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Interactive effects of elevated CO_2 and precipitation change on leaf nitrogen of dominant *Stipa* L. species

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Abstract

Nitrogen (N) serves as an important mineral element affecting plant productivity and nutritional quality. However, few studies have addressed the interactive effects of elevated CO_2 and precipitation change on leaf N of dominant grassland genera such as *Stipa* L. This has restricted our understanding of the responses of grassland to climate change. We simulated the interactive effects of elevated CO_2 concentration and varied precipitation on leaf N concentration (N_{mass}) of four *Stipa* species (*Stipa baicalensis*, *Stipa bungeana*, *Stipa grandis*, and *Stipa breviflora*; the most dominant species in arid and semiarid grassland) using open-top chambers (OTCs). The relationship between the N_{mass} of these four *Stipa* species and precipitation well fits a logarithmic function. The sensitivity of these four species to precipitation change was ranked as follows: *S. bungeana* > *S. breviflora* > *S. baicalensis* > *S. grandis*. The N_{mass} of *S. bungeana* was the most sensitive to precipitation change, while *S. grandis* was the least sensitive among these *Stipa* species. Elevated CO_2 exacerbated the effect of precipitation on N_{mass}. N_{mass} decreased under elevated CO_2 due to growth dilution and a direct negative effect on N assimilation. Elevated CO_2 reduced N_{mass} only in a certain precipitation range for *S. baicalensis* (163–343 mm), *S. bungeana* (164–355 mm), *S. grandis* (148–286 mm), and *S. breviflora* (130–316 mm); severe drought or excessive rainfall would be expected to result in a reduced impact of elevated CO_2. Elevated CO_2 affected the N_{mass} of *S. grandis* only in a narrow precipitation range. The effect of elevated CO_2 reached a maximum when the amount of precipitation was 253, 260, 217, and 222 mm for *S. baicalensis*, *S. bungeana*, *S. grandis*, and *S. breviflora*, respectively. The N_{mass} of *S. grandis* was the least sensitive to elevated CO_2. The N_{mass} of *S. breviflora* was more sensitive to elevated CO_2 under a drought condition compared with the other *Stipa* species.

Introduction

The atmospheric CO_2 concentration has been rising from preindustrial values of approximately 280–390 ppm at present and is expected to reach approximately 450 and 560 ppm under low (RCP2.6) and medium (RCP4.5) scenarios, respectively, in the 21st century (IPCC, 2013). Accompanied with an increase in greenhouse gases, many midlatitude arid and semiarid regions will likely experience less precipitation, and more extreme weather events may arise (IPCC, 2013). Elevated atmospheric CO_2 concentration and simultaneous precipitation change directly or indirectly affect plant physiology and growth (Reich et al. 2001; Xu and Zhou 2006; Sun et al. 2009; Ghannoum et al. 2010; Albert et al. 2011; Tian et al. 2013). Grassland is an important part of the terrestrial ecosystem and plays a significant role in the functioning and structure of the Earth's ecosystems; grasslands are generally thought to be very vulnerable and sensitive to climate change (Weltzin et al. 2003; Ji et al. 2005; Zhang et al. 2007). Leaf nitrogen (N) is closely related to photosynthesis, and leaf N concentration is also one of the key traits of the economic spectrum of leaves (Wright et al. 2004; Feng et al. 2009). The leaf N concentration (N_{mass}) of a plant is determined by both genetic characteristics and environmental factors (precipitation, temperature, CO_2 and O_3), and reflects the ability of a plant to adapt to the environment. Many studies have addressed the effects of elevated atmospheric CO_2 concentrations or precipitation change on plant N_{mass}. These studies have shown that elevated atmospheric CO_2 concentrations can result in a decrease in N_{mass}, while drought stress can increase N_{mass} (Ainsworth and Long 2005; Teng et al. 2006; Bloom et al. 2010; Lee et al. 2011; Zhou et al. 2011; Housman et al. 2012). However, some researchers found that elevated CO_2 did not affect N_{mass} (Watling et al. 2000; Novriyanti et al. 2012; Li et al. 2013), and others indicated that N_{mass} decreased with decreasing rainfall (Xu and Zhou 2006; Galmés et al. 2007), possibly depending on plant species.

