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Nitrogen Fertilization Modified the Responses of *Schima superba* Seedlings to Elevated CO_2 in Subtropical China

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Abstract: There are very few studies about the effects of relatively higher CO_2 concentration (e.g., 1000 $\mu mol \cdot mol^{-1}$) or plus N fertilization on woody plants. In this study, *Schima superba* seedings were exposed to ambient or eCO_2 (550, 750, and 1000 $\mu mol \cdot mol^{-1}$) and N fertilization (0 and 10 $g \cdot m^{-2} \cdot yr^{-1}$, hereafter: low N, high N, respectively) for one growth season to explore the potential responses in a subtropical site with low soil N availability. N fertilization strongly increased leaf mass-based N by 118.38%, 116.68%, 106.78%, and 138.95%, respectively, in different CO_2 treatments and decreased starch, with a half reduction in leaf C:N ratio. Leaf N was significantly decreased by eCO_2 in both low N and high N treatments, and N fertilization stimulated the decrease of leaf N and mitigated the increase of leaf C:N by eCO_2. In low N treatments, photosynthetic rate (Pn) was maximized at 733 $\mu mol \cdot mol^{-1}$ CO_2 in August and September, while, in high N treatments, Pn was continuously increased with elevation of CO_2. N fertilization significantly increased plant biomass especially at highly elevated CO_2, although no response of biomass to eCO_2 alone. These findings indicated that N fertilization would modify the response of *S. superba* to eCO_2.

Keywords: elevated CO_2; N fertilization; leaf N; photosynthesis; biomass

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1. Introduction

Elevated atmospheric CO_2 concentration (eCO_2) and N deposition are always important issues in the research of global change. Until today, amounts of previous studies reported the effects of eCO_2 alone or eCO_2 combined with N deposition on plants [1–6]. While, according to the IPCC (Intergovernmental Panel on Climate Change) Fifth Evaluation Report, the CO_2 concentrations will be between 430–480, 580–720, 720–1000 $\mu mol \cdot mol^{-1}$, and more than 1000 $\mu mol \cdot mol^{-1}$ in 2100 without additional mitigation efforts, respectively, in the four levels of Representative Concentration Pathways (RCPs), RCP2.6, RCP4.5, RCP6.0, and RCP8.5 [7]. RCPs are used for making projections based on these factors, described four different 21st century pathways of greenhouse gas emissions and atmospheric concentrations, air pollutant emissions, and land use in this report [7]. N_2O emission will also increase with increasing CO_2 concentration, and will reach up to more than 20 T $g \cdot yr^{-1}$ until 2100 for scenario RCP8.5 [7]. And total reactive-N emissions were predicted to reach 83 and 114 Tg N yr^{-1} by 2100 under the RCP4.5 and RCP8.5, respectively [8]. A model analysis over the globe indicated that large increases have occurred for all soluble reactive N fractions and will continuously occur mainly in Asia in the future, and NO_y deposition will be increased only in Asia [9]. Across all forms of deposition, rates of N deposition in Eastern Asia are among the highest in the world, in which China is a hotspot of N deposition with the greatest number of urban sites mentioned in 174 publications focusing on N deposition over the world [10].

Most of the previous experiments studied the effects of double ambient CO_2 concentration (about 700 $\mu mol \cdot mol^{-1}$) on plants, which could not predict the potential responses of the plants to the future CO_2 concentration based on RCP6.0 and RCP8.5. Although a few reports studied the effects of the higher CO_2 concentration like 1000 μmol·mol^{-1} on the crops [11–17], to the best of our knowledge, there have been no studies on the effects of about 1000 μmol·mol^{-1} CO_2 combined with N fertilization on woody plants.

