Effects of Elevated CO_2 and N Addition on Growth and N_2 Fixation of a Legume Subshrub (*Caragana microphylla* Lam.) in Temperate Grassland in China

Lin Zhang[1], Dongxiu Wu[1]*, Huiqiu Shi[1,2], Canjuan Zhang[1], Xiaoyun Zhan[1], Shuangxi Zhou[1]

1 State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing, China, 2 Graduate School of Chinese Academy of Sciences, Yuquanlu, Beijing, China

Abstract

It is well demonstrated that the responses of plants to elevated atmospheric CO_2 concentration are species-specific and dependent on environmental conditions. We investigated the responses of a subshrub legume species, *Caragana microphylla* Lam., to elevated CO_2 and nitrogen (N) addition using open-top chambers in a semiarid temperate grassland in northern China for three years. Measured variables include leaf photosynthetic rate, shoot biomass, root biomass, symbiotic nitrogenase activity, and leaf N content. Symbiotic nitrogenase activity was determined by the C_2H_2 reduction method. Elevated CO_2 enhanced photosynthesis and shoot biomass by 83% and 25%, respectively, and the enhancement of shoot biomass was significant only at a high N concentration. In addition, the photosynthetic capacity of *C. microphylla* did not show down-regulation under elevated CO_2. Elevated CO_2 had no significant effect on root biomass, symbiotic nitrogenase activity and leaf N content. Under elevated CO_2, N addition stimulated photosynthesis and shoot biomass. By contrast, N addition strongly inhibited symbiotic nitrogenase activity and slightly increased leaf N content of *C. microphylla* under both CO_2 levels, and had no significant effect on root biomass. The effect of elevated CO_2 and N addition on *C. microphylla* did not show interannual variation, except for the effect of N addition on leaf N content. These results indicate that shoot growth of *C. microphylla* is more sensitive to elevated CO_2 than is root growth. The stimulation of shoot growth of *C. microphylla* under elevated CO_2 or N addition is not associated with changes in N_2-fixation. Additionally, elevated CO_2 and N addition interacted to affect shoot growth of *C. microphylla* with a stimulatory effect occurring only under combination of these two factors.

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* E-mail: wudx@ibcas.ac.cn

Introduction

Increasing atmospheric carbon dioxide (CO_2) concentration caused by combustion of fossil fuels and enhanced nitrogen (N) deposition by human activities are two factors associated with global climate change. These factors are likely to have a widespread influence on individual plant communities and their interactions with each other [1]. It is well documented that the increase in atmospheric CO_2 concentration stimulates photosynthesis, plant biomass and plant water-use efficiency in many plant species, and that these effects are dependent on plant species as well as nutrient availability [2,3]. It is hypothesized that the sustainability of ecosystem response to CO_2 will be constrained by the progressive N limitation induced by the growth stimulation of increased CO_2 [4,5]. Therefore, the interaction between CO_2 and soil N availability has attracted intense interest, and varying results have been reported in different studies [3,6,7]. For example, the results from a six-year field study of perennial grassland species showed that the positive effect of CO_2 without N addition is reduced substantially [5]. However, plant growth in scrub-oak woodland showed a sustained increase after 11 years of

atmospheric CO_2 enrichment with enhanced inorganic N absorption from deep soil [6].

Differential sensitivities of different plant species or functional groups in response to elevated CO_2 are often observed. Legumes show greater response to elevated CO_2 through symbiotic N_2 fixation to counteract the progressive N limitation than any other functional types in most cases [8]. Lee *et al* (2003) reported that the legume species *Lupinus perennis* showed a stronger response to elevated CO_2 than non-leguminous species independent of N status, and that a 47% greater proportion of N was derived from stimulated N_2 fixation relative to other sources of N at elevated CO_2 [9]. The significant stimulation of N_2 fixation by elevated CO_2 is also reported in *Trifolium repens* in a fertilized Swiss grassland [10], *Galactia elliottii* in Florida scrub oak [11], soybean [8] and alfalfa [12]. The positive effect of elevated CO_2 on N_2 fixation may also contribute to the positive response of the co-occurring non-leguminous plants in response to elevated CO_2 [13]. However, stimulation of N_2 fixation by CO_2 can only occur under conditions in which other nutrients (e.g. P, K, and Mg) are not limited [14,15,16]. Furthermore, the stimulatory effect of elevated CO_2 on N_2 fixation has been found to diminish with the

extended period of CO$_2$ enrichment in oak woodland [15]. In addition, the response of legumes and symbiotic N$_2$ fixation to elevated CO$_2$ is species-specific and dependent on N availability in the soil [17,18]. Fixation of N$_2$ is often suppressed by N fertilization [19,20], but not in all cases [21]. It is predicted that plants would fix N$_2$ by symbiosis under conditions where it is less costly than soil N uptake [22], and show a significant yield response to N addition when the N$_2$ fixation apparatus unable to meet plant N demand [20,23]. Furthermore, how elevated CO$_2$ affects the suppression of N fixation with N addition remains unclear, but varies from no effect [16] to a positive effect [19]. In addition, elevated CO$_2$ partly promotes shrub encroachment in arid or semiarid grasslands [24]. As most shrubs are C$_3$ plants, they may benefit relatively more from higher levels of CO$_2$ compared to many C$_4$ grasses [25]. Elevated CO$_2$ may slow soil water depletion by herbaceous vegetation, thus promoting the establishment of deeper-rooted shrubs, especially in semiarid and/ or arid areas [26,27,28]. Although an increase in woody plant density was observed after CO$_2$ enrichment for five years in semiarid shortgrass steppe in Colorado [24], it remains unclear whether elevated atmospheric CO$_2$ plays a widespread role in encroachment of C$_3$ shrub and woody plants into grasslands [26,29].