The responses of plant growth and physiology to climatic change, in a multifactor context, may not be predictable from a single factor experiment. However, most experiments have focused on the effects of an individual factor; therefore, multifactorial experiments are urgently needed to reveal the integrated responses of plants to environmental changes (Albert et al. 2011; Vile et al. 2012; Hou et al. 2013; Xu et al. 2014). Grassland dominated by *Stipa*, a group of species with good palatability and high forage value, is widespread in North China as the part of the Euro-Asia steppe, an ecosystem that has experienced severe degradation during recent decades (Bai et al. 2004; Zhang et al. 2007; Xu et al. 2014). Previous studies were mainly concerned about the effect of precipitation change; the interaction with CO_2 concentration was unclear. The increase in the CO_2 concentration and changes in precipitation will occur simultaneously in the future (IPCC, 2013), and the responses of *Stipa* to changing precipitation may vary in an environment with a higher CO_2 concentration. Leaf N_{mass} affects the decomposition rate of plant litter and is closely related to forage quality (Gorissen and Cotrufo 2000; Vitousek et al. 2002; Pleijel and Uddling 2012). In this study, open-top chambers (OTCs) were used to (1) investigate the interactive effects of elevated CO_2 and precipitation change on N_{mass}; (2) quantify the relationship between N_{mass} and precipitation; and (3) elucidate the mechanisms involved the N_{mass} response to elevated CO_2 and precipitation change.

Materials and Methods

Plant materials and experimental design

Four *Stipa* species (*Stipa baicalensis*, *Stipa bungeana*, *Stipa grandis*, and *Stipa breviflora*), which are the most typical species in the arid and semiarid grassland of China, were chosen for this experiment. The experiment was conducted at the Institute of Botany, Chinese Academy of Sciences, in 2011, using OTCs. *S. baicalensis*, *S. bungeana*, *S. grandis,* and *S. breviflora* seeds were collected from natural grasslands in Hulunber (49°190′N, 119°55′E), Ordos (39°29′N, 110°11′E), Xilinhot (44°08″N, 117°05″E), and Ulanqab (41˚43’’N, 111˚52′E) in the autumn of 2010. The seeds were sterilized in a 0.5% potassium permanganate solution for 8 min before sowing. The soil (N_{mass}: 1.45 g·kg^{-1}) had been collected from the original grassland in Xilinhot, Inner Mongolia, and was placed into plastic pots (0.56 L).

Three CO_2 concentration treatments (ambient, 450 and 550 ppm) with three replications were tested in a total of nine OTCs. The hexagonal structure of the OTCs, which were fabricated using an aluminum frame lined with colorless transparent glass, had a length and height of 0.85 and 1.8 m, respectively. Pure CO_2 gas was released through a PVC tube connected to an air-exhaust blower mounted at the base of the OTCs. The input of CO_2 gas was automatically controlled, and an air sample from the middle of the chamber was drawn into a CO_2 sensor (eSENSE-D, SenseAir, Delsbo, Sweden) to monitor the concentration change every minute. The natural precipitation of the seed provenances was similar for pairs of species, that is, (1) *S. baicalensis* and *S. bungeana* and (2) *S. grandis* and *S. breviflora*. To facilitate a comparison of the species pairs, the baseline precipitation (June, July, and August) data from Hulunber (240 mm) and Xilinhot (217 mm) were used for calculating the experimental precipitation rates. That is, two sets of five precipitation levels (−30%, −15%, control, +15%, and +30%) were used. These were based on the average monthly precipitation (June, July, and August) in different regions of the two pairs of species from 1978 to 2007. Every precipitation level had two replicates in each OTC. The monthly precipitation (mm) of each level (Table 1) was converted into an irrigation amount (ml), and this was supplied every 3 days.

Table 1. Average monthly precipitation from 1978 to 2007 in the provenances of the four species.

Species	Month	Precipitation (mm) −30%	−15%	Control	+15%	+30%
S. baicalensis	June	36	44	51	59	67
S. bungeana	July	62	75	88	101	114
	August	70	85	100	115	130
	Total	168	204	240	275	311
S. grandis	June	39	47	56	64	72
S. breviflora	July	65	79	93	107	121
	August	47	57	68	78	88
	Total	151	183	217	249	281

After sowing on 18 April 2011, the seedlings were first cultured in a greenhouse (day/night temperature 26–28°C/18–20°C, maximum photosynthetic photon flux density of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Four healthy seedlings with a uniform growth pattern were retained in each pot when the fourth leaf appeared. A total of 360 pots (90 pots for each species) were randomly selected and moved into the OTCs (10 pots for each species in each chamber) on 23 May. Thus, there were six replicates (six pots, each with four plants) per treatment for each species. Before CO_2 enrichment and irrigation started on 31 May, we weighed every pot with soil and plants to ensure that initial soil moisture was consistent. During the experiment, we monitored the CO_2 supply system every day, watered at 16:00 every 3 days, and kept the glass walls clean.

Sampling and analysis

After harvesting on 2 September 2011, the leaves were dried at 65°C to a constant weight and leaf biomass was measured using an electronic balance. The leaf N concentration (N_{mass}) was determined using a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Total leaf N (N_{total}) = leaf biomass × N_{mass}. The relative effects of N_{mass} (α_{Nmass}), leaf biomass ($\alpha_{biomass}$), and N_{total} (α_{Ntotal}) can be expressed using the following equation:

$$\alpha_{i,j} = A_{i,j}/A_{\mathrm{ref},j} - 1,$$

where $\alpha_{i,j}$ is the relative effect on variable j of treatment i in relation to the control, $A_{i,j}$ is the value of variable j of treatment i, and $A_{\mathrm{ref},j}$ is the value of variable j of the control. Controls were only used to calculate experimental effects; by definition, α is zero for all variables in the control (Pleijel and Uddling 2012).

