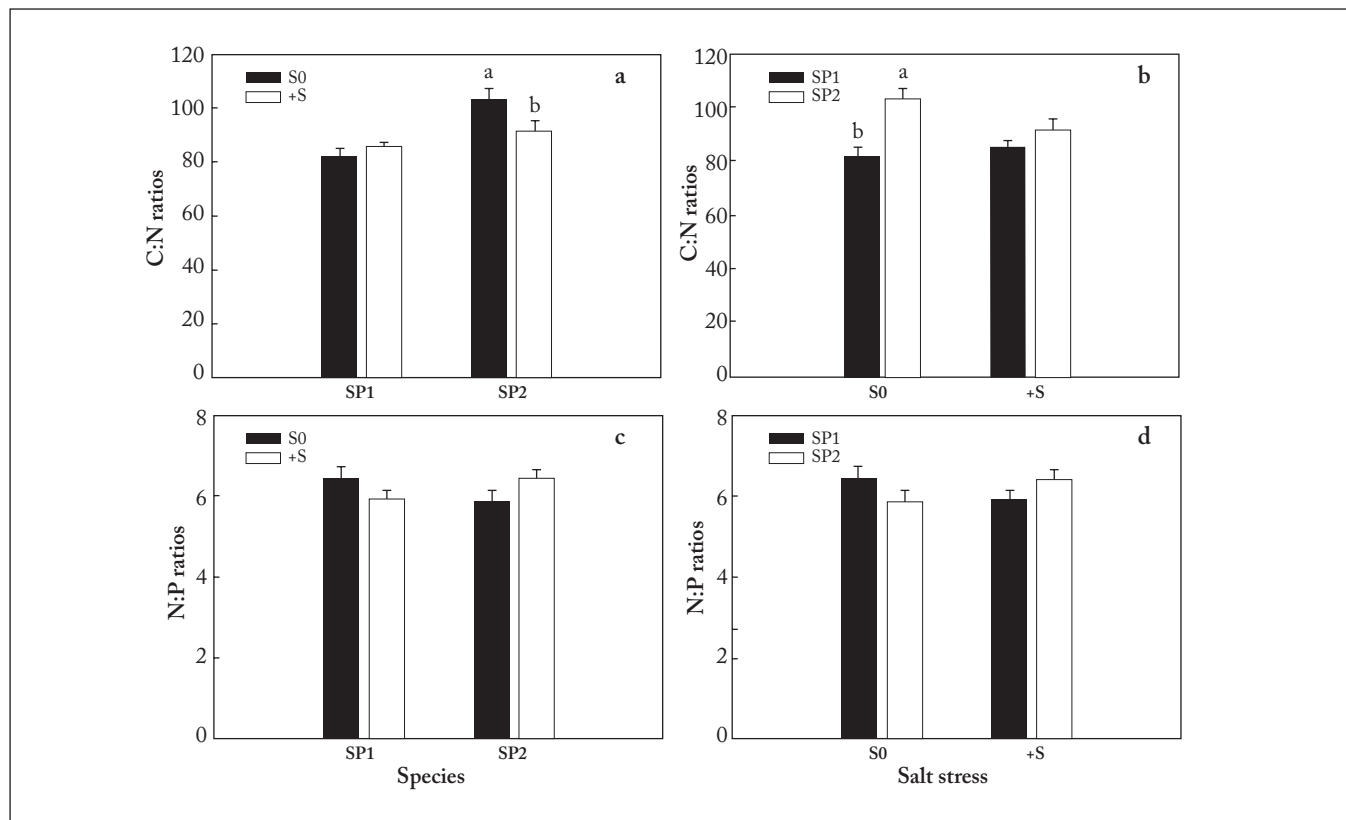
## DISCUSSION

Salt stress can have significant influences on plant nutrient concentrations, and their stoichiometric ratios in various ecosystems (Loupassaki et al., 2002; Song et al., 2015; van Dijk et al., 2015; Gong et al., 2017; Sun et al., 2017). Our findings suggested that salt stress significantly decreased plant C concentrations but had no influences on N and P concentrations and C:N:P ratios. These results were just partly in accordance with our hypothesis.

Plant C is mainly derived from the atmosphere through photosynthesis (Wang et al., 2015). Whereas, plant transpiration and photosynthetic rates can be declined due to the reduction of stomatal conductance under salt stress. As a result, carbon dioxide assimilation would be hindered which might lead to a decreasing of C concentration in plants (Sardans et al., 2012). Consistent with our result, Song et al. (2015) observed that the C contents in both shoot and root of *Hordeum brevisubulatum* declined significantly as salt stress was more severe.

Based on the results of the four-way ANOVAs, on average of the two plant species, all of the C:N:P ratios were unchanged under salt stress. Wang et al. (2015) demonstrated that salinity was not a potential driver of leaf C:N:P stoichiometry in an arid-saline environment which supports our results. Inconsistently, there were also many studies which showed that variation in soil salt concentrations have profound influences on plant stoichiometry in natural ecosystems and pot experiments (Song et al. 2015; Gong et al., 2017). The inconsistencies among different studies might be due to the differences in experimental environments, such as soil nutrient availability and salt types, as well as variations in the plant species selectivity for nutrients, and plant physiological adaptation strategies in response to salinity (Sun et al., 2017).

In natural ecosystems, drought can alter plant nutrient concentrations and stoichiometry through different ways, such as changing soil moisture and nutrient availability (An et al., 2005; Sardans & Peñuelas, 2008a). However, in this study, we observed that drought had no significant influences on plant C, N and P concentrations and C:N:P ratios. Our results were partly in line with the findings of Sardans et al. (2008a), who observed that N and P in *Hypnum cupressiforme* showed no significant responses to moderate drought in a Mediterranean forest. There were many studies on the responses of plant stoichiometry to drought, but the results differed among ecosystems and species (Sardans et al., 2012). It has been reported that drought increased C:N ratio in temperate heathlands (Larsen et al., 2011), but decreased this ratio in wet-temperate ecosystems (Sardans et al., 2012). Sardans et al. (2008b) found that C:N:P stoichiometry of *Quercus ilex* and *Arbutus unedo* were more sensitive to drought than that of *Phillyrea latifolia* which was not affected by drought. Nutrient stoichiometry could be maintained in some species while in other species



**Fig. 4.** The responses of C:N and N:P ratios to the interaction of salt stress and species identity (SP1, *Allium polyrhizum*; SP2, *Stipa krylovii*). Data are adjusted means + 1 SE ( $n=5$ ). Bars within species with dissimilar letters represent significance difference ( $P<0.05$ ). Absence of letters above the bars indicate no significant differences ( $P>0.05$ ).

**Fig. 4.** Respuestas de las relaciones C:N y N:P a la interacción de estrés salino e identidad de la especie (SP1, *Allium polyrhizum*; SP2, *Stipa krylovii*). Los datos son el promedio + 1 EE de  $n=5$ . Dentro de cada especie, letras minúsculas (SP1) o mayúsculas (SP2) diferentes indican diferencias significativas ( $P<0,05$ ). La ausencia de letras sobre las barras indican diferencias no significativas ( $P>0,05$ ).

it might be affected by drought as observed in this and other studies mentioned above. It is possible that competitive capacity among species might be changed by increased drought in a natural ecosystem. Given the importance of water availability for ecosystem processes (e.g. biogeochemical cycles) (Yuan et al., 2015), further studies are still needed to examine the effects of drought on plant stoichiometry.

Partly in line with our hypothesis, N addition significantly increased N concentration and decreased C:N and C:P ratios in the plants we observed. N fertilization always results in higher soil N availability and thus in enhanced N concentrations and declined C:N ratios in many plant species (Galloway et al., 2002; Xia & Wan, 2008). Similar to our results, a previous study also reported that N addition led to a higher foliar N concentrations, unchanged C concentrations and a lower C:N ratio (Cui et al., 2010). A recent meta analysis showed that N fertilization increased leaf N concentrations in all studied vegetation types (Sardans et al., 2017). Therefore, it seems that the responses of plants to N fertilization with increased N concentrations and reduced C:N ratios are universal in various ecosystems (Cui et al., 2010). However, large

uncertainties still remained on the effects of N enrichment on plant C:N:P stoichiometry. For the two species we studied, we found that C:N and C:P were decreased by N addition which were only consistent with certain prior studies (Heyburn et al., 2017), while many other studies reported inconsistent results. Zhang et al. (2016) reported that N addition had no impacts on C:N:P ratios of *Phragmites communis*. Ai et al. (2017) observed that N addition had no influence on C:N and C:P ratios, but significantly increased N:P ratios in shoots of *Bothriochloa ischaemum*. These inconsistent results may be contributed by the various N addition rates, species-specific responses to N addition and different ecosystem types in individual studies (Cui et al., 2010). Due to these inconsistencies, a meta-analysis using existing data to assess the underlying mechanisms accounting for the differences in previous studies is needed (Menge & Field, 2007).

Though the interactions among salinity, drought and N addition in affecting plant nutrient contents and stoichiometric ratios were not observed in this study, our data demonstrated that there were significant interactions between salt stress and species identity to affect certain variables of plant nutrient

chemistry, such as C and N concentrations and C:N ratios.

Salt stress significantly reduced C concentrations of *Allium polyrhizum*, but never affected C concentrations of *Stipa krylovii*. In addition, there were no significant differences between C concentrations of the two species under ambient salt conditions. However, C concentrations of *Stipa krylovii* were much higher than those of *Allium polyrhizum* under salt conditions. Plants can have different physiological adaptation strategies in response to environmental stress, such as salinity (Sun et al., 2017). The responses to salt stress might be species-specific. We considered that *Stipa krylovii* would be more resistant to salinity conditions than *Allium polyrhizum* according to our results.

Salinity significantly decreased N concentrations of *Allium polyrhizum* while enhanced N concentrations of *Stipa krylovii*. Under salt conditions, nitrogen uptake by plant roots would be lowered due to Na<sup>+</sup> ion competition (Sun et al., 2017) and salt-induced low transpiration rates (Eallonardo et al., 2013). Moreover, the presence of excess salt might make non-available some essential elements (such as N), inhibit protein synthesis, and alter the composition of membrane phospholipids which would change the membrane permeability to various nutrients (Nathawat et al., 2007). All of these mechanisms may finally contribute to the decreased N concentrations on *Allium polyrhizum*. Similarly, Nathawat et al. (2007) also reported that different nutrient elements, such as N, in different parts of *Brassica juncea* (leaves, stem, and root) decreased under saline conditions. On the other hand, our results showed that the response of N concentrations of *Stipa krylovii* to salt stress was positive. This could also be attributed to different adaptive mechanisms of the two plant species in response to salinization (Sun et al., 2017). Our finding was consistent with that of Song et al. (2015) which found that N content increased in wild barley (*Hordeum brevisubulatum*) as NaCl concentrations increased. In this study, the increased N concentration of *Stipa krylovii* may partly be due to the increased antioxidant enzyme synthesis that required the use of N under salt stress (Song et al., 2015). It has been reported that plant photosynthetic capacity correlated strongly with the allocation of N to the photosynthetic machinery, so some plant species might allocate the majority of their N to their leaves and stems in order to resist the negative influence that salt stress exerted on photosynthesis (Song et al., 2015). This might be another reason for the increased N concentration. In addition, salinity suppressed plant growth (Sun et al., 2017) and decreased water uptake by shoots (Nathawat et al., 2007). This may have caused the lower plant biomass as well as the relatively higher N concentrations due to the smaller dilution effect. This result also indicated that *Stipa krylovii* could tolerate salt stress to some extent because of its increased N concentrations under saline conditions in comparison to *Allium polyrhizum*.

Salt stress also interacted with species identity to influence plant C:N ratios. The C:N ratio of *Stipa krylovii* showed a

negative response to salt stress. The increased N concentration and unchanged C concentration of *Stipa krylovii* under saline conditions resulted in decreased C:N ratios (Fig. 2). However, there was no significant response of C:N ratio of *Allium polyrhizum* to salt stress. We attributed the unchanged C:N ratio to the simultaneous reduction of C and N under saline conditions (Fig. 2). Species-specific responses of stoichiometric ratios to salt addition were observed in this study. As a result, we considered that salt stress might alter species competition and community composition by changing species-specific nutritional status as well as stoichiometric ratios in natural grassland ecosystems. Therefore, the interactive effects of salt stress and species identity should be well studied in the future to demonstrate their influence on grassland nutrient cycling.

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## CONCLUSIONS

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Our study showed that both salt stress and N addition had direct effects on certain chemical and stoichiometric variables of the two study plant species. Drought had no significant influences on all of these variables. Moreover, our data showed that there were interactions between salt stress and species identity that affected plant C and N concentrations as well as C:N and N:P ratios. We observed the profound influences of salinity and N addition on plant stoichiometry at the species level. This is important when we model the interactions of C, N and P cycles in terrestrial ecosystems under global change scenarios. This study further enriches the knowledge of plant stoichiometry in response to multiple environmental stresses. However, further research is still needed to investigate the responses of plant nutrient chemistry to environmental factors due to the inconsistencies found among the existing studies.

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