The leguminous sub-shrub *Caragana microphylla* is a common species that dominates an important plant successional stage in the semiarid grasslands in northern China. It is reported that the distribution of *C. microphylla* shrubs in the Xilin River Basin in northern China has increased substantially in recent years [30,31,32]. This study was conducted to determine how the growth and symbiotic N$_2$ fixation of *C. microphylla* respond to elevated CO$_2$ and N addition in a semiarid temperate grassland over three growing seasons.

Materials and Methods

Research site

The experiment was conducted at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) (43°38'N, 116°42'E; 1100 m altitude) in the Xilin River Basin, Inner Mongolia, China. The site is located in the Eurasian steppe region, which is the largest contiguous grassland in the world. The site has a continental, moderate temperate, semiarid climate characterized by long, cool, dry winters and short, warm, moist summers. The mean annual temperature is 0.8°C, and the mean annual precipitation is 340.2 mm, with the majority (86%) of the rainfall

occurring during the growing season (May to September) during the previous 24 years (1982–2005). During the three experimental years (2006–2008), the mean annual temperature was 1.4°C, 2.2°C, and 1.6°C, respectively, and the annual total rainfall was 304.1 mm, 240.1 mm and 363.5 mm, respectively [33].

Plant materials, experimental design and treatments

The experiment was conducted on *C. microphylla* (Fig. S1). There were four treatment groups: ambient CO$_2$ without N addition (C), elevated CO$_2$ without N addition (E), ambient CO$_2$ with N addition (CN), and elevated CO$_2$ with N addition (EN). The experiment followed a split-plot design, with the CO$_2$ treatment applied at the whole plot level (with three chambers for each of the two CO$_2$ levels) and the N addition treatments applied at the split-plot level (pot-within-chamber). The experiment was conducted for three years (2006–2008) and most variables were measured in each year.

Six field open-top chambers (3 m in diameter, 3 m in height) were used. Three of the chambers contained the current CO$_2$ concentration (380 μmol mol^{-1}) and the other three contained an elevated CO$_2$ concentration (760 μmol mol^{-1}) (Fig. S2) [33]. For N addition treatment, 17.5 g m^{-2} of N [34] was added in each year by applying (NH$_4$)$_2$SO$_4$ solution at the beginning and in the middle of the growing season.

The experiment site (15 m×15 m) was established in 2005. Pots (30 cm diameter, 30 cm deep) were filled with the universal native dark chestnut soil, and then buried underground with the pot mouth positioned at the ground surface. The soil organic carbon concentration was 0.081% and total N concentration was 0.704%. Seeds of *C. microphylla*, collected in the vicinity of the research station, were sown in pots in late autumn in 2005. At the beginning of the experiment in 2006, plants were thinned to 20 plants per pot.

Shoot biomass was harvested and oven dried at 65°C to constant mass and weighed in 2006–2007. In 2008, the whole plant was harvested, and shoot and root biomass processed separately. Soil was carefully removed from roots, which were temporarily stored at 4°C until all nodules could be removed for determination of symbiotic nitrogenase activity (see below). The remaining root biomass and shoot biomass were oven dried at 65°C to constant mass and weighed.

Symbiotic nitrogenase activity was determined by the C$_2$H$_2$ reduction method [35] using nodules within 48 h of collection. Nodules were placed in a 25 ml closed culture bottle and sealed with rubber. Three ml C$_2$H$_2$ gas was injected into the culture

Table 1. Results (*P*-values) of mixed model ANOVA for the effects of elevated CO$_2$ (CO$_2$), N addition (N) and their interactions on shoot biomass and leaf nitrogen content (Leaf N) in three growing years (Y; 2006 to 2008), and light-saturated leaf net photosynthetic rate (A$_{sat}$) and down-regulation of photosynthesis (D) in 2007 and 2008, and root biomass, root/shoot ratio (R/S), specific symbiotic nitrogenase activity (SNA) and symbiotic nitrogenase activity per plant (PNA) in 2008.

