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 baikalensis, Stipa bungeana, Stipa grandis, and Stipa brevilflora; 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 each of these species to precipitation change was ranked as follows: S. bungeana > S. brevilflora > S. baikalensis > 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. baikalensis (163–343 mm), S. bungeana (164–355 mm), S. grandis (148–286 mm), and S. brevilflora (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. baikalensis, S. bungeana, S. grandis, and S. brevilflora, respectively. The N$_{mass}$ of S. grandis was the least sensitive to elevated CO$_2$. The N$_{mass}$ of S. brevilflora 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

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traits of the economic spectrum of leaves (Wright et al. 2004; Feng et al. 2009). The leaf N concentration (N\textsubscript{mass}) of a plant is determined by both genetic characteristics and environmental factors (precipitation, temperature, CO\textsubscript{2} and O\textsubscript{3}), and reflects the ability of a plant to adapt to the environment. Many studies have addressed the effects of elevated atmospheric CO\textsubscript{2} concentrations or precipitation change on plant N\textsubscript{mass}. These studies have shown that elevated atmospheric CO\textsubscript{2} concentrations can result in a decrease in N\textsubscript{mass} while drought stress can increase N\textsubscript{mass} (Ainsworth and Long 2005; Teng et al. 2006; Bloom et al. 2010; Lee et al. 2011; Zhou et al. 2011; Hou et al. 2013; Xu et al. 2014). However, some researchers found that elevated CO\textsubscript{2} did not affect N\textsubscript{mass} (Watling et al. 2000; Novriyanti et al. 2012; Li et al. 2013), and others indicated that N\textsubscript{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\textsubscript{2} concentration was unclear. The increase in the CO\textsubscript{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\textsubscript{2} concentration. Leaf N\textsubscript{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\textsubscript{2} and precipitation change on N\textsubscript{mass}; (2) quantify the relationship between N\textsubscript{mass} and precipitation; and (3) elucidate the mechanisms involved the N\textsubscript{mass} response to elevated CO\textsubscript{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°19′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\textsubscript{mass}: 1.45 g kg\textsuperscript{-1}) had been collected from the original grassland in Xilinhot, Inner Mongolia, and was placed into plastic pots (0.56 L).

Three CO\textsubscript{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\textsubscript{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\textsubscript{2} gas was automatically controlled, and an air sample from the middle of the chamber was drawn into a CO\textsubscript{2} sensor (eSENSE-D, Sense-Air, 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       | −30% | −15% | Control | +15% | +30% |
|------------------|-------------|------|------|---------|------|------|
| S. baicalensis   | June        | 36   | 44   | 51      | 59   | 67   |
|                  | July        | 62   | 75   | 88      | 101  | 114  |
|                  | August      | 70   | 85   | 100     | 115  | 130  |
|                  | Total       | 168  | 204  | 240     | 275  | 311  |
| S. bungeana      | June        | 39   | 47   | 56      | 64   | 72   |
|                  | 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 μmol·m⁻²·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 CO₂ 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 CO₂ 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 (N⁰mass) was determined using a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Total leaf N (N⁰total) = leaf biomass × N°mass. The relative effects of N°mass (x°Nmass), leaf biomass (x°biomass), and N°total (x°Ntotal) can be expressed using the following equation:

\[
\epsilon_{ij} = A_{ij}/A_{ref,j} - 1,
\]

where \( \epsilon_{ij} \) is the relative effect on variable \( j \) of treatment \( i \) in relation to the control, \( A_{ij} \) is the value of variable \( j \) of treatment \( i \), and \( A_{ref,j} \) is the value of variable \( j \) of the control. Controls were only used to calculate experimental effects; by definition, \( \epsilon \) 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 CO₂ and precipitation change were analyzed using ANOVA (\( P = 0.05 \)). Differences between the means of the elevated

| Species       | CO₂ 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**      | \( y = -2.061\ln(x) + 14.52 \) | 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 |
CO₂ or precipitation changes were compared using Duncan’s multiple range test at a 0.05 probability level.

**Results and Analysis**

**Responses of N\textsubscript{mass} to elevated CO₂ and precipitation changes**

The relationship between N\textsubscript{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\textsubscript{mass}, \( x: \) precipitation). The slope \( (a) \) reflected the degree of influence of the precipitation change on N\textsubscript{mass}. A larger \( |a| \) indicated a greater effect of precipitation change on N\textsubscript{mass}. Under the same CO₂ concentration conditions, the sensitivities of the N\textsubscript{mass} of the four species to precipitation change were ranked as: *S. bungeana* > *S. breviflora* > *S. baicalensis* > *S. grandis*. The N\textsubscript{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 CO₂ concentration intensified the effect of precipitation change on N\textsubscript{mass}.

An elevated CO₂ concentration led to a lower N\textsubscript{mass} in the four *Stipa* species. However, the effect of elevated CO₂ was closely related to the precipitation rate (Fig. 1). The relative effect of elevated CO₂ (550 ppm) on N\textsubscript{mass} (\( \Delta N\textsubscript{mass} \)) showed a quadratic relationship with the precipitation level (Fig. 2, Table 3). This meant that the effect of elevated CO₂ would be obvious within a particular precipitation range, but would disappear outside of this range. The effective precipitation ranges in which the N\textsubscript{mass} of the four *Stipa* species responded to elevated CO₂ (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 CO₂ (550 ppm) reached the maximum (Table 3).

