Nitrogen Fertilization Modified the Responses of *Schima superba* Seedlings to Elevated CO₂ in Subtropical China

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**Abstract:** There are very few studies about the effects of relatively higher CO₂ concentration (e.g., 1000 µmol·mol⁻¹) or plus N fertilization on woody plants. In this study, *Schima superba* seedlings were exposed to ambient or eCO₂ (550, 750, and 1000 µmol·mol⁻¹) and N fertilization (0 and 10 g·m⁻²·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 CO₂ treatments and decreased starch, with a half reduction in leaf C:N ratio. Leaf N was significantly decreased by eCO₂ 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 eCO₂. In low N treatments, photosynthetic rate (Pn) was maximized at 733 µmol·mol⁻¹·CO₂ in August and September, while, in high N treatments, Pn was continuously increased with elevation of CO₂. N fertilization significantly increased plant biomass especially at highly elevated CO₂, although no response of biomass to eCO₂ alone. These findings indicated that N fertilization would modify the response of *S. superba* to eCO₂.

**Keywords:** elevated CO₂; N fertilization; leaf N; photosynthesis; biomass

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1. Introduction

Elevated atmospheric CO₂ concentration (eCO₂) and N deposition are always important issues in the research of global change. Until today, amounts of previous studies reported the effects of eCO₂ alone or eCO₂ combined with N deposition on plants [1–6]. While, according to the IPCC (Intergovernmental Panel on Climate Change) Fifth Evaluation Report, the CO₂ concentrations will be between 430–480, 580–720, 720–1000 µmol·mol⁻¹, and more than 1000 µmol·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]. N₂O emission will also increase with increasing CO₂ concentration, and will reach up to more than 20 T·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 NOₓ 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 CO₂ concentration (about 700 µmol·mol⁻¹) on plants, which could not predict the potential responses of the plants to the future CO₂ concentration based on RCP6.0 and RCP8.5. Although a few
reports studied the effects of the higher CO₂ 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 CO₂ combined with N fertilization on woody plants.

This study aimed to explore the responses of native tree species to highly elevated CO₂ 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 eCO₂ (about 700 μmol·mol⁻¹) and N deposition [18–20]. Photosynthetic rate of *S. superba* seedlings was reduced, while height growth was enhanced by eCO₂, after the exposure of 6 months [20]. Although eCO₂ did not affect photosynthetic rate in high N treatment, *S. superba* grown in ambient N treatment had 23% and 47% greater photosynthetic rate (Pn) in ambient CO₂ than those in eCO₂ after exposure of 20 and 31 months, and leaf N was also reduced by eCO₂ in both low and high N treatment [21]. If this species similarly responds to high CO₂ concentration as to about 700 μmol·mol⁻¹ CO₂ in previous studies, how would it respond to high CO₂ and N fertilization? In this present study, we tried to explore the responses of gas exchange, carbohydrates and biomass to different levels of CO₂ concentration and N fertilization. We hypothesized that: 1) Pn, leaf N would be reduced much more at 1000 μmol·mol⁻¹ CO₂ than at 700 μmol·mol⁻¹ CO₂; and 2) nitrogen fertilization would affect the response of *S. superba* to eCO₂.

2. Results

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

Leaf N was decreased with CO₂ concentration increasing at both low N and high N treatments (Figure 1, R² = 0.9968, p = 0.003 and R² = 0.8798, p = 0.034, respectively). High N significantly improved leaf N (p ≤ 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 CO₂. Although soluble sugar content was not significantly affected by elevated CO₂ at low N, while it was linearly decreased with CO₂ enrich at high N (Figure 1, R² = 0.8580, p = 0.016). The starch content was linearly increased by eCO₂ 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 CO₂. 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 CO₂ concentration under N fertilization (Figure 1). Although leaf C:N was both increased with CO₂ 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 gs, both decreased from July to September (Figures 2 and 3). In July, Pn was increased with CO₂ enrich at both low N and high N, with greater slope at high N (Figure 2). Pn was also linearly increased by eCO₂ 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 CO₂ and then decreased when CO₂ concentration continuously increased (Figure 2). Pn from plants exposed to 1000 μmol·mol⁻¹ CO₂ at high N was increased of 73.68%, 119.90%, and 106.78%, and 118.38%, 116.68%, 106.78%, and 138.95% compared to low N, while the increment at high N was slower than that at low N (Figure 1). CO₂ and N, CO₂ and measuring time, N and measuring time, while no significant impacts of CO₂, N and measuring time on Pn. No matter under low N or high N, gs, was significantly negatively correlated with eCO₂ 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, gs 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 gs, 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.
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).

| 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    | 8.63 ± 1.52     | 9.55 ± 1.52     | 25.73 ± 3.60     |
|                            | (0.519)        | (0.135)         | (0.144)         | (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    | 7.10 ± 0.40     | 10.13 ± 0.52    | 26.62 ± 2.83     |
|                            | (0.130)        | (0.063)         | (0.019)         | (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   | 12.42 ± 2.65    | 12.17 ± 2.44    | 36.28 ± 6.51     |
|                            | (0.30)         | (0.077)         | (0.056)         | (0.043)          |