Source of variation	A$_{sat}$	D	Shoot biomass	Leaf N	Root biomass	R/S	SNA	PNA
CO$_2$	0.01	0.94	0.03	0.52	0.19	0.69	0.86	0.74
N	<0.01	0.01	0.30	<0.01	0.11	<0.01	0.89	0.01
CO$_2$×N	0.01	0.05	0.04	0.53	0.39	<0.01	/	0.64
Y	0.55	0.07	<.0001	<.001				
Y×CO$_2$	0.78	0.17	0.16	0.41				
Y×N	0.70	0.94	0.57	0.02				
Y×CO$_2$×N	0.21	0.61	0.20	0.34				

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bottles and incubated for 2 hours at 28°C, after which 1 ml gas samples were collected from each bottle and analyzed for production of C_2H_4 using gas chromatography (Shimadzu GC-7AG gas chromatograph, Shimadzu Corp., Japan). The parameters used for spectrometer determination of symbiotic nitrogenase activity with the flame ionization detector (FID) were as follows: column temperature 60°C, detector temperature 250°C, sample temperature 120°C, gas flow of H_2 0.7 kg cm^{-2}, N_2 35 ml min^{-1}, and air 0.6 kg cm^{-2}. Production of C_2H_4 (μmol g^{-1} h^{-1}) was used to calculate symbiotic nitrogenase activity. Specific symbiotic nitrogenase activity (SNA) represented the symbiotic nitrogenase activity per unit weight of nodule. Plant symbiotic nitrogenase activity (PNA) represented the symbiotic nitrogenase activity per plant.

$$SNA = \frac{\text{sample } C_2H_4 \text{ peak area} \times \text{standard air content} \times \text{culture bottle volume}}{\text{standard air peak area} \times \text{sample volume} \times \text{incubation time} \times \text{nodules drymass}}$$

$$PNA = \frac{SNA \times \text{nodules drymass per pot}}{\text{plant number per pot}}$$

Specific symbiotic nitrogenase activity for the CN treatment was treated as missing data since live root nodules collected from this treatment were insufficient for measurement.

Leaf gas exchange (μmol CO_2 m^{-2} s^{-1}) measurements were conducted *in situ* using a portable, steady-state gas exchange
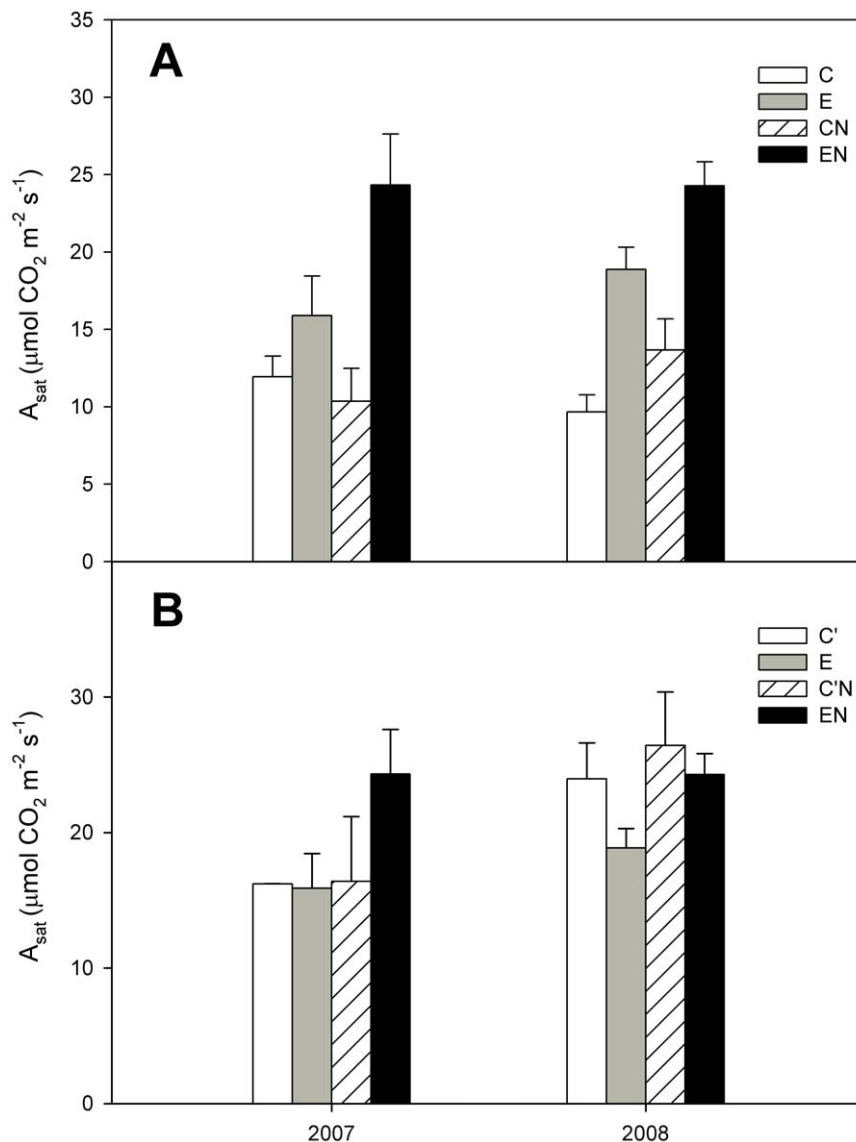
Figure 1. Light-saturated leaf net photosynthetic rate (A$_{sat}$, μmol m^{-2} s^{-1}) and down-regulation of photosynthesis of *C. microphylla*. Plants were grown and measured under ambient (380 μmol mol^{-1}) or elevated (760 μmol mol^{-1}) CO_2 concentrations (A), and plants were grown under ambient or elevated CO_2 and measured at a common CO_2 concentration of 760 μmol mol^{-1} (B) at two nitrogen-amended levels (0 and 17.5 g N m^{-2} year^{-1} applied to native N-deficient soil) in the 2007 and 2008 growing seasons. Treatments: ambient CO_2 without N addition (C); elevated CO_2 without N addition (E); ambient CO_2 with N addition (CN); elevated CO_2 with N addition (EN); grown at ambient but measured in elevated CO_2 without N addition (C'); grown at ambient but measured in elevated CO_2 with N addition (C'N). Bars represent means and error bars the standard error.
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system, incorporating an infrared gas analyzer (LI-6400, Li-Cor, Lincoln, NE, USA), in late August 2007 and mid-July 2008. Measurements were made on three leaves of randomly chosen individual plants in each pot on sunny days. The second healthy leaf from the top of the plants was selected. Photosynthetic rates were determined under light-saturating conditions (PAR 1500 μmol m^{-2} s^{-1}), constant leaf air temperature (25°C), and at the CO_2 concentration under which the plants were grown (i.e., 380 or 760 μmol mol^{-1}) as long-term responses. In addition, for plants grown under ambient CO_2 photosynthetic rates were determined at 760 μmol mol^{-1} CO_2 to assess the short-term response of the species to instantaneous CO_2 enrichment [36]. Following gas exchange measurement, leaf areas were measured with a leaf area meter (LI-3100, Li-Cor) and photosynthetic rates were calibrated with the known area.

Statistical analysis

Statistical analysis of data was conducted using the Statistical Analysis System 9.2 (SAS Institute Inc., Cary, NC, USA). There were three replicates for each treatment group. Split-plot analysis of variance (ANOVA) was performed using mixed model procedures with CO_2 concentration as the whole-plot factor and N as the split-plot factor. Year was taken as a repeated factor for indices measured in multiple years, such as photosynthetic rate, shoot biomass, and leaf N content. Tukey's studentized range test was conducted to make pairwise comparisons of means for those indices in which ANOVA showed a significant effect. Results were considered to be significant at $P \leq 0.05$, and highly significant at $P \leq 0.01$.

Results

Effects of elevated CO_2 and N addition on net photosynthesis

Both elevated CO_2 and N addition had significant effects on leaf-level gas exchange of *C. microphylla* ($P < 0.05$, Table 1), and their interactions also had a significant effect ($P < 0.01$, Table 1). Elevated CO_2 markedly increased the light-saturated leaf net photosynthetic rate (A_{sat}) under both N levels across the three years, with average increases of 83% (Fig. 1A). Plant grown under high N showed an average 29% higher photosynthetic rate than those grown under low N. However, the stimulatory effect of N addition on A_{sat} was different between the two CO_2 concentrations, with a 40% increase occurring at the elevated CO_2 concentration. The responses of A_{sat} to elevated CO_2, N addition or both did not vary significantly between the 2 years (Table 1).

In addition, when measured with the same elevated CO_2, A_{sat} did not differ significantly between C plants and E plants, as well as between CN plants and EN plants. This implied that no acclimation of photosynthesis occurred in *C. microphylla* (Table 1, Fig. 1B).

Effects of elevated CO_2 and N addition on aboveground growth

Elevated CO_2 significantly stimulated shoot biomass by 25% when all treatments were considered ($P < 0.05$, Table 1). The stimulation of elevated CO_2 was significant only under high N concentration (Fig. 2). Addition of N significantly stimulated shoot biomass by 32% ($P < 0.05$) at elevated CO_2, but showed no significant effect on shoot biomass at ambient CO_2 across the three years.

Shoot biomass in 2006 was significantly lower than in 2007 and 2008. The shoot biomass increased by 62% ($P < 0.001$) in 2007 and 69% ($P < 0.001$) in 2008 compared with that in 2006. Annual variation in shoot biomass was not significantly affected by CO_2 concentration, N addition or their interaction.

Effects of elevated CO_2 and N addition on root biomass and root/shoot ratio

Neither elevated CO_2, N addition nor their interaction significantly affected root biomass (Fig. 3A, Table 1). Elevated
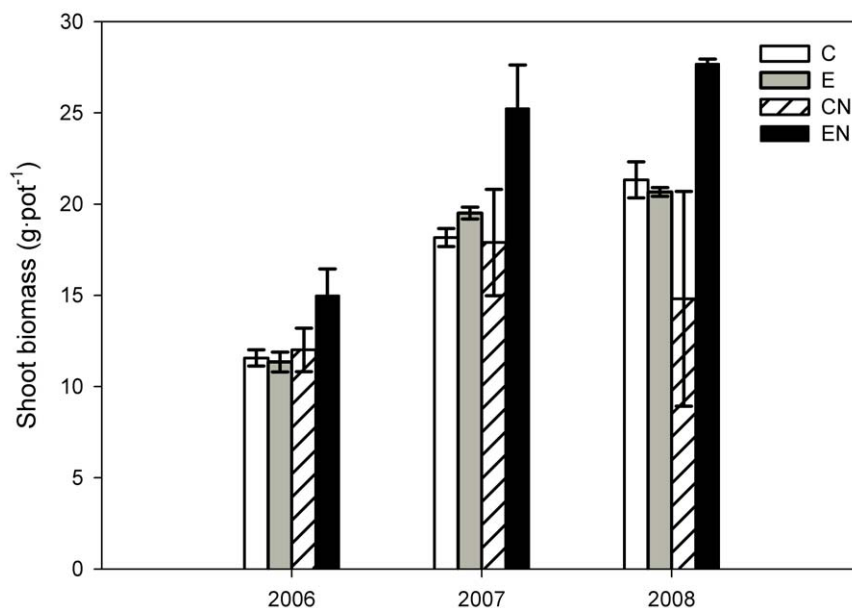
Figure 2. Shoot biomass per pot of *C. microphylla*. Plants were grown in open-top chambers under ambient (380 μmol mol^{-1}) and elevated (760 μmol mol^{-1}) atmospheric CO_2 concentrations at two nitrogen levels (0 and 17.5 g N m^{-2} year^{-1}) in the growing seasons from 2006 to 2008. Treatments: ambient CO_2 without N addition (C); elevated CO_2 without N addition (E); ambient CO_2 with N addition (CN); elevated CO_2 with N addition (EN). Bars represent means and error bars the standard error.
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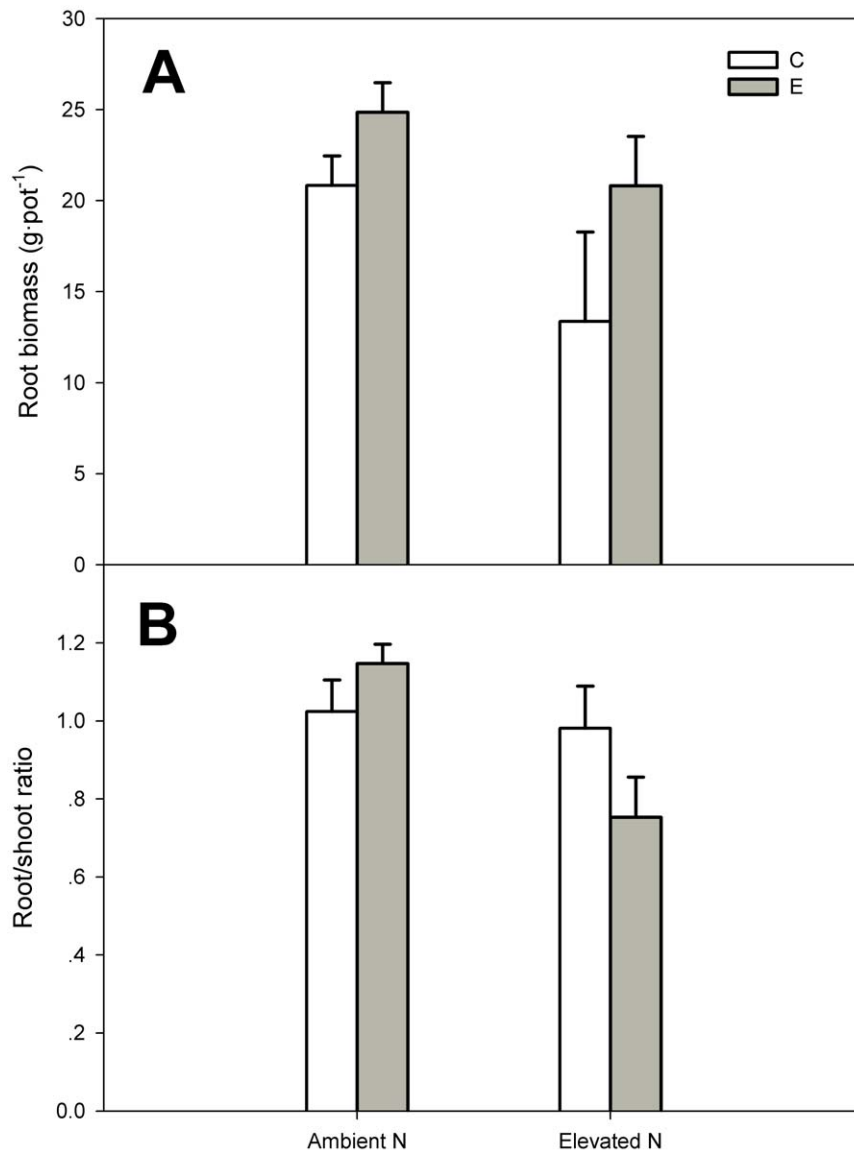
Figure 3. Root biomass (A) and root/shoot ratio (B) per pot of *C. microphylla*. Plants were grown under ambient (380 µmol mol^{-1}) and elevated (760 µmol mol^{-1}) atmospheric CO_2 concentrations at two nitrogen levels (0 and 17.5 g N m^{-2} year^{-1}) in 2008. Treatments: ambient CO_2 without N addition (C); elevated CO_2 without N addition (E); ambient CO_2 with N addition (CN); elevated CO_2 with N addition (EN). Bars represent means and error bars the standard error.
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CO_2 had no significant effect on root/shoot ratio either. However, N addition significantly decreased the root/shoot ratio by 34% at elevated CO_2 ($P<0.01$, Table 1), but had no significant effect on the root/shoot ratio at ambient CO_2 (Table 1, Fig. 3B).

Effects of elevated CO_2 and N addition on specific symbiotic nitrogenase activity and symbiotic nitrogenase activity per plant

Elevated CO_2 did not significantly affect specific symbiotic nitrogenase activity (Fig. 4A) and symbiotic nitrogenase activity per plant (Fig. 4B, Table 1). Addition of N had no significant effect on specific symbiotic nitrogenase activity, but markedly decreased symbiotic nitrogenase activity per plant by over 95% ($P<0.05$, Table 1, Fig. 4).

Effects of elevated CO_2 and N addition on leaf N content

Elevated CO_2 had no significant effect on leaf N content, whereas N addition significantly enhanced leaf N content by 10% in all treatments over the three years ($P<0.01$, Table 1, Fig. 5). No interaction effect between CO_2 and N addition on leaf N content was detected. The increase in leaf N content induced by N addition differed between the three years: 1% in 2006 (ns), 13% in 2007 ($P<0.01$) and 18% in 2008 ($P<0.01$).

Discussion

Effect of elevated CO_2 on N_2 fixation and growth of *C. microphylla*

Our finding that elevated CO_2 has a stimulatory effect on photosynthetic rates in the leguminous subshrub *C. microphylla* is
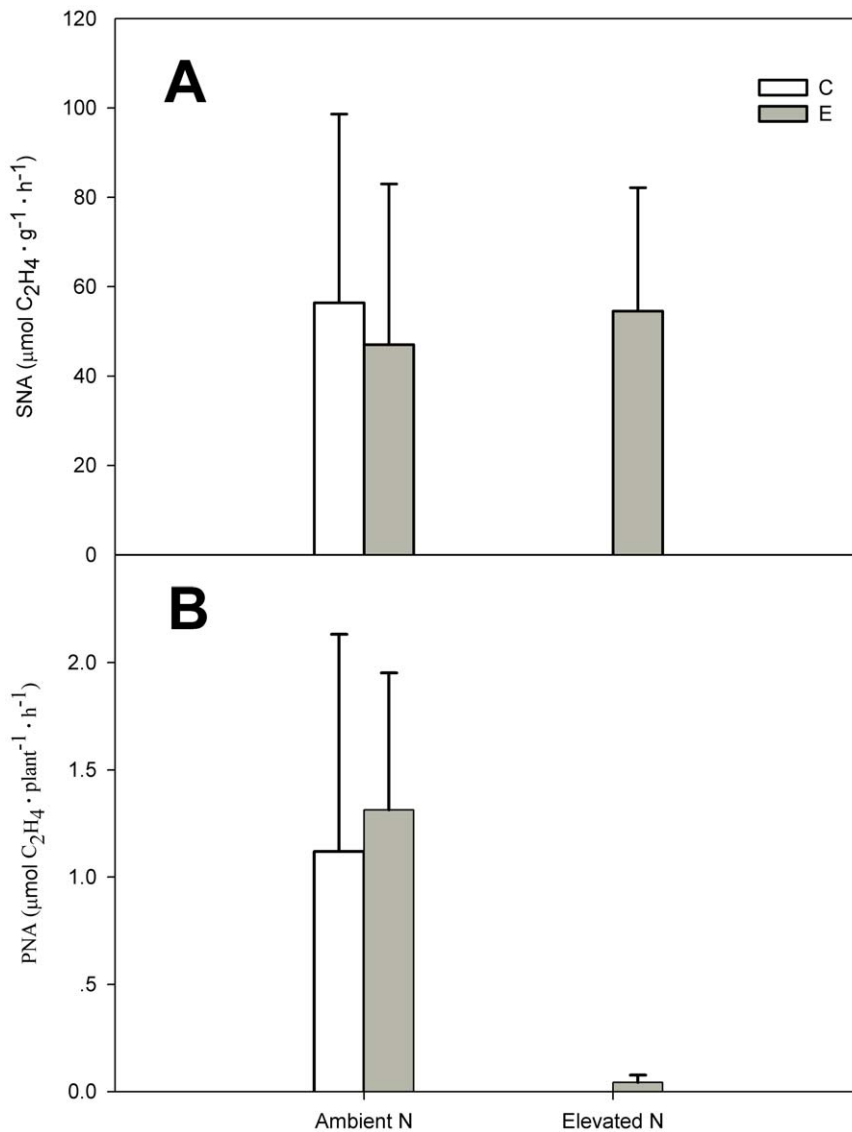
Figure 4. The specific symbiotic nitrogenase activity (SNA, μmol C_2H_2 g^{-1} h^{-1}) (A), and symbiotic nitrogenase activity per plant (PNA, μmol C_2H_2 g^{-1} h^{-1}) (B) of *C. microphylla*. Plants were grown under ambient (380 μmol mol^{-1}) and elevated (760 μmol mol^{-1}) atmospheric CO_2 concentrations at two nitrogen levels (0 and 17.5 g N m^{-2} $year^{-1}$) in 2008. Treatments: ambient CO_2 without N addition (C); elevated CO_2 without N addition (E); ambient CO_2 with N addition (CN); elevated CO_2 with N addition (EN). Bars represent means and error bars the standard error.
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consistent with most previous studies on other plant species [37,38,39]. Numerous studies have found that stimulation of photosynthetic rates induced by elevated CO_2 will decrease or even disminished over time as plants acclimate to elevated CO_2 concentrations through a process known as down-regulation [36,38,40]. Acclimation of photosynthesis can be partly explained by N scarcity or progressive reduction in N availability, since the down-regulation of photosynthesis induced by elevated CO_2 is always highly associated with reduction of leaf N content [5,39]. Results in the present study revealed that acclimation of photosynthesis did not occur in *C. microphylla*. This is in line with the hypothesis proposed by many previous studies that species capable of symbiosis with N_2-fixing organisms may sustain longer stimulation [2,9]. Accordingly, leaf N content of *C. microphylla* in this study did not vary with CO_2 concentration.

In the present study, symbiotic nitrogenase activity did not respond significantly to elevated CO_2 concentrations. The effect of elevated CO_2 on N_2-fixation may be positive, neutral or negative, depending on the species examined, soil N acquisition [18], or availability of other soil resources, such as phosphorus [14,22]. Thus, it can be inferred that the symbiotic N_2-fixation of *C. microphylla* is not sensitive to changes in CO_2 concentration. Another possible explanation is that other limited nutrients, such as P, constrain the responses of N_2-fixation of *C. microphylla* to elevated CO_2. Availability of P is reported to be more limited than that of N in our experimental area [41]. Although the N_2-fixation was not enhanced by elevated CO_2, *C. microphylla* could utilize soil N more efficiently at elevated CO_2 (unpublished data).

The finding that elevated CO_2 had a significant positive effect on shoot biomass, but not on root biomass, implies that shoot
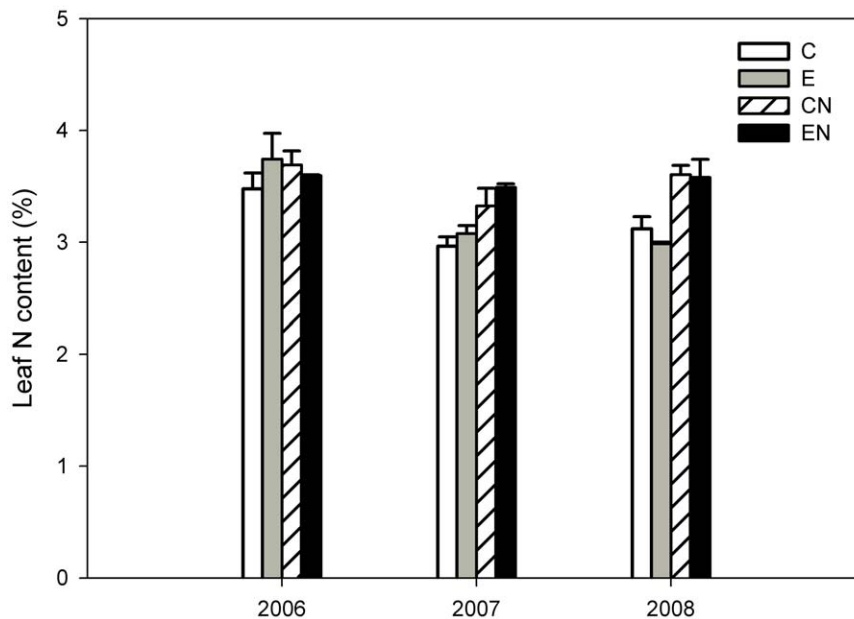
Figure 5. The leaf N content (Leaf N, %) of *C. microphylla*. Plants were grown under ambient (380 µmol mol^{-1}) and elevated (760 µmol mol^{-1}) atmospheric CO$_2$ concentrations at two nitrogen levels (0 and 17.5 g N m^{-2} year^{-1}) in the growing seasons from 2006 to 2008. Treatments: ambient CO$_2$ without N addition (C); elevated CO$_2$ without N addition (E); ambient CO$_2$ with N addition (CN); elevated CO$_2$ with N addition (EN). Bars represent means and error bars the standard error.
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growth of *C. microphylla* is more sensitive to elevated CO$_2$ than that of root growth. This finding is in contrast to the non-leguminous species *Leymus chinensis* in the same experiment field [33]. Previous studies have shown that the relative responses of root systems to elevated CO$_2$ are species specific and dependent on experimental conditions [33,42,43]. For example, Arnone *et al.* found that among 12 grassland studies in different areas, seven showed little or no change in root-system size under elevated CO$_2$ [44]. On the other hand, pots may constrain growth of the root system and may therefore partly depress the root response to elevated CO$_2$ when plants are grown in pots [33,45]. However, root growth of *C. microphylla* was not significantly suppressed by pot containment in this study because *C. microphylla* is a slow-growing shrub and was in early seedling stage. Additionally, the shoot response of *C. microphylla* to elevated CO$_2$ is much higher than *L. chinensis* (25% vs. 9%) in the same experimental field [33]. This is in line with most previous studies, which reported that legume species show a stronger response to elevated CO$_2$ than non-leguminous species [39]. This result implies that the relative competitiveness of the legume *C. microphylla* with *L. chinensis*, the dominant temperate grassland species in the study area, will increase in the future under elevated CO$_2$ conditions.