Statistical tests

All statistical analyses on the N_{mass} and N_{total} values were performed using SPSS 16.0 (SPSS Institute Incorporated, Chicago, IL, USA). The effects of elevated CO_2 and precipitation change were analyzed using ANOVA ($P = 0.05$). Differences between the means of the elevated

Table 2. Relationship between N_{mass} and precipitation under different CO_2 concentrations.

Species	CO_2 concentration	Equation	R^2	P
S. baicalensis	Ambient	$y = -1.526\ln(x) + 11.677$	0.6749	<0.01
	450 ppm	$y = -1.963\ln(x) + 13.954$	0.7634	<0.01
	550 ppm	$y = -1.853\ln(x) + 13.144$	0.6778	<0.01
S. bungeana	Ambient	$y = -2.06\ln(x) + 14.52$	0.8892	<0.01
	450 ppm	$y = -2.262\ln(x) + 15.482$	0.6861	<0.01
	550 ppm	$y = -2.531\ln(x) + 16.73$	0.7836	<0.01
S. grandis	Ambient	$y = -0.765\ln(x) + 7.5102$	0.5719	<0.01
	450 ppm	$y = -0.877\ln(x) + 7.9664$	0.5036	<0.01
	550 ppm	$y = -0.869\ln(x) + 7.7904$	0.4344	<0.01
S. breviflora	Ambient	$y = -1.816\ln(x) + 13.078$	0.7649	<0.01
	450 ppm	$y = -2.214\ln(x) + 14.991$	0.6602	<0.01
	550 ppm	$y = -1.906\ln(x) + 12.978$	0.6439	<0.01

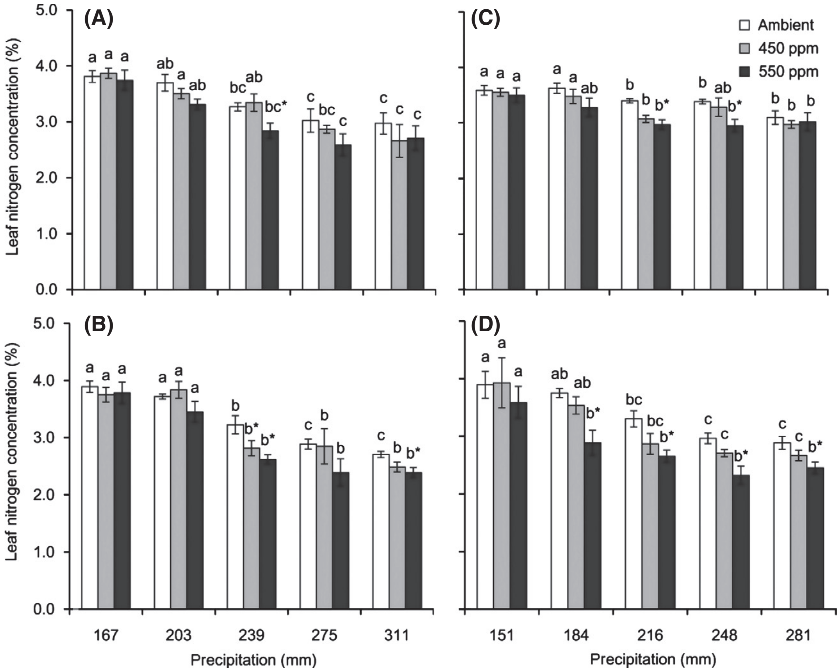

Figure 1. Interactive effects of changing precipitation and CO_2 on the N_{mass} of four *Stipa* species: *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D). Different lower case letters indicate significant differences among precipitation treatments for the same CO_2 concentration ($P < 0.05$); * indicates significant differences between CO_2 concentrations for the same level of precipitation ($P < 0.05$).

CO_2 or precipitation changes were compared using Duncan's multiple range test at a 0.05 probability level.

Results and Analysis

Responses of N_{mass} to elevated CO_2 and precipitation changes

The relationship between N_{mass} and precipitation for the four *Stipa* species was better observed using a logarithmic function (Fig. 1, Table 2). The equations in Table 2 showed a better linear relationship between y and $\ln x$ (y: N_{mass}, x: precipitation). The slope (a) reflected the degree of influence of the precipitation change on N_{mass}. A larger $|a|$ indicated a greater effect of precipitation change on N_{mass}. Under the same CO_2 concentration conditions, the sensitivities of the N_{mass} of the four species to precipitation change were ranked as: *S. bungeana* > *S. breviflora* > *S. baicalensis* > *S. grandis*. The N_{mass} of *S. bungeana* was the most sensitive to precipitation change, while *S. grandis* was the least sensitive among these *Stipa* species. Compared with the ambient level, high CO_2 concentration intensified the effect of precipitation change on N_{mass}.