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 CO\(_2\) or N fertilization, and the interaction between CO\(_2\) and N fertilization, \(p\)-values indicate significant effects of that factor on 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.
Table 1. Cont.

| CO₂ (µmol·mol⁻¹) | Biomass (g dw) |   |   |   |
|------------------|---------------|---|---|---|
|                  | Root          | Stem | Leaf | Total |
| 1000             | 6.98 ± 1.02   | 5.95 ± 1.2  | 6.12 ± 0.91 | 19.05 ± 2.96 |
|                  | 13.60 ± 1.94  | 12.45 ± 0.60 | 13.27 ± 0.15 | 39.32 ± 1.32 |
| 700              |               | (0.039)     | (0.008)    | (0.014)    | (0.003)    |
| 1000             |               | NS          | NS         | NS         | NS         |
|                  | NS            | NS          | NS         | NS         |

3. Discussion

Many studies have shown that eCO₂ caused a reduced N concentration, a decrease in gs, and an increase of starch accumulation [22,23], all of which were all confirmed by this present study. In this study, eCO₂ 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 CO₂ treatments, which partially confirmed our first hypothesis that the reduction of leaf N would be much greater at 1000 µmol·mol⁻¹ CO₂ than at 700 µmol·mol⁻¹ CO₂. The reduction rate of leaf N induced by eCO₂ 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 CO₂ inhibition of nitrate assimilation [25]. While the rapid reduction of leaf N by eCO₂ 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 eCO₂ 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 eCO₂. The different responses of S. superba to eCO₂ 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 CO₂ concentration at different treatments during the growth season.](image-url)

The reduction of leaf N caused by eCO₂ led to imbalance of C and N, and the C:N ratio increased with the increase of CO₂ 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 eCO₂ on imbalance of C and N. Although eCO₂ could still increase leaf C:N under high N treatments, the slope of linear regression across CO₂ 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 CO₂ 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 eCO₂ 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 eCO₂, and the plant biomass was greatly increased when N was applied at high CO₂ concentration (Figure 4).

Although there are exceptions to the general rule that gs declines under eCO₂, in Free Air CO₂ Enrichment experiments, gs was reduced by 22% on average at eCO₂ without any significant change in stomatal density, indicating that the change in stomatal aperture rather than density determines the response of gs to eCO₂ [34]. Our study also confirmed that gs was linearly decreased by eCO₂ in both low N and high N treatments. Compared with low N, high N increased the slope and intercept of the relationship between gs and CO₂ concentration, indicating that the responses of gs of S. superba seedlings to eCO₂ 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 µmol·mol⁻¹ CO₂ than at 700 µmol·mol⁻¹ CO₂ was well verified in high N treatments, while, in low N treatments, Pn would decrease when CO₂ concentration exceeded 733 µmol·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 eCO₂ increased Pn in most treatments, Pn of S. superba was decreased by eCO₂ 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 CO₂ concentration corresponding to the treatment, whereas, in the previous studies, Pn was measured in ambient air without considering experimentally set CO₂ concentration [20,21]. When plants growing in eCO₂ were measured in ambient air, the change of background CO₂ concentration may lead to underestimation of Pn. In this study, Pn of low N treatments was linearly increased by eCO₂ 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 eCO₂, 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 eCO₂ 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 eCO₂ and N fertilization above. In August and September, Pn increased and then decreased with elevation of CO₂ in low N treatments. Interestingly the maximum of Pn was both under 733 µmol·mol⁻¹ CO₂, although the regression relationship was different (Figure 3), which may indicate that 733 µmol·mol⁻¹ CO₂ was a certain threshold concentration for the growth of S. superba without N fertilization, while there was no threshold of CO₂ concentration for the positive linear correlation between CO₂ concentration and Pn when N was applied.

Although it is commonly reported that eCO₂ 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 eCO₂ reaching 1000 µmol·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 µmol·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 µmol·mol$^{-1}$), 550 µmol·mol$^{-1}$, 750 µmol·mol$^{-1}$, and 1000 µmol·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·m$^{-2}$·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·kg$^{-1}$, total nitrogen of 500 mg·kg$^{-1}$, available $p$ of 1.58 mg·kg$^{-1}$, available K of 20.1 mg·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, 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·m$^{-2}$·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.

4.3. 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 µmol·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 CO₂ 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, µmol·m⁻²·s⁻¹), stomatal conductance (gs, µmol·m⁻²·s⁻¹).

4.4. Statistics

CO₂ 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 CO₂ 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 CO₂ concentration and Pn and gs. 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 CO₂ concentration, biomass was analyzed by using one-way ANOVA, Tukey’s HSD to determine the effects of CO₂ treatments, respectively, at low N and high N, and the effects of N fertilization were determined by t-test at each CO₂ level. The single and internal effects of eCO₂ and N fertilization on leaf nutrition and biomass, and eCO₂, N fertilization, and measuring date on Pn and gs were determined by a multi-way ANOