

Original Research

How Plant Functional Traits of Dominant Species Respond to Fencing and Water-Nitrogen Addition in Horqin Grassland, China

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Abstract

Plant functional traits can reflect the response and adaptation of plant species to environmental changes. However, how plant functional traits of dominant species respond to the fencing, water and nitrogen additions in a sandy grassland ecosystem is still unclear. Here, a manipulative field experiment was conducted to investigate the effects of fencing, water and nitrogen addition on the functional traits of four dominant species (*Pennisetum centrasiaticum*, *Cleistogenes squarrosa*, *Chenopodium acuminatum* and *Salsola collina*) in Horqin sandy grassland. The results showed that nitrogen addition had a significant effect on plant height, specific leaf area (SLA), leaf nitrogen content (LNC), and leaf dry matter content (LDMC). Nitrogen addition increased plant height for *C. squarrosa* as well as SLA for *P. centrasiaticum* and *S. collina*, while reducing plant height for *P. centrasiaticum* and LDMC for *S. collina*. Also, nitrogen addition increased LNC for the four dominant species. Water addition reduced LDMC in grazing treatment, as well as plant height in fencing and nitrogen addition treatments. Compared to control treatment, nitrogen addition increased the strength of negative associations of LDMC with plant height and LNC. The results suggest that nitrogen addition plays an important role in determining the growth of the four dominant species, and water addition increases the competition of resource use among species in fencing and nitrogen addition treatments. Plants in sandy grassland can mediate the key functional traits to cope with alterations of water and nitrogen under the future global change scenarios.

Keywords: fencing, water and nitrogen addition, plant functional traits, dominant species, sandy grassland

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Introduction

Grassland accounts for 41% of the terrestrial ecosystems in China [1], and also plays an important role in sustaining ecosystem function [2, 3]. However, the degradation of grassland is still severe due to climate change and unreasonable human activities such as grazing [4, 5], and the negative effect of grazing on plant community diversity and primary productivity has been reported [4, 6]. Moreover, Huang et al. [7] reported that precipitation decreased in Inner Mongolia, whereas nitrogen deposition increased due to agricultural and industrial activities [8]. Nitrogen supply not only increased aboveground biomass [9] but also impacted plant functional traits [10]. Species have different strategies to cope with environmental change [11], such as the different responses of functional traits of *Leymus chinensis* and *Cleistogenes squarrosa* to grazing in Inner Mongolia typical steppe [12]. However, the response of plant functional traits for perennials and annuals to fencing, water and nitrogen addition is still unclear in the field, especially in Horqin sandy grassland.

Plant functional traits are important in ecology, because they can respond to changes in environmental conditions at the individual level and also affect ecosystem structure and function [13, 14]. Recently, plant functional traits have been documented in response to climate changes [15] and grazing [16, 17]. The key plant functional traits such as plant height, specific leaf area (SLA), leaf dry matter content (LDMC) and leaf nitrogen content (LNC) not only strongly correlated with the relative growth rate and photosynthesis [18] but also linked to resource acquisition and environmental adaptation [19]. Moreover, plant height, SLA, LDMC and LNC are easy to be measured [15, 19, 20]. Here, these key functional traits were chosen in our study.

Horqin sandy grassland is located in the agro-pastoral zone [21], and the sandy grassland is very vulnerable to climate change and human activities [22]. The degradation of sandy grassland is mainly due to unreasonable land use management and heavy grazing [23, 24]. In recent years, more attempts had been adopted to restore the degraded sandy grassland. It has been reported that conservation practices such as fencing and decreased grazing intensity help to restore the degraded grassland ecosystem [23, 25]. Su et al. [26] showed that fencing not only increased the aboveground biomass, but also enhanced vegetation recovery in Horqin sandy land. However, species have different growth strategies in response to grazing [27], and the effect of fencing and grazing on plant functional traits is still rare in Horqin sandy grassland. Here, we selected two dominant perennial species (*Pennisetum centrasiaticum* and *C. squarrosa*) and two dominant annual species (*Chenopodium acuminatum* and *Salsola collina*) because these species are widespread in the

sandy grassland and sensitive to environmental change [28].

In order to provide theoretical support for the sustainable development of sandy grassland in Horqin, we established a fencing, water and nitrogen addition experiment in the field. The object of this study was to investigate (1) how the functional traits of four dominant species respond to fencing, water and nitrogen addition treatments and (2) do nitrogen addition affect the relationships between functional traits.

Materials and Methods

Study Area

This study was conducted in Horqin sandy grassland near the Naiman Desertification Research Station of the Chinese Academy of Science (42°57.83'N, 120°40.03'E, 364 m a.s.l.). The climate is characterized as typical semiarid continental. The mean annual precipitation is 360 mm, and nearly 75% of the precipitation falls during the growing season (June to September) [21]. The average annual temperature is about 6.4°C [21]. The vegetation is dominated by *P. centrasiaticum*, *C. squarrosa*, *C. acuminatum* and *S. collina*. The soil is aeolian sandy soil according to the Chinese soil taxonomy classification system (<http://www.resdc.cn>).

Experiment Design

We set up a fencing, nitrogen and water addition experiment in a homogeneous sandy grassland in 2014. There were eight treatments: grazing (G), fencing (F), N addition (N), water addition (W), F+N, F+W, G+W+N and F+W+N. Forty-eight 8 m×8 m plots were randomly arranged (six replicates for each treatment), and plots were separated from each other by a 1 m wide aisle. The grazing plots were grazed by cattle in early July and winter, respectively. The fenced plots were fenced with barbed wire. The plots with N addition treatment receive urea 10 g N m⁻² month⁻¹ in April and July, respectively. N addition was applied by hand in the rainy days. The water addition treatment was watered by 30% of the monthly precipitation (from May to August) using the underground water nearby.

Sampling

We carried out the sampling at the peak of growing season (late August) in 2016. Four dominant plant species – two perennial species (*P. centrasiaticum* and *C. squarrosa*) and two annual species (*C. acuminatum* and *S. collina*) – were chosen as the study material. However, *P. centrasiaticum*, *C. squarrosa* and *S. collina* were not present in all plots. Sampled subplots were set in the center of each plot. We focused

Table 1. ANOVA analysis of functional traits of dominant species responding to fencing, water and nitrogen additions.

	Plant height		SLA		LDMC		LNC	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Fencing	3.09	ns	0.45	ns	0.88	ns	0.00	ns
Nitrogen	5.19	*	13.44	**	6.77	*	272.72	**
Water	0.56	ns	0.34	ns	0.37	ns	0.90	ns
Species	93.94	**	44.27	**	323.08	**	30.44	**
Fencing × Nitrogen	0.02	ns	0.18	ns	0.04	ns	3.28	ns
Fencing × Water	7.04	*	4.65	*	7.32	*	0.35	ns
Nitrogen × Water	5.67	*	0.26	ns	0.07	ns	0.63	ns
Species × Fencing	2.37	ns	0.38	ns	0.43	ns	0.44	ns
Species × Nitrogen	18.05	**	1.72	ns	3.06	*	14.79	**
Species × Water	0.45	ns	0.07	ns	0.46	ns	0.46	ns

Trait abbreviations are specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC). *F*, *F*-values. *P*, Probabilities. *represents $P<0.05$, **represents $P<0.01$, ns represents no significant ($P>0.05$).

on four functional traits, including plant height, specific leaf area (SLA), leaf dry matter content (LDMC), and leaf nitrogen content (LNC) because these traits related to different plant functional strategies [29, 30]. The maximum plant height was measured using steel tape. 5 to 10 fully mature and healthy leaves of each dominant species were selected from 3-5 individuals in the plot in order to measure plant functional traits. The leaves were placed between moist papers in sealed plastic bags, and then stored in a refrigerator for laboratory analysis. SLA, LDMC and LNC were measured according to standard protocols [19]. LNC was determined using an elemental analyser (Costech ECS 4010, Italy), and LNC was expressed in mass (%).

Data Analysis

The effect of species, fencing, water and nitrogen addition on plant functional traits (plant height, SLA, LDMC and LNC) was performed using analysis of variance (ANOVA) under the general linear models (GLM), with species, fencing, water and nitrogen addition as fixed factors, and the plant functional traits as dependent variables. Multiple comparisons with least-significant difference (LSD) test procedures were made only when ANOVA tests were considered significant ($P<0.05$). To illustrate the responses of plant functional traits to N addition, Pearson correlation analysis was used to analyze relationships between plant functional traits. The results were considered significant when $P<0.05$. Data analysis and mapping were performed by using software of SPSS19.0 and SigmaPlot12.0, respectively.