An elevated CO_2 concentration led to a lower N_{mass} in the four *Stipa* species. However, the effect of elevated CO_2 was closely related to the precipitation rate (Fig. 1). The relative effect of elevated CO_2 (550 ppm) on N_{mass} (α_{Nmass}) showed a quadratic relationship with the precipitation level (Fig. 2, Table 3). This meant that the effect of elevated CO_2 would be obvious within a particular precipitation range, but would disappear outside of this range. The effective precipitation ranges in which the N_{mass} of the four *Stipa* species responded to elevated CO_2 (550 ppm) were calculated from the equations in Table 3: *S. baicalensis* (163–343 mm), *S. bungeana* (164–355 mm), *S. grandis* (148–286 mm), and *S. breviflora* (130–316 mm). When the precipitation amount was 253, 260, 217, and 222 mm for *S. baicalensis*, *S. bungeana*, *S. grandis*, and *S. breviflora*, respectively, the effect of elevated CO_2 (550 ppm) reached the maximum (Table 3).

Table 3. Relationship between the effect of 550 ppm CO_2 on α_{Nmass} and precipitation.

Species	Equation	R^2	P	OP (mm)	ERP (mm)
S. baicalensis	$y = 1.62E\text{-}05x^2 - 0.0082x + 0.9071$	0.4314	0.0338	253	163–343
S. bungeana	$y = 1.83E\text{-}05x^2 - 0.0095x + 1.0658$	0.4130	0.0409	260	164–355
S. grandis	$y = 2.58E\text{-}05x^2 - 0.0112x + 1.0935$	0.4093	0.0425	217	148–286
S. breviflora	$y = 2.58E\text{-}05x^2 - 0.0115x + 1.0574$	0.4066	0.0437	222	130–316

OP, optimum precipitation represents the amount of precipitation when elevated CO_2 had a maximal effect on N_{mass}; ERP, effective range of precipitation shows the range of precipitation in which elevated CO_2 affected N_{mass}.

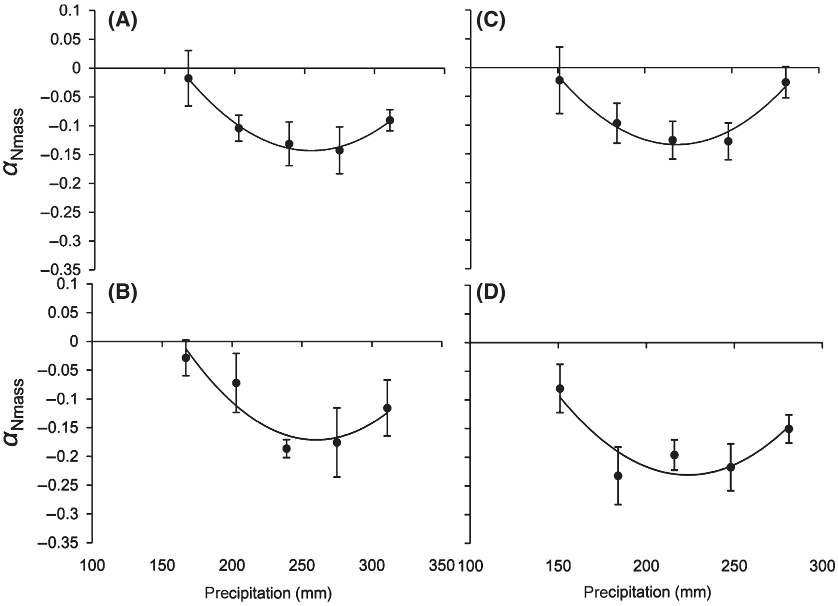

Figure 2. Relationship between α_{Nmass} under elevated CO_2 (550 ppm) and precipitation. *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D).

Responses of leaf biomass and N_{total} to elevated CO_2 and precipitation changes

Changes in precipitation significantly affected leaf biomass (Fig. 3). Compared with the control, the leaf biomass of *S. baicalensis*, *S. bungeana*, *S. grandis*, and *S. breviflora* decreased 30.4%, 44.4%, 35.5%, and 49.8% (precipitation −30%) and increased 52.2%, 65.1%, 79.0%, and 19.8% (precipitation +30%), respectively, under ambient CO_2 conditions. When the CO_2 concentration elevated from ambient to 550 ppm, leaf biomass significantly increased. However, the effect of elevated CO_2 on leaf biomass was also closely related to the precipitation rate, similar to N_{mass}. Severe drought (precipitation −30%) restricted the effect of elevated CO_2 concentration on leaf biomass (Fig. 3).