This study aimed to explore the responses of native tree species to highly elevated CO_2 and N fertilization. *S. superba* is a representative tree species of subtropical forests in China and widely used in urban landscaping. Previous studies showed that photosynthesis, N:P ratio, biomass accumulation and allocation, and soil respiration of *S. superba* were affected by eCO_2 (about 700 μmol·mol^{-1}) and N deposition [18–20]. Photosynthetic rate of *S. superba* seedlings was reduced, while height growth was enhanced by eCO_2, after the exposure of 6 months [20]. Although eCO_2 did not affect photosynthetic rate in high N treatment, *S. superba* grown in ambient N treatment had 23% and 47% greater photosynthate rate (Pn) in ambient CO_2 than those in eCO_2 after exposure of 20 and 31 months, and leaf N was also reduced by eCO_2 in both low and high N treatment [21]. If this species similarly responds to high CO_2 concentration as to about 700 μmol·mol^{-1} CO_2 in previous studies, how would it respond to high CO_2 and N fertilization? In this present study, we tried to explore the responses of gas exchange, carbohydrates and biomass to different levels of CO_2 concentration and N fertilization. We hypothesized that: 1) Pn, leaf N would be reduced much more at 1000 μmol·mol^{-1} CO_2 than at 700 μmol·mol^{-1} CO_2; and 2) nitrogen fertilization would affect the response of *S. superba* to eCO_2.

2. Results

2.1. Leaf N, C:N Ratio, and Carbohydrates

Leaf N was decreased with CO_2 concentration increasing at both low N and high N treatments (Figure 1, $R^2 = 0.9968$, $p = 0.003$ and $R^2 = 0.8798$, $p = 0.034$, respectively). High N significantly improved leaf N ($p \leq 0.05$) (Figure 1) by 118.38%, 116.68%, 106.78%, and 138.95% compared to low N, respectively, at ambient air, 550, 750, and 1000 μmol·mol^{-1} CO^2. Although soluble sugar content was not significantly affected by elevated CO_2 at low N, while it was linearly decreased with CO_2 enrich at high N (Figure 1, $R^2 = 0.8580$, $p = 0.016$). The starch content was linearly increased by eCO_2 under both low N and high N, while the increment at high N was slower than that at low N (Figure 1). N fertilization decreased the starch contents by 31.52%, 49.36%, 39.32%, and 52.00%, respectively, at ambient, 550, 750, and 1000 μmol·mol^{-1} CO_2. Simultaneously, the accumulation of leaf N and reduction of sugar and starch by high N leaded to reduction in plant C:N ratio by 51.64%, 54.79%, 50.92%, and 59.67%, respectively, at ambient and elevated CO_2 concentration under N fertilization (Figure 1). Although leaf C:N was both increased with CO_2 concentration increasing at low and high N, the slope of the regression equation at high N was lower than that at low N.

2.2. Gas Exchange

In general, Pn, and g_s both decreased from July to September (Figures 2 and 3). In July, Pn was increased with CO_2 enrich at both low N and high N, with greater slope at high N (Figure 2). Pn was also linearly increased by eCO_2 at high N in August and September ($p < 0.001$), while, at low N, Pn was increased to the maximum at 733 μmol·mol^{-1} CO_2 and then decreased when CO_2 concentration continuously increased (Figure 2). Pn from plants exposed to 1000 μmol·mol^{-1} CO_2 at high N was increased of 73.68%, 119.90%, and 183.46%, respectively, measured in July, August, and September compared to ambient CO_2 at low N. CO_2 concentration, N fertilization and measuring time all had significant effects on Pn ($p < 0.001$), and there were significant interactions between CO_2 and N, CO_2 and measuring time, N and measuring time, while no significant impacts of CO_2, N and measuring time on Pn. No matter under low N or high N, g_s was significantly negatively correlated with eCO_2 in all measuring time ($p < 0.001$ at both low N and high N in July and August; $p = 0.009$ and 0.015, respectively, at low N and high N in September. In July, the two lines were almost parallel at both low N and high N with almost equal slopes (0.02259 and 0.02300, respectively, at low N and high N), while, in August and September, g_s decreased more quickly with CO_2 concentration increasing at high N than that at low N, with steeper lines at high N. Whether it is CO_2 concentration, N fertilization, or measuring time, all had significant impacts on g_s, while there was no interaction among them (Figure 3).