Results

Functional Traits Respond to Fencing and Water-Nitrogen Addition

N addition has a significant effect on all the measured functional traits compared to other treatments ($P<0.05$, Table 1). N addition increased plant height, SLA and LNC while decreasing LDMC (Fig. 1). Plant functional traits were significantly different among species ($P<0.01$, Table 1). Plant height was higher in

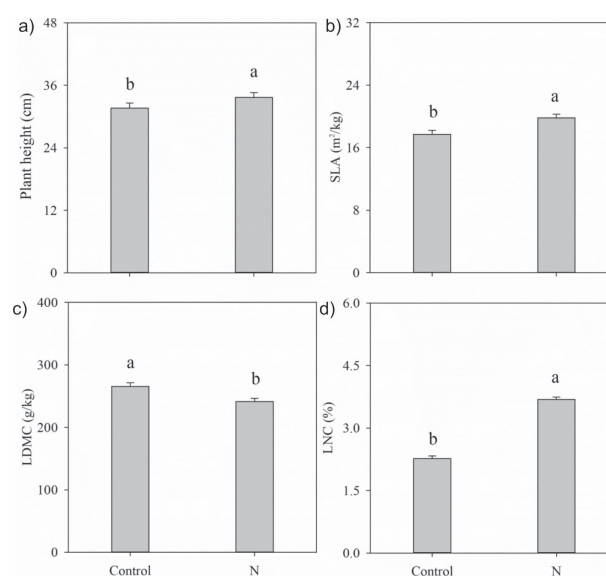


Fig. 1. Effects of N addition on plant functional traits. Trait abbreviations are specific leaf area (SLA), leaf dry matter content (LDMC), and leaf nitrogen content (LNC). Different letters represent significant differences at $P<0.05$.

P. centrasiaticum and *C. acuminatum* than in *C. squarrosa* and *S. collina*. SLA and LDMC were higher in perennials (*P. centrasiaticum* and *C. squarrosa*) than in annuals (*C. acuminatum* and *S. collina*). LNC was higher in *C. acuminatum* than in perennials and *S. collina* (Fig. 2).

There was a significant interaction between fencing and water addition on plant height, SLA and LDMC,

while a significant interactive effect between N addition and water addition on plant height was found ($P < 0.05$, Table 1). Water addition decreased LDMC in grazing treatment, and also decreased plant height in fencing and N addition treatments. Grazing increased LDMC in control (Fig. 2). Additionally, the significant interactive effects of N addition and species on plant height, LDMC and LNC were observed ($P < 0.01$, Table 1).