In the present study, the stimulatory effect of elevated CO$_2$ on *C. microphylla* was dependent on N status. This finding is similar to that with other shrubs [46], but in contrast to herbaceous legume species, which always show a strong response to elevated CO$_2$ independent of N status [9]. On the other hand, the effect of CO$_2$ on photosynthesis, growth, and leaf N content of *C. microphylla* did not show any annual variation, even though the weather conditions varied among the study years.

Effect of N addition and its interaction with elevated CO$_2$ on growth and N$_2$ fixation of *C. microphylla*

The stimulatory effects of N addition on photosynthesis and shoot biomass, as well as its inhibitory effect on the root/shoot

ratio, were only observed under elevated CO$_2$ in the present study. These results indicate that accumulation of photosynthate and biomass allocation in response to soil N supply was affected by elevated CO$_2$. The finding that N addition had no significant effect on biomass production of *C. microphylla* at ambient CO$_2$ is consistent with some previous studies on legumes [47], but is inconsistent with the effect on biomass production by the grass species *L. chinensis* in the same experimental field, which was greatly increased by N addition. This indicated the competitiveness of *C. microphylla* with the herbaceous species *L. chinensis* would decrease with N addition at ambient CO$_2$. However, when plants were grown under elevated CO$_2$, N addition had a positive effect on *C. microphylla* growth and with a similar degree of enhancement on *L. chinensis*. This indicates that the depressive effect of N fertilization or deposition on the competitiveness of *C. microphylla* with *L. chinensis* would be alleviated by elevated CO$_2$. This is in line with indications from other studies that elevated CO$_2$ may reduce the increased risk of legume species loss due to the N fertilization or deposition [3,48].

The most obvious effect of N addition on *C. microphylla* in the present study is its inhibitory effect on symbiotic nitrogenase activity per plant. This inhibitory effect is in agreement with most previous reports on other plant species, while the detrimental effect of N addition on symbiotic nitrogenase activity per plant in the current study is more serious than that of other reports [9,19]. The greater effect may be attributed to the relatively high N concentration applied in the present study. Given that no changes in specific symbiotic nitrogenase activity and reduction in root nodule number under the high N level in the current study [49], it can be concluded that the strong decrease in symbiotic nitrogenase activity per plant under the high N level mainly resulted from the inhibitory effect of N addition on nodule formation.

In the present study, the effect of N addition on photosynthesis and shoot biomass of *C. microphylla* did not show interannual variation. However, the stimulatory effect of N addition on leaf N

content was increased across years. This implies that the responses of *C. microphylla* to N addition may be more affected by interannual climatic variation than elevated CO_2.

In conclusion, we demonstrated that elevated CO_2 stimulates leaf-level photosynthesis of *C. microphylla* and no acclimation of photosynthesis occurred over the three experimental years. Elevated CO_2 stimulates shoot growth of *C. microphylla*, but only under a high N concentration. Shoot growth of *C. microphylla* is more sensitive to elevated CO_2 than is root growth. Elevated CO_2 has no effect on symbiotic nitrogenase activity. Addition of N markedly inhibits N_2 fixation capacity, but stimulates photosynthesis and shoot growth, of *C. microphylla*. However, the stimulatory effect of N addition occurred only under elevated CO_2 condition. Interaction between CO_2 and N significantly affected photosynthesis, shoot biomass and biomass allocation of *C. microphylla*. When compared to responses of the grass species *L. chinensis* grown in the same experimental field, N addition tends to decrease the relative competitiveness of *C. microphylla*, whereas elevated CO_2 tends to increase competitiveness. These results indicate that elevated CO_2 will interact with N deposition in the future to benefit the growth of the leguminous subshrub *C. microphylla*.

Supporting Information

Figure S1 The photograph of the *C. microphylla* in the Xilin River Basin.
(TIF)

Figure S2 The photograph of the six open-top chambers.
(TIF)

Author Contributions

Conceived and designed the experiments: DW LZ. Performed the experiments: LZ HS CZ XZ SZ. Analyzed the data: LZ. Contributed reagents/materials/analysis tools: LZ. Wrote the paper: LZ DW.

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