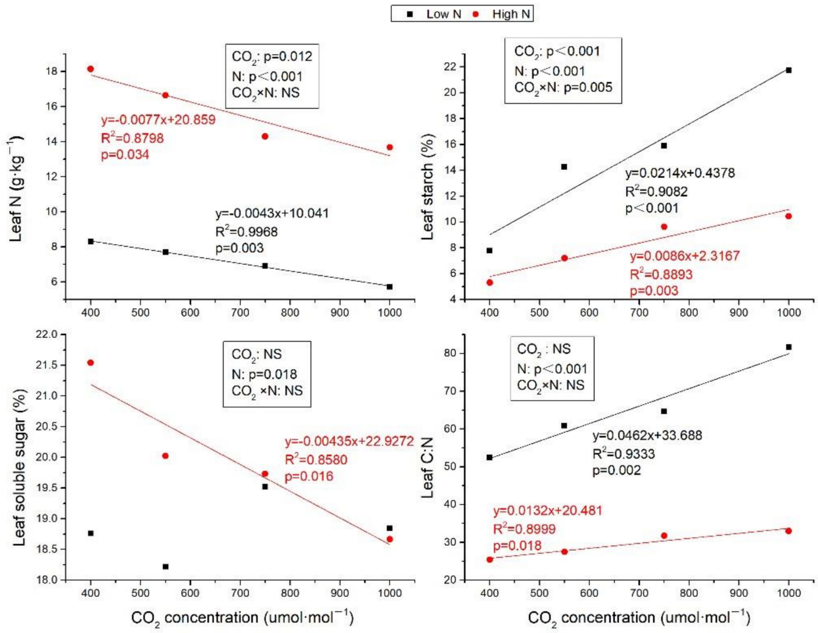

Figure 1. The different effects of elevated CO_2 on leaf N, carbohydrates and leaf C:N between low N and high N treatments. The results of two-factor analysis were also shown. NS: not significant. Leaf N, soluble sugar, and starch were all calculated by dry mass. Values are means ± SE (n = 6).

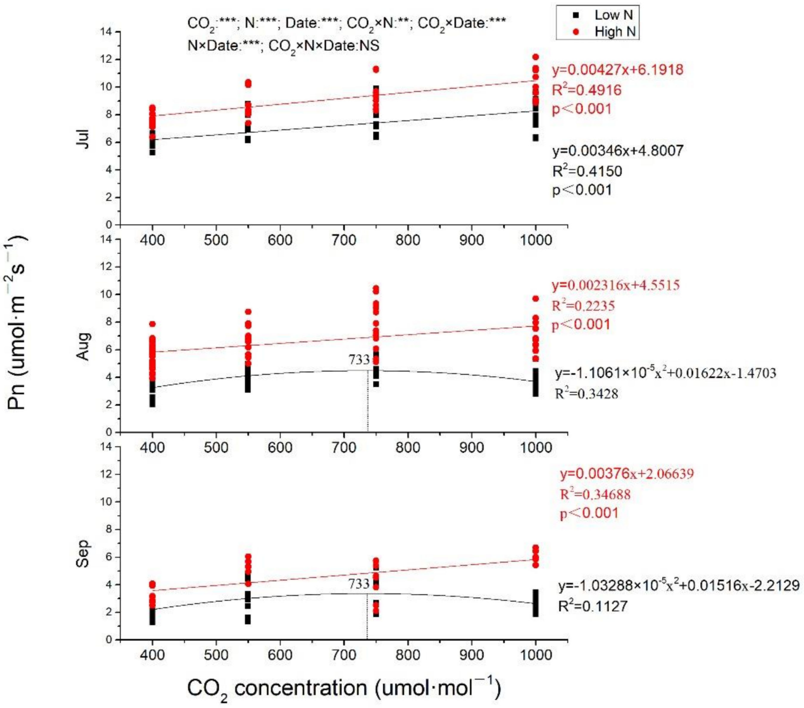

Figure 2. The different responses of photosynthate rate to elevated CO_2 at low N and high N treatments. Pn, photosynthate rate. CO_2: different CO_2 concentrations; N: N fertilization; Date: measuring time; ***, **: significant at 0.001 and 0.01 level; NS: not significant; Jul: July; Aug: August; Sep: September.