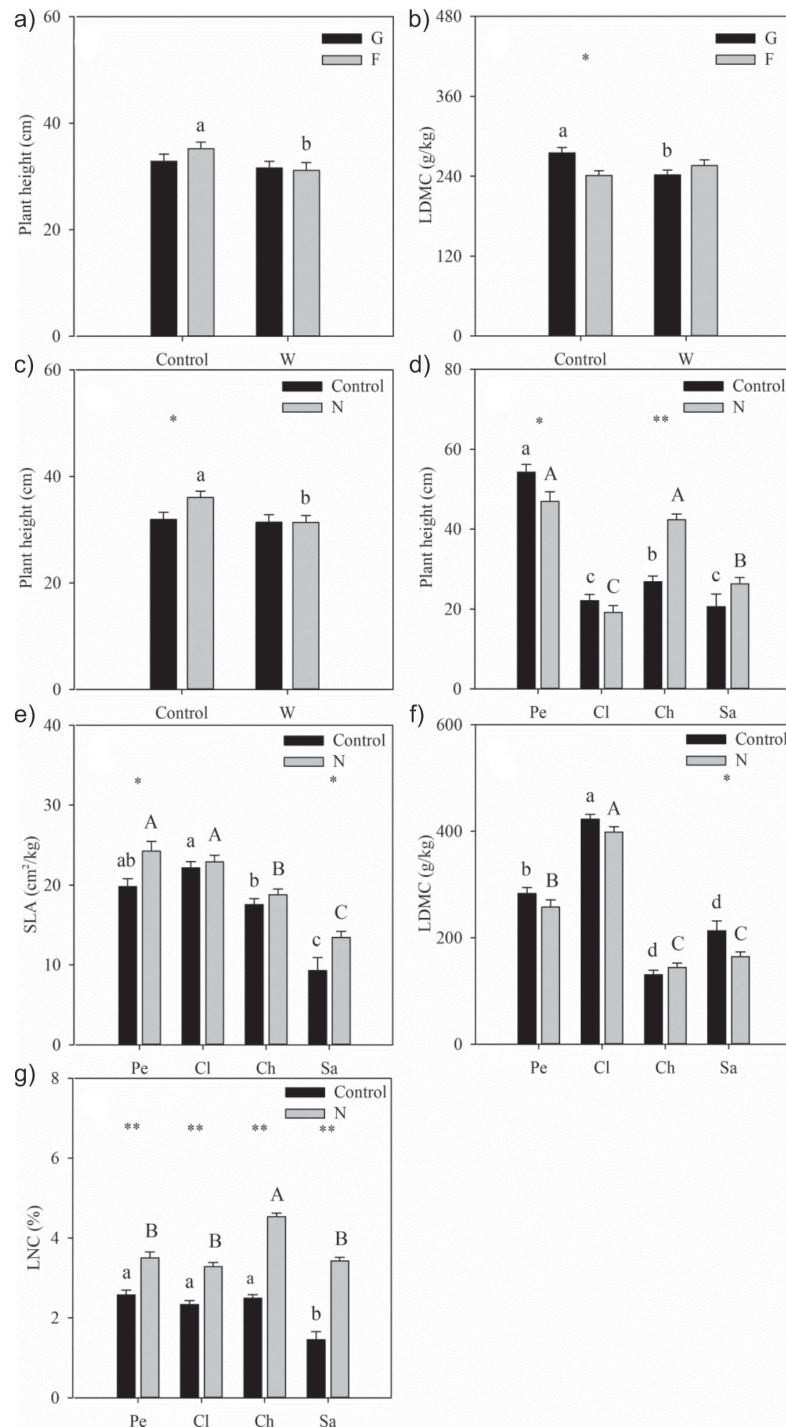


Fig. 2. Effects of fencing \times water, nitrogen \times water and species \times nitrogen on plant functional traits. (a and b, fencing \times water; c and d, nitrogen \times water; e-g, species \times nitrogen.). F, fencing; G, grazing; W, water addition; N, nitrogen addition. Pe, *P. centrasiaticum*; Cl, *C. squarrosa*; Ch, *C. acuminatum*; Sa, *S. collina*. Trait abbreviations are specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC). Different letters represent significant differences at $P < 0.05$. *represents $P < 0.05$, **represents $P < 0.01$.

N addition significantly increased plant height for *C. acuminatum*, while decreasing plant height for *P. centasiaticum* ($P < 0.05$, Fig. 2). N addition increased SLA for perennials and annuals, and SLA of *P. centasiaticum* and *S. collina* was significantly higher in N addition treatment than in control ($P < 0.05$). N addition decreased LDMC for *S. collina*, while it increased LNC for the four dominant species (Fig. 2).

Relationships Between Functional Traits

Correlation analysis showed that plant height was significantly and negatively correlated with LDMC in N addition treatment, while it was positively correlated with LNC in control and N addition treatments, respectively ($P < 0.01$, Fig. 3). SLA was positively associated with LDMC in control and