Compared with the control, reduced precipitation increased the N_{mass} (Fig. 1) but decreased the N_{total} of the four *Stipa* species (Fig. 4). The N_{total} of *S. baicalensis*, *S. bungeana, S. grandis,* and *S. breviflora* decreased 19.3%,

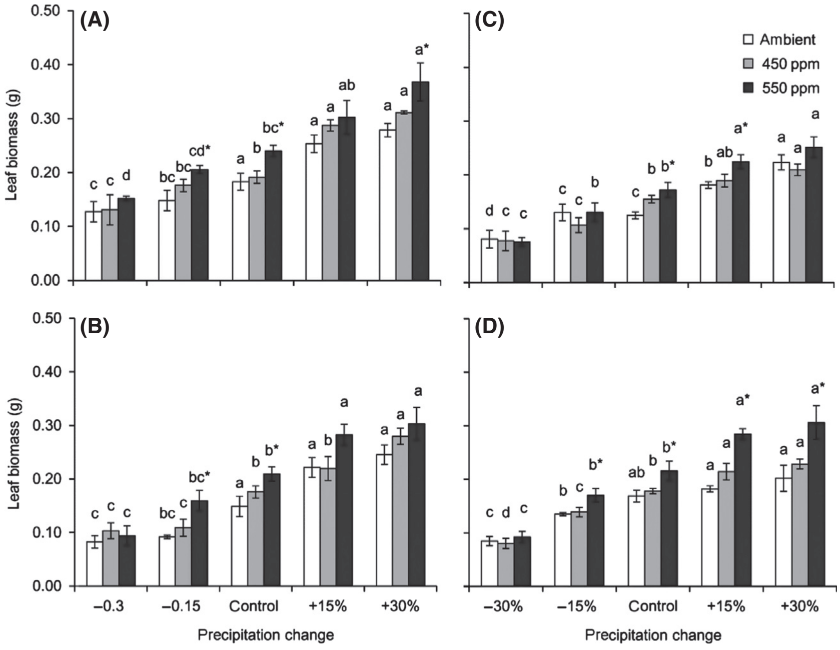

Figure 3. Interactive effects of changing precipitation and CO_2 on leaf biomass of the four *Stipa* species: *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D). See Fig. 1 for notes.

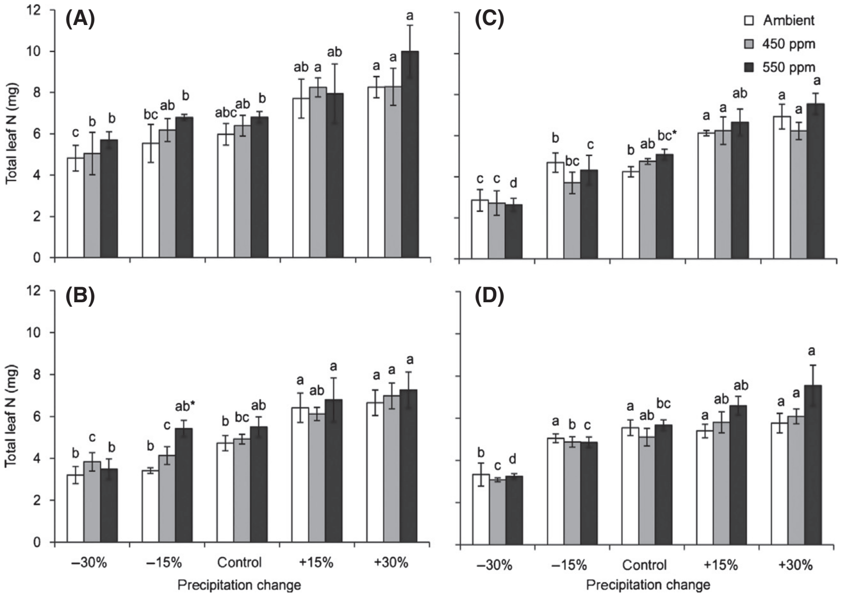

Figure 4. Interactive effects of changing precipitation and CO_2 on the N_{total} of the four *Stipa* species: *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D). See Fig. 1 for notes.

32.3%, 32.6%, and 40.0% (precipitation −30%), respectively, under ambient CO_2 conditions compared with the control. Although elevated CO_2 increased the N_{total} of the four *Stipa* species, the effect was not significant expect under the −15% (*S. bungeana*) and control (*S. grandis*) precipitation conditions.