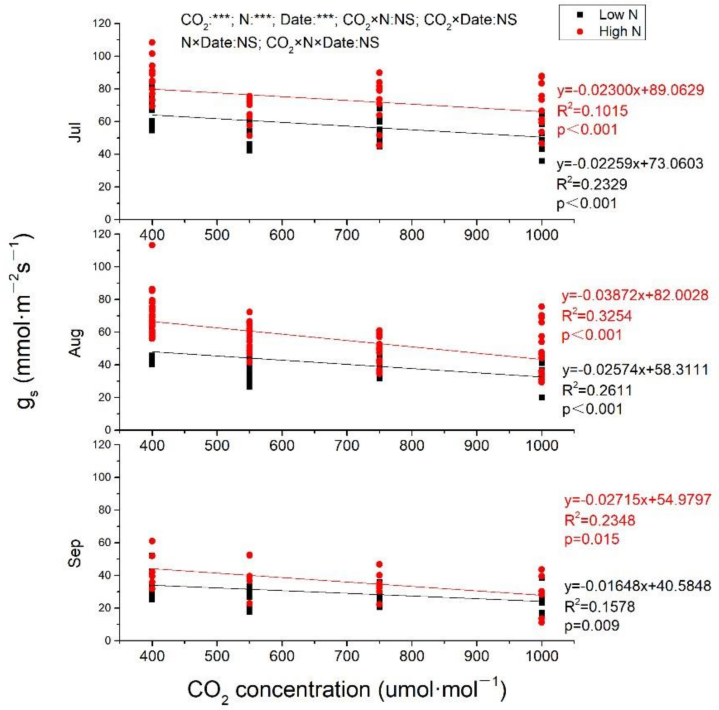

Figure 3. The effects of elevated CO_2 and N fertilization on stomatal conductance. g_s, stomatal conductance. CO_2: different CO_2 concentrations; N: N fertilization; Date: measuring date; ***: significant at 0.001 level; NS: not significant. Jul: July; Aug: August; Sep: September.

2.3. Plant Biomass

Elevated CO_2 alone even up to 1000 μmol·mol^{-1} had no effects on plant biomass, including different issue and total biomass, whether at low N or high N. While N fertilization impacted the biomass at eCO_2. In ambient CO_2 treatments, N fertilization had no effect on biomass. High N significantly increased leaf biomass and total biomass compared to plants in low N treatments at 550 μmol·mol^{-1} CO_2. When plants exposed to 750 μmol·mol^{-1}, N fertilization increased root and total biomass. Notably, N fertilization significantly increased the biomass of each issue and also the total biomass when plants exposed to 1000 μmol·mol^{-1}. The results of two-way ANOVA indicated that eCO_2 had not any significant effects on plant biomass, while N fertilization significantly affected biomass ($p < 0.001$), without interaction between CO_2 and N fertilization (Table 1).

Table 1. Effects of elevated CO_2 and N fertilization on plant biomass. *p*-Values (in the brackets) indicate significant differences between mean values of low N and high N at the same CO_2 concentration. For the main effect of either CO2 or N fertilization, and the interaction between CO_2 and N fertilization, *p*-values indicate significant effects of that factor on these biomass variables; "NS" indicate no significant effects of a factor on biomass. dw, dry weight; Root, root biomass; Stem, stem biomass; Leaf, leaf biomass; Total, total biomass. CO_2, different CO_2 concentration treatments; N, different N fertilization treatments; CO_2 × N, the interaction of CO_2 concentration and N fertilization.

CO_2 (μmol·mol^{-1})		**Biomass (g dw)**			
		Root	**Stem**	**Leaf**	**Total**
ambient	Low N	6.77 ± 0.89	5.37 ± 0.87	6.17 ± 1.08	18.30 ±2.73
	High N	7.55 ± 0.66 (0.519)	8.63 ± 1.52 (0.135)	9.55 ± 1.52 (0.144)	25.73 ±3.60 (0.176)
550	Low N	5.50 ± 1.05	5.18 ± 0.45	5.30 ± 1.15	15.98 ±2.60
	High N	9.38 ± 1.75 (0.130)	7.10 ± 0.40 (0.063)	10.13 ± 0.52 (0.019)	26.62 ± 2.83 (0.05)
750	Low N	5.42 ± 0.83	5.85 ± 0.81	5.53 ± 0.49	16.80 ± 1.35
	High N	11.70 ± 1.70 (0.30)	12.42 ± 2.65 (0.077)	12.17 ± 2.44 (0.056)	36.28 ± 6.51 (0.043)

Table 1. *Cont.*

CO_2 (μmol·mol^{-1})		**Biomass (g dw)**			
		Root	**Stem**	**Leaf**	**Total**
1000	Low N	6.98 ± 1.02	5.95 ± 1.2	6.12 ± 0.91	19.05 ± 2.96
	High N	13.60 ± 1.94	12.45 ± 0.60	13.27 ± 0.15	39.32 ± 1.32
		(0.039)	(0.008)	(0.014)	(0.003)
CO_2		NS	NS	NS	NS
N		<0.001	<0.001	<0.0001	<0.0001
CO_2 × N		NS	NS	NS	NS

3. Discussion

Many studies have shown that eCO_2 caused a reduced N concentration, a decrease in g_s and an increase of starch accumulation [22,23], all of which were all confirmed by this present study. In this study, eCO_2 had a significant negative effect on leaf N in both low and high N treatments, and high N markedly increased leaf N compared to low N in all CO_2 treatments, which partially confirmed our first hypothesis that the reduction of leaf N would be much greater at 1000 μmol·mol^{-1} CO_2 than at 700 μmol·mol^{-1} CO_2. The reduction rate of leaf N induced by eCO_2 under high N treatments was quicker and the slope was larger than that under low N treatments (Figure 1). The possible reasons for leaf N decline in low N treatments include less N available [24] and CO_2 inhibition of nitrate assimilation [25]. While the rapid reduction of leaf N by eCO_2 in high N treatments could be interpreted as a dilution effect [26,27], because high N highly stimulated plant biomass (Figure 4). The study conducted in tropical China showed that eCO_2 increased leaf N of *S. superba*, while N fertilization had no effect leaf N [28], which was contrasted with our results that N fertilization affected the response of leaf N to eCO_2. The different responses of *S. superba* to eCO_2 and N fertilization between the two studies were attributed to N availability. At tropical site in China, N was not a limiting factor due to high ambient N deposition [28], while, in our experimental site, plants were constrained by N and P [29].

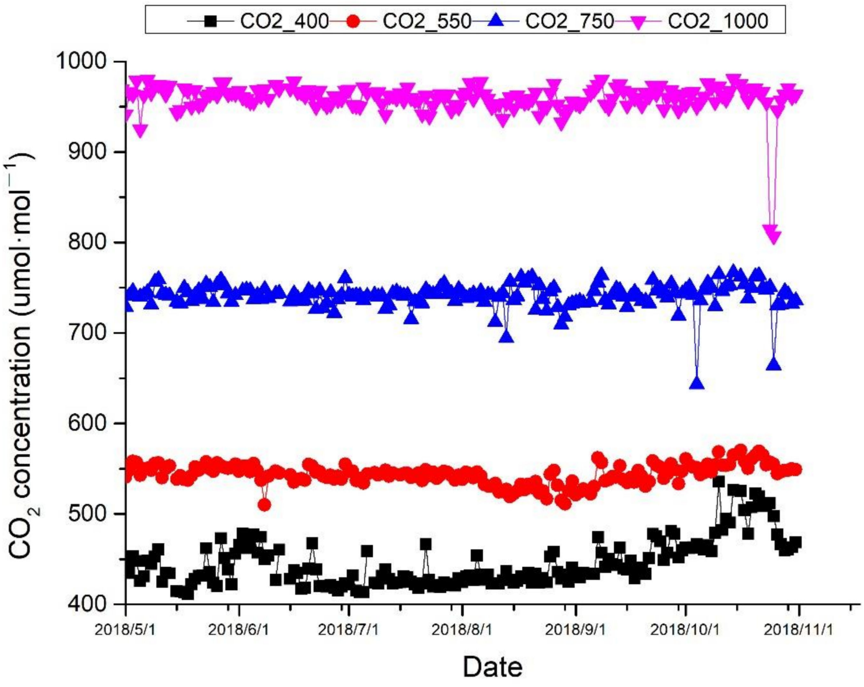

Figure 4. The actual CO_2 concentration at different treatments during the growth season.

The reduction of leaf N caused by eCO_2 led to imbalance of C and N, and the C:N ratio increased with the increase of CO_2 concentration. While, it is pivotal to maintain the C:N ratio for various growth and development processes in plants productivity [22].

N fertilization alleviated the effects of eCO_2 on imbalance of C and N. Although eCO_2 could still increase leaf C:N under high N treatments, the slope of linear regression across CO_2 concentration was much smaller than that under low N treatments (Figure 1). The increment of C:N ratio could increase a large accumulation of carbohydrates [30–32] that cannot be used for structural growth, which was also confirmed in this present study that starch content was significantly accumulated under higher CO_2 concentration in both low N and high N treatments, and the slope of high N treatment was much lower than that of low N (Figure 1). Conversely, lower starch concentrations in high N seedlings than in low N seedlings imply that N fertilization mitigate the inhibition of growth by eCO_2 because starch accumulation is a passive response to decreased rates of growth [33]. This was confirmed by the plant biomass in our study, which was not affected by eCO_2, and the plant biomass was greatly increased when N was applied at high CO_2 concentration (Figure 4).

Although there are exceptions to the general rule that g_s declines under eCO_2, in Free Air CO_2 Enrichment experiments, g_s was reduced by 22% on average at eCO_2 without any significant change in stomatal density, indicating that the change in stomatal aperture rather than density determines the response of g_s to eCO_2 [34]. Our study also confirmed that g_s was linearly decreased by eCO_2 in both low N and high N treatments. Compared with low N, high N increased the slope and intercept of the relationship between g_s and CO_2 concentration relationship, indicating that the responses of g_s of *S. superba* seedlings to eCO_2 was modified by N fertilization as expected in hypothesis 2.

Photosynthetic stimulation was maintained despite stomatal conductance was decreased especially in high N treatments, which is consistent with the previous finding [35]. The first hypothesis that Pn would be reduced much more at 1000 $\mu mol \cdot mol^{-1}$ CO_2 than at 700 $\mu mol \cdot mol^{-1}$ CO_2 was well verified in high N treatments, while, in low N treatments, Pn would decrease when CO_2 concentration exceeded 733 $\mu mol \cdot mol^{-1}$. Pn of *S. superba* showed downward trend in the whole measurement time, with September < August < July, which could be due to the change of environmental factors, such as the highest temperature in August and the lowest humidity in September in open top chambers (OTCs). Different from our findings that eCO_2 increased Pn in most treatments, Pn of *S. superba* was decreased by eCO_2 in both subtropical [20] and tropical sites [21]. The variation of Pn responses of the same species may be caused by the measurement conditions. In fact, in our experiment, Pn was measured in an OTC with the same CO_2 concentration corresponding to the treatment, whereas, in the previous studies, Pn was measured in ambient air without considering experimentally set CO_2 concentration [20,21]. When plants growing in eCO_2 were measured in ambient air, the change of background CO_2 concentration may lead to underestimation of Pn. In this study, Pn of low N treatments was linearly increased by eCO_2 only in July, while Pn of high N treatments increased linearly throughout July, August, and September, indicating that N modified the response of Pn to eCO_2, which confirmed our second hypothesis. This was different to the findings of the previous study [28]; that is, N fertilization did not affect the effects of eCO_2 on photosynthesis of *S. superba* seedlings. This is due to N-limiting in our study site and not N-limiting in their study, which was clarified when discussing the different responses of leaf N of *S. superba* seedlings to eCO_2 and N fertilization above. In August and September, Pn increased and then decreased with elevation of CO_2 in low N treatments. Interestingly the maximum of Pn was both under 733 $\mu mol \cdot mol^{-1}$ CO_2, although the regression relationship was different (Figure 3), which may indicate that 733 $\mu mol \cdot mol^{-1}$ CO_2 was a certain threshold concentration for the growth of *S. superba* without N fertilization, while there was no threshold of CO_2 concentration for the positive linear correlation between CO_2 concentration and Pn when N was applied.