**Table 3.** Relationship between the effect of 550 ppm CO₂ on \( \Delta N\textsubscript{mass} \) and precipitation.

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

OP, optimum precipitation represents the amount of precipitation when elevated CO₂ had a maximal effect on N\textsubscript{mass}; ERP, effective range of precipitation shows the range of precipitation in which elevated CO₂ affected N\textsubscript{mass}.

**Figure 2.** Relationship between \( \Delta N\textsubscript{mass} \) under elevated CO₂ (550 ppm) and precipitation. *S. baicalensis* (A), *S. bungeana* (B), *S. grandis* (C), and *S. breviflora* (D).
Responses of leaf biomass and N\textsubscript{total} to elevated CO\textsubscript{2} and precipitation changes

Changes in precipitation significantly affected leaf biomass (Fig. 3). Compared with the control, the leaf biomass of \textit{S. baicalensis}, \textit{S. bungeana}, \textit{S. grandis}, and \textit{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\textsubscript{2} conditions. When the CO\textsubscript{2} concentration elevated from ambient to 550 ppm, leaf biomass significantly increased. However, the effect of elevated CO\textsubscript{2} on leaf biomass was also closely related to the precipitation rate, similar to N\textsubscript{mass}. Severe drought (precipitation $-30\%$) restricted the effect of elevated CO\textsubscript{2} concentration on leaf biomass (Fig. 3).

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

![Figure 3](image1.png)

**Figure 3.** Interactive effects of changing precipitation and CO\textsubscript{2} on leaf biomass of the four \textit{Stipa} species: \textit{S. baicalensis} (A), \textit{S. bungeana} (B), \textit{S. grandis} (C), and \textit{S. breviflora} (D). See Fig. 1 for notes.

![Figure 4](image2.png)

**Figure 4.** Interactive effects of changing precipitation and CO\textsubscript{2} on the N\textsubscript{total} of the four \textit{Stipa} species: \textit{S. baicalensis} (A), \textit{S. bungeana} (B), \textit{S. grandis} (C), and \textit{S. breviflora} (D). See Fig. 1 for notes.
32.3%, 32.6%, and 40.0% (precipitation −30%), respectively, under ambient CO₂ conditions compared with the control. Although elevated CO₂ increased the Nₜₐₜₜ of the four Stipa species, the effect was not significant except under the −15% (S. bunęeana) and control (S. grandis) precipitation conditions.

Impacts of elevated CO₂, precipitation changes and their interactions on Nₘₐₜₙ, leaf biomass and Nₜₐₜₜ

Precipitation changes generally resulted in significant effects on the Nₘₐₜₙ, leaf biomass, and Nₜₐₜₜ of the four Stipa species (P < 0.001). The Nₘₐₜₙ and leaf biomass changed with elevated CO₂ concentration, but Nₜₐₜₜ was not significant. The interaction between elevated CO₂ and precipitation changes had no significant effect on the Nₘₐₜₙ, leaf biomass, and Nₜₐₜₜ of the four Stipa species except for the leaf biomass of S. breviflora (Table 4).

Discussion

Impacts of elevated CO₂ and precipitation changes and their interactions on Nₘₐₜₙ

Nitrogen serves as one of the major mineral elements affecting plant growth, and leaves are the largest N sinks in plants. Leaf Nₘₐₜₙ is closely related not only to the photosynthetic capacity of grass species (Sicher and Bunce et al. 2011). A reduction in Nₘₐₜₙ 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.

To date, there are limited reports on the interactive effect of changing precipitation and elevated CO₂ on the Nₘₐₜₙ of Stipa. This experiment showed that the elevated CO₂ effect on leaf Nₘₐₜₙ depended on the precipitation pattern. The changes in precipitation rates changed the sensitivity of Nₘₐₜₙ to elevated CO₂ 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 CO₂ concentrations.

In this study, the patterns of leaf Nₘₐₜₙ of the four Stipa species (S. baicalensis, S. bungeana, S. grandis and S. breviflora) in response to elevated CO₂ and precipitation change were similar. However, elevated CO₂ reduced Nₘₐₜₙ in different precipitation ranges for the four Stipa species. The sensitivities of leaf Nₘₐₜₙ 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 Nₘₐₜₙ 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$_{\text{mass}}$ 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$_{\text{mass}}$ response to elevated CO$_2$ and precipitation changes**

Three hypotheses have been proposed in relation to the mechanisms by which N$_{\text{mass}}$ 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$_{\text{mass}}$ 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$_{\text{mass}}$ decreased, total leaf N (N$_{\text{total}}$) 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 (a$_{\text{biomass}}$) and total leaf N (a$_{\text{Ntotal}}$). If the a$_{\text{Ntotal}}$ data are plotted on the y-axis and the a$_{\text{biomass}}$ 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 a$_{\text{Ntotal}}$ and a$_{\text{biomass}}$ for *Stipa* under elevated CO$_2$ showed that N$_{\text{mass}}$ 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$_{\text{mass}}$. The sensitivity of the four *Stipa*

![Graphs showing the relationship between N$_{\text{total}}$ and N$_{\text{biomass}}$ for different *Stipa* species under elevated CO$_2$](https://example.com/graphs.png)

**Figure 5.** Relationship between a$_{\text{Ntotal}}$ and a$_{\text{biomass}}$ for *Stipa* under elevated CO$_2$.

*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 $N_{\text{total}}$ (as y-axis) and $N_{\text{biomass}}$ (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 CO2 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 CO2 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 CO2 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 CO2 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 CO2 on S. breviflora were the most obvious among the four species.

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Conflict of Interest
None declared.

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