Impacts of elevated CO_2, precipitation changes and their interactions on N_{mass}, leaf biomass and N_{total}

Precipitation changes generally resulted in significant effects on the N_{mass}, leaf biomass, and N_{total} of the four *Stipa* species ($P < 0.001$). The N_{mass} and leaf biomass changed with elevated CO_2 concentration, but N_{total} was not significant. The interaction between elevated CO_2 and precipitation changes had no significant effect on the N_{mass}, leaf biomass, and N_{total} of the four *Stipa* species except for the leaf biomass of *S. breviflora* (Table 4).

Discussion

Impacts of elevated CO_2 and precipitation changes and their interactions on N_{mass}

Nitrogen serves as one of the major mineral elements affecting plant growth, and leaves are the largest N sinks in plants. Leaf N_{mass} is closely related not only to the photosynthetic capacity of grass species (Sicher and Bunce 1997; Gerdol et al. 2000; Long et al. 2006; Duval et al. 2012) but also to the forage quality (Vitousek et al. 2002; Pleijel and Uddling 2012). This study confirmed earlier results that showed elevated CO_2 concentrations decreased the N_{mass} of *Stipa* plants compared with those growing under ambient CO_2 conditions (Ellsworth et al. 2004; Ainsworth and Long 2005; Crous et al. 2010; Lee et al. 2011). A reduction in N_{mass} is unfavorable for photosynthesis because it leads to a photosynthetic adaption phenomenon (Taub and Wang 2008; Lei et al. 2011) and is unfavorable for forage quality, which would cause a problem in the nutrition of animals.

Table 4. F-values and significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) from two-way ANOVAs for the main effects of CO_2, precipitation and their interactions on N_{mass}, leaf biomass and N_{total}

	CO_2		Precipitation		CO_2 × Precipitation	
	F-values	df	*F*-values	df	*F*-values	df
S. baicalensis						
N_{mass}	4.877***	2	21.98***	4	0.584	8
Biomass	11.80***	2	50.91***	4	0.534	8
N_{total}	2.001	2	10.47***	4	0.240	8
S. bungeana						
N_{mass}	7.077**	2	43.66***	4	0.847	8
Biomass	10.70***	2	57.15***	4	0.775	8
N_{total}	2.696	2	20.39***	4	0.551	8
S. grandis						
N_{mass}	7.648**	2	11.42***	4	0.877	8
Biomass	4.810*	2	58.73***	4	1.190	8
N_{total}	1.376	2	34.83***	4	0.635	8
S. breviflora						
N_{mass}	12.10***	2	20.70***	4	0.495	8
Biomass	23.58***	2	60.24***	4	2.295*	8
N_{total}	3.056	2	25.90***	4	1.147	8

Compared with the control precipitation rate used in this study, drought increased the N_{mass} of *Stipa*, which is consistent with previous reports (Luo et al. 2005; Huang et al. 2009). However, increased precipitation had no notable effect on N_{mass}. The relationship between the N_{mass} of the four *Stipa* species and precipitation well fit a logarithmic function. N_{mass} gradually decreased with an increase in precipitation and was close to a constant value. A possible explanation for this phenomenon is that an element such as N must reach a certain concentration to allow plants to maintain their normal physiological activities. The increase in N_{mass} might enhance the number and activity of photosynthetic enzymes and improve the photosynthetic rate when plants are grown under drought conditions (Wright et al. 2001; Knight and Ackerly 2003; Huang et al. 2009). In addition, a higher N_{mass} level could increase intracellular osmotic pressure, which would strengthen the ability of plants to survive during drought, improve their water use efficiency and alleviate water-related stress (Wright et al. 2001; Huang et al. 2009; Novriyanti et al. 2012).

To date, there are limited reports on the interactive effect of changing precipitation and elevated CO_2 on the N_{mass} of *Stipa*. This experiment showed that the elevated CO_2 effect on leaf N_{mass} depended on the precipitation pattern. The changes in precipitation rates changed the sensitivity of N_{mass} to elevated CO_2 concentrations. Precipitation is the most important factor in arid and semiarid ecosystems and plays a critical role in plant growth and physiological processes (Noy-Meir 1973; Morgan et al. 2004; Heisler-White et al. 2009). Precipitation limits the effect of elevated CO_2 concentrations.

In this study, the patterns of leaf N_{mass} of the four *Stipa* species (*S. baicalensis*, *S. bungeana*, *S. grandis* and *S. breviflora*) in response to elevated CO_2 and precipitation change were similar. However, elevated CO_2 reduced N_{mass} in different precipitation ranges for the four *Stipa* species. The sensitivities of leaf N_{mass} of these four species to precipitation change were also different. The differential performance of the four *Stipa* species indicated that there may be species-specific leaf N_{mass} responses to precipitation change. This phenomenon might be related to the different biogeographic environments where the four *Stipa* species are distributed in nature. The leaf N_{mass} of *S. grandis* was the least sensitive to elevated CO_2 and precipitation change among the four species. *S. grandis* is a principal species in typical steppe ecosystems (Zhang et al. 2007); it is more widely distributed than the other three species in the North China grassland in which *S. grandis* is better able to adapt to environmental change. Thus, *S. grandis* showed insensitivity to elevated CO_2 and precipitation change in this experiment. *S. breviflora* thrives as a dominant species in desert steppe ecosystems (Zhang et al. 2007). This study showed that *S. breviflora* exposed to elevated CO_2 was more sensitive than the other three species under drought conditions. *S. baicalensis* is as an important species in meadow steppe ecosystems in eastern Inner Mongolia (Zhang et al. 2007), which may explain why it was readily influenced by elevated CO_2 under higher precipitation.

Mechanisms of N_{mass} response to elevated CO_2 and precipitation changes

Three hypotheses have been proposed in relation to the mechanisms by which N_{mass} responds to elevated CO_2. (1) The growth dilution hypothesis: If the increase in the accumulation of leaf biomass is more than the increase in N acquisition under high CO_2 concentration, N_{mass} will decrease (Yamakawa et al. 2004; Johnson 2006; Taub and Wang 2008; Duval et al. 2012). (2) The inhibition of N absorption and transport capacity hypothesis. Initially, elevated CO_2 results in lower transpiration rates and increased water use efficiency; secondly, elevated CO_2 affects the exudates of roots and changes soil pH, thus influencing N assimilation. Additionally, decreased N assimilation has also been explained as a result of an increase in N use efficiency and a decrease in N demand under elevated CO_2 (Zerihun et al. 2000; Teng et al. 2006; Taub and Wang 2008; Bloom et al. 2010; Duval et al. 2012). (3) Both (1) and (2) coexist (Pleijel and Uddling 2012). Our results showed that although N_{mass} decreased, total leaf N (N_{total}) increased under high CO_2 concentration (Fig. 3), which was consistent with previous results (Yin et al. 2011). We can test the mechanisms using the data of the relative effects of leaf biomass ($\alpha_{biomass}$) and total leaf N (α_{Ntotal}). If the α_{Ntotal} data are plotted on the *y*-axis and the $\alpha_{biomass}$ data are plotted on the *x*-axis and the result is a linear regression with a slope between 0 and 1, this can be interpreted as a significant growth dilution effect. If a direct negative effect on N uptake exists that is unrelated to the effect on leaf biomass, in addition to the growth dilution effect, there will be a significant intercept on the *x*- and *y*-axes (Taub and Wang 2008; Pleijel and Uddling 2012). The relationship between α_{Ntotal} and $\alpha_{biomass}$ for *Stipa* under elevated CO_2 showed that N_{mass} decreased because of the combined effect of growth dilution (the slope was between 0 and 1) and assimilation inhibition (the intercept on the *y*-axis was smaller than 0) (Fig. 5), which is the same as the results of previous studies (Teng et al. 2006; Taub and Wang 2008; Pleijel and Uddling 2012). The slope (k) and *y*-axis intercept ($|b|$) reflect the respective degree to which the growth dilution and assimilation capacity affect N_{mass}. The sensitivity of the four *Stipa*

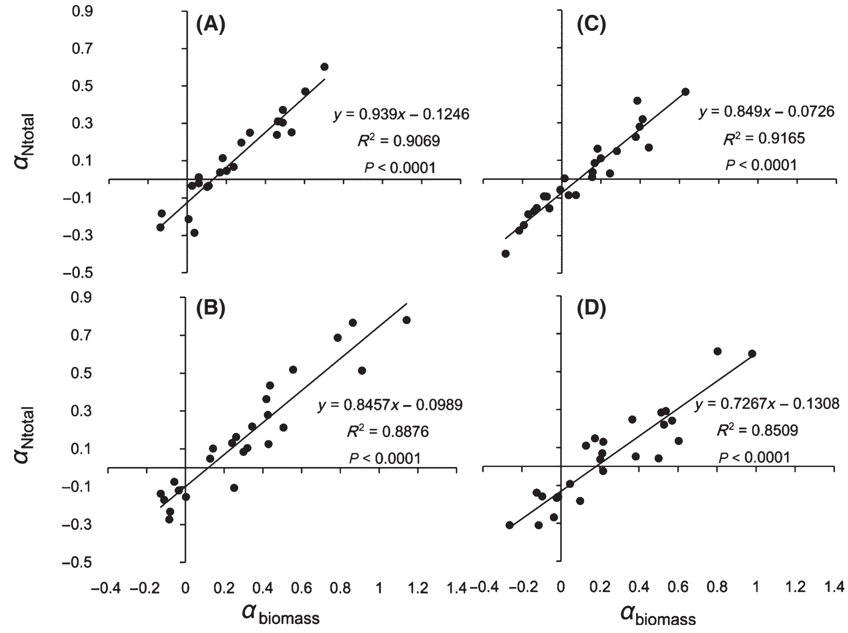

Figure 5. Relationship between α_{Ntotal} and $\alpha_{biomass}$ for *Stipa* under elevated CO_2. *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D).