Although it is commonly reported that eCO_2 increase the rate of photosynthesis, low N availability could limit the enhancement of biomass accumulation [36]. In this study site, the soil total N content was 0.5%, which was relatively low. It was confirmed in this study that low N availability limited biomass accumulation and even eCO_2 reaching 1000 $\mu mol \cdot mol^{-1}$ could not increase each organ and total biomass. Compared with plants treated in low N, when plants exposed to eCO_2, high N could alleviate low N availability and significantly increase total biomass. N fertilization also increased the biomass of root, stem and leaf when plants were exposed to 1000 $\mu mol \cdot mol^{-1}$ CO_2. Other studies related to *S. superba* have also obtained similar results: eCO_2 had no effect on biomass, N fertilization promoted the growth [21] and biomass accumulation, and eCO_2 and N fertilization had a synergistic effect on biomass [37].

4. Materials and Methods

4.1. Experimental Site and Design

The experiments were carried out in open top chambers (OTCs) of 2 m in diameter and 2.2 m in height, which was used to study the effects of elevated O_3 on plants in the past few years, and the details of the OTCs could be found in Chen et al. [38] and Yu et al. [39]. These field OTCs were set up in 2013, located in the Qianyanzhou ecological station of the Chinese Academy of Sciences (115°04′13″E, 26°44′48″N), with a subtropical monsoon climate. The mean annual temperature, mean annual precipitation, and mean relative humidity was 17.8 °C, 1471.2 mm, and 83%, respectively. Soil of the region, weathered from red sandstone and mudstone, is classified as Typic Dystrudepts Udepts Inceptisols using US soil taxonomy [40].

Four different CO_2 treatments were set according to RCPs of IPCC, as ambient air (about 400 $\mu mol \cdot mol^{-1}$), 550 $\mu mol \cdot mol^{-1}$, 750 $\mu mol \cdot mol^{-1}$, and 1000 $\mu mol \cdot mol^{-1}$, with three respective OTCs for each, and in total 12 OTCs. The concentration of CO_2 inside the OTCs was monitored with an CO_2 analyzer (FGD2-C-CO_2, Shenzhen Xin Hairui Science and Technology Development Co., Ltd., Shenzhen, China). The actual CO_2 concentration was shown in Figure 4. In each OTC, there were two nitrogen fertilization treatments, no nitrogen fertilization (low N), and 10 $g \cdot m^{-2} \cdot yr^{-1}$ (high N). In total, there were eight treatments, and, for each treatment, there were five replicated seedlings and a total of ten seedling in each OTC. The mean light intensity in OTCs was 25,050 Lux, and the mean air temperature and humidity was 26.56 °C and 83.6% in OTCs, during the experiments.

4.2. Plant Growth

One-year-old seedlings of *S. superba* were transplanted to flower pots (diameter: 30 cm) containing local soil under ambient air condition in April 2018. The baseline soil nutrients were analyzed before the experiment, and the soils contained organic matter content of 8.63 $g \cdot kg^{-1}$, total nitrogen of 500 $mg \cdot kg^{-1}$, available p of 1.58 $mg \cdot kg^{-1}$, available K of 20.1 $mg \cdot kg^{-1}$, and soil pH was 4.70. In the end of April, the seedlings with similar height and basal stem diameter were selected and moved in to OTCs. And seven days later, the plants were fumigated with ambient or eCO_2 air. In each OTC, five plants were selected to receive nitrogen fertilization, and, on June 15, July 20, August 24, and September 20, 2018, 150 mL of KNO_3 solution (6.8 $g \cdot L^{-1}$) per pot was added. The same volume of tap water was applied to the control plants as low N treatment at each time. The seedlings were watered with tap water as needed during the experiment.

4.3. Sampling, Physiological and Biochemical Measurements

To investigate the responses of *S. superba* to eCO_2 and N fertilization, two last years′ fully expanded leaves (third to fifth leaf position from the apex) of the main stem per seedling, and these two leaves were developed before treatments began. Two seedlings per treatment were randomly selected to measure gas exchange parameters. Gas exchange parameters were measured once a month from July to September. On 26 October 2018, about five last year′s fully expanded leaves (third to eighth leaf position from the apex) of the main stem per seedling and three seedlings per treatment in each OTC were randomly selected to determine plant nutrient. Finally, two seedlings without sampling leaves were harvested, and different tissues were dried at 70 °C to constant weight for dry biomass determination.

4.3.1. Plant C, N, and Carbohydrates

Oven-dried samples of different tissues were grounded to powder through a 2-mm sieve for C, N, and carbohydrates analyses. Plant N was decided by an automated Kjeldahl apparatus (KD310, Opsis, Sweden). To extract soluble sugars, powdered leaf (0.5 g, dry weight (dw)) was added to 50 mL distilled water, and then high-pressure steamed for 2 h. Starch was extracted with 0.1 g (dw) powdered material added 10 mL distilled water and 1 mL hydrochloric acid (2:1), and then incubated at 100 °C in a water bath for 8 h. After chilling to room temperature, the mixture was adjusted to neutral pH with 40% NaOH solution. Both of the mixtures were filtrated and diluted to a constant volume at a room temperature. Carbohydrates were represented by water-soluble sugar and starch. Carbohydrates were determined by injection of 10-uL sample volume into a high-performance liquid chromatography system using a Sugar-Pak 1 chromatographic column and a refractive index detector (Waters HPLC 2695, Milford, MA, USA). The column temperature was 70 °C, and distilled water was used as mobile phase (flow rate 0.6 mL/min).

4.3.2. Gas Exchange Measurement

Gas exchange was determined by a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The system controlled photosynthetically active radiation (PAR) at light-saturating 1000 $\mu mol \cdot m^{-2}\ s^{-1}$ using 6400-2B red/blue light-emitting diode (LED) light source. The block temperature was set to the ambient average (25–30 °C). Relative humidity was controlled at 50–65%. The parameters were measured in OTC with ambient or elevated CO_2 between 9:00 a.m. and 11:30 a.m. The air temperature was 29.2, 32.6, and 31.1 °C, and relative humidity was 81.4%, 71.7%, and 66.2%, respectively, in July, August, and September in OTCs before gas-exchange data collection. The mean light intensity was 26533, 28333, and 30,033 Lux in July, August, and September in OTCs before data collection. The gas exchange parameters included photosynthate rate (Pn, $\mu mol \cdot m^{-2}\ s^{-1}$), stomatal conductance (g_s, $\mu mol \cdot m^{-2}\ s^{-1}$).

4.4. Statistics

CO_2 treatment means were statistically compared, respectively, at low N and high N using the statistical package SPSS (SPSS Inc., Chicago, IL, USA) for leaf nutrition. The relationship between CO_2 concentration and means of leaf N, starch, soluble sugar, and C:N were analyzed using Pearson correlation, respectively, at low N and high N. Pearson correlation was also used to determine the relationship between CO_2 concentration and Pn and g_s. If there was significant correlation, the linear equation was generated by OriginPro 9.0. While, for Pn at low N in August and September, quadratic regression equation was used to explain the relationship between Pn and CO_2 concentration, biomass was analyzed by using one-way ANOVA, Tukey's HSD to determine the effects of CO_2 treatments, respectively, at low N and high N, and the effects of N fertilization were determined by *t*-test at each CO_2 level. The single and internal effects of eCO_2 and N fertilization on leaf nutrition and biomass, and eCO_2, N fertilization, and measuring date on Pn and g_s were determined by a multi-way ANOVA. All the figures were produced by OriginPro 9.0.

5. Conclusions

The responses of *S. superba* to eCO_2 are mediated by N fertilization. Although N fertilization increased leaf N of *S. superba*, leaf N was decreased by eCO_2 in both low N and high N, and N fertilization accelerated this reduction with larger slope of eCO_2 and leaf N relationship. High N decreased leaf C:N compared to low N, and the slope and intercept of leaf C:N and eCO_2 relationship were both decreased, indicating N fertilization mitigated the negative effects of eCO_2 on the imbalance of leaf C and N. N fertilization also modified the responses of Pn and g_s to eCO_2. In low N treatments, Pn was improved with CO_2 concentration increasing in July, while, in August and September, Pn was increased and then decreased by eCO_2 with a threshold of 733 $\mu mol \cdot mol^{-1}$ CO_2, and, in high N treatments, Pn was consistently increased by eCO_2 in all of July, August, and September. Through these ways, N fertilization application in this low N availability site significantly accumulated the biomass of the *S. superba* seedlings. Although this present study lasted only one growth season, the results indicated that global change with multi-factors, like both eCO_2 and N deposition, could have stronger effects on growth of woody plants, which should be paid continuous attention.

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