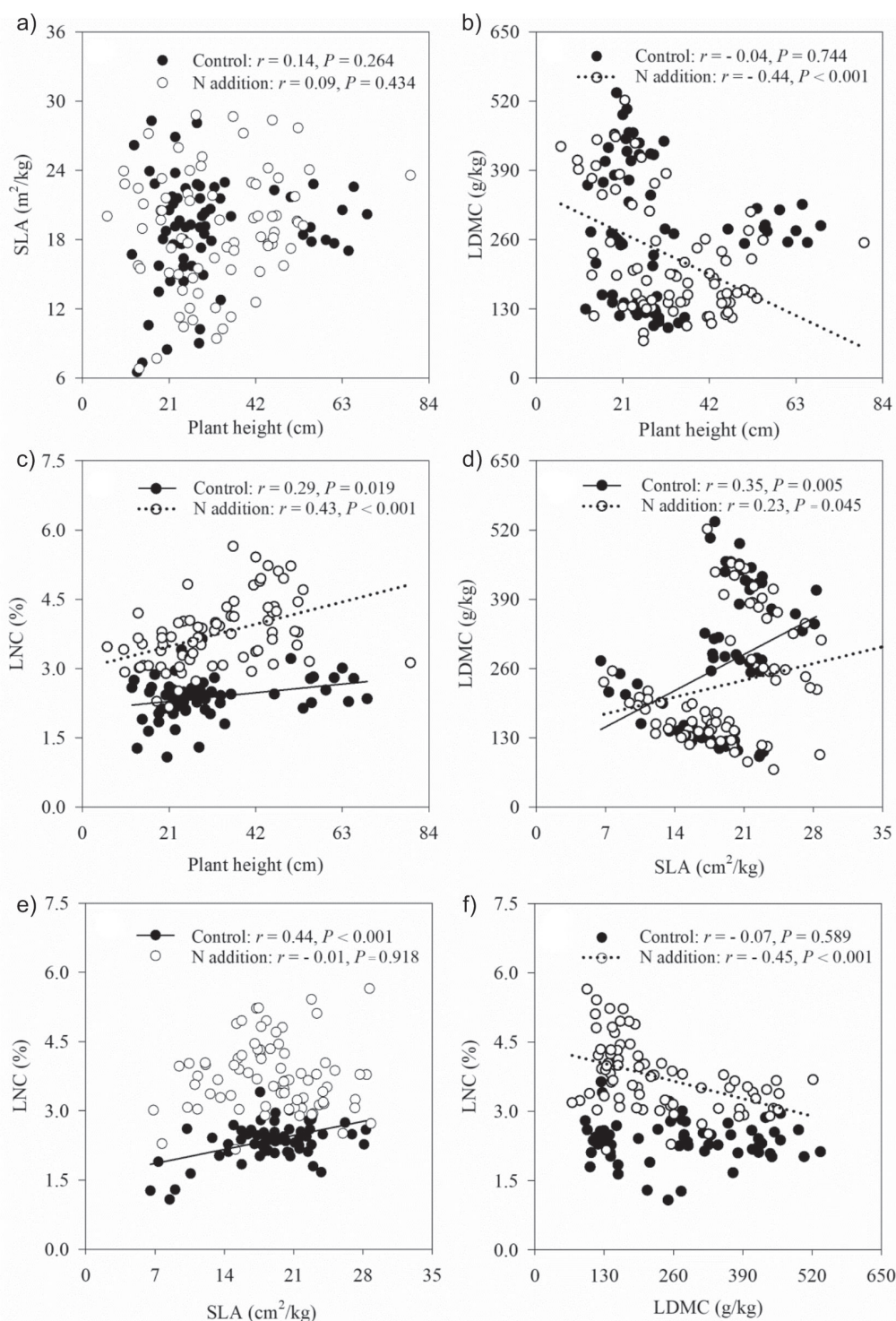


Fig. 3. Correlations between functional traits in control and N addition treatments. Trait abbreviations are specific leaf area (SLA), leaf dry matter content (LDMC), and leaf nitrogen content (LNC).

N addition treatments, respectively ($P < 0.05$), and positively correlated with LNC in control ($P < 0.01$). Negative correlation between LDMC and LNC was found in N addition treatment ($P < 0.01$, Fig. 3).

Discussion

We found that N addition increases plant height, SLA and LNC while reducing LDMC in Horqin sandy grassland. The result confirmed our previous finding that N addition has diverse effects on plant functional traits [31], and also in accordance with previous findings showing that SLA [32] and LNC [10, 31] increased with N supply. Because nitrogen is one of the important resources for plants to grow [33, 34], and N addition can improve soil nitrogen availability which promotes the photosynthesis of plants [10, 35]. Additionally, SLA was positively correlated with plant growth rates and reflected light capture and plant growth [36], and high SLA suggests that plants have a strong ability to maintain nutrition [14, 37]. N supply promotes the photosynthesis of plants and results in high SLA, and accordingly low LDMC was observed. It is an important strategy for plants to adapt to environmental changes, especially N deposition.

SLA and LDMC were higher in perennials (*C. squarrosa* and *P. centrasiatricum*) than in annuals (*C. acuminatum* and *S. collina*) in our study. The higher SLA in perennials than in annuals is related to the differences of leaf area and leaf water content [38]. The result is in agreement with previous studies showed that SLA varied between leaves on the same plant and different species [39, 40]. However, the difference between LDMC is mainly due to the leaf water content of specific species. Because *C. acuminatum* and *S. collina* are annuals with succulent leaves [37], thus low LDMC for these two annuals.