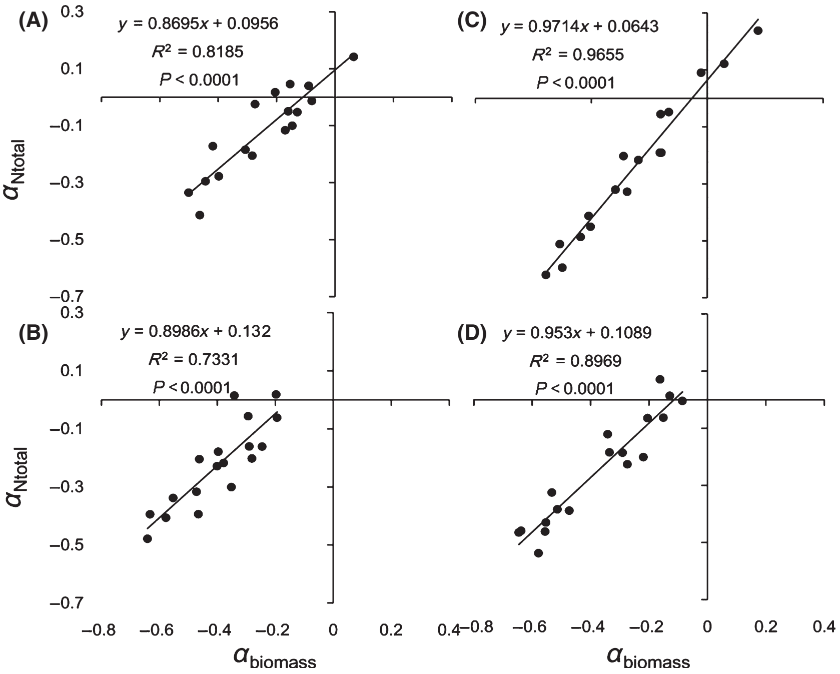

Figure 6. Relationship between α_{Ntotal} and $\alpha_{\mathrm{biomass}}$ for *Stipa* under reduced precipitation. *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D).

species can be listed as: *S. breviflora* > *S. bungeana* > *S. grandis* > *S. baicalensis* for growth dilution, and *S. breviflora* > *S. baicalensis* > *S. bungeana* > *S. grandis* for decreased N assimilation capacity.

Compared with the control, reduced precipitation increased the N_{mass} but decreased the N_{total} of *Stipa* L. (Fig. 3). Based on the relationship between α_{Ntotal} (as *y*-axis) and $\alpha_{\mathrm{biomass}}$ (as *x*-axis) of *Stipa* (Fig. 6), the increase in N_{mass} under drought can be explained in two ways. First, the decrease in leaf biomass accumulation was larger than the decrease in N_{total} accumulation. Second, drought strengthened N uptake and transport (the intercept on the *y*-axis was >0). It is possible that N_{mass} increased because more N was needed to maintain a high osmotic pressure or because drought increased the root–shoot ratio and more roots transported N to the same volume of leaves (Jiang et al. 2004; Pan et al. 2008; Duval et al. 2012).

Conclusions

In this experiment, we studied the interactive effects of CO_2 concentration (ambient, 450 and 550 ppm) and precipitation (−30%, −15%, control, +15%, and +30% based on average monthly precipitation from 1978 to 2007 in the provinces that support the populations of the four species) on leaf N of four species: *S. baicalensis S. bungeana*, *S. grandis,* and *S. breviflora*. The results suggested the following: (1) Elevated CO_2 decreased the N_{mass} but increased the N_{total} of *Stipa* L. The decrease in N_{mass} was caused by the combination of growth dilution and assimilation inhibition. The effect of elevated CO_2 was influenced by precipitation: Within a precipitation range, the effect was obvious; however, the effect disappeared outside of that range. (2) Compared with the control precipitation, reduced precipitation increased the N_{mass} of the four *Stipa* species, but increased precipitation had no significant effect on N_{mass}. The increase in N_{mass} under drought conditions might have resulted from two causes: The decrease in leaf biomass accumulation was greater than the decrease in N_{total} accumulation, and drought strengthens N uptake and transport. The relationship between the N_{mass} of the four *Stipa* species and precipitation was described using a logarithmic function. Elevated CO_2 exacerbated the effect of precipitation on N_{mass}. (3) The sensitivity of the N_{mass} of the four species to precipitation was ranked as: *S. bungeana* > *S. breviflora* > *S. baicalensis* > *S. grandis*. The N_{mass} of *S. grandis* was the least sensitive among these four species. Under drought conditions, the effects of elevated CO_2 on *S. breviflora* were the most obvious among the four species.

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Conflict of Interest

None declared.

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