Water addition had an insignificant effect on plant functional traits in our experiment, suggesting that functional traits were not altered by the water supply in the sandy grassland. However, previous findings showed that water addition has an effect on plant functional traits in a typical steppe [10], and plant height was low in dry conditions [41]. It is probably attributed to plants having been acclimated themselves to the local environment in Horqin sandy grassland, further suggesting that water addition is not a proper way to maintain the sustainable development of sandy grassland in Horqin because of the decline of groundwater caused by unreasonable irrigation and climate change in Horqin sandy land [22, 42]. Moreover, water addition reduced plant height in fencing and N addition treatments in our study, indicating that water addition interacted with fencing, and N supply is not favor plant growth in the sandy grassland. One explanation for this result is that water and N addition improved the soil resource availability, which enhanced competition among species in the use of available water and nitrogen. This result further supported the

hypothesis that competition among species would be more profound under high resource conditions compared to less favorable conditions [36].

This study showed that N addition reduced the plant height of perennials (*P. centrasiatricum* and *C. squarrosa*), while increasing the plant height of annuals (*C. acuminatum* and *S. collina*). Likewise, previous studies reported that high and low N addition treatments reduced the plant height of *Mosla cavaleriei* [32], while there was no significant effect from nitrogen addition on *Mosla dianthera* [32] and *Pelargonium sidoides* [43]. The result confirmed the different response of plant functional traits to N addition treatment [31, 32], and indicated that plant functional traits are strongly species-dependent [44]. This result further suggests that N addition favors the growth of annuals over perennials in the sandy grassland. We also found that N addition increased SLA and LNC for the four dominant species, and the result is in agreement with previous findings that N addition increased LNC for *C. acuminatum* in Horqin sandy grassland [45]. This is mainly due to N being the main limiting factor that determines plant growth in Horqin sandy grassland [45], and N addition may lessen N limitation by improving soil nitrogen availability [8]. N addition significantly reduced LDMC for *S. collina* in our experiment. The reason is that *S. collina* is an annual plant with succulent leaves, and N addition increased SLA for *S. collina*, which induced the reduction of LDMC.

Our study found a significant negative association between plant height and LDMC in N addition treatment. Such an association can be attributed to N addition promoting plant growth, and plants invest less dry matter content in the leaves, especially for *S. collina*. SLA was strongly and positively associated with LDMC in control and N addition treatments, indicating that plants exhibited high SLA and high LDMC in Horqin sandy grassland. LDMC reflects the investment of plants in dry matter content in leaves, and plants tend to accumulate more dry matter in poor-nutrition environmental conditions [20, 46]. The proportion of species with succulent leaves may also affect the relationships between SLA and LDMC [11], because these succulent species have low LDMC in control and N addition treatments compared to perennials. Thus, it is possible that the high SLA and high LDMC for plants in the control and nitrogen addition treatments. In addition, different relationships between SLA and LDMC have been found in previous experiments [46, 47], and the heterogeneity between different habitats is an important factor affecting the relationships [48, 49]. Previous studies have reported that SLA was positively related to LNC [20, 38, 50], and the relationships in control treatment confirmed this finding in our study. LDMC negatively associated with LNC in N addition treatment, suggesting that N supply favors the increase of LNC and the improvement of photosynthesis, and plants accumulated low dry matter content in leaves. Therefore, these results indicate that

different species and their functional traits varied in different environmental conditions [46, 51], and plants use a combination of traits to cope with changing environments [52] – especially the alteration of nitrogen availability.

Conclusions

Our results clearly reveal that the fencing, water and nitrogen addition affect the different aspects of functional traits for the dominant species in Horqin sandy grassland. N addition is the main factor in regulating the growth of species in the sandy grassland, as it significantly increased LNC for the four dominant species. Moreover, water addition interacted with fencing and nitrogen addition decreased plant height, indicating that competition among species is more profound under high resource conditions than under unfavorable conditions. Importantly, nitrogen addition contributes to the significant association of LDMC with plant growth and LNC, and plants can alter the key functional traits to cope with N supply. These results further suggest that N deposition favors the growth of dominant species in the sandy grassland under future global change.

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Conflicts of Interest

The authors declare no conflict of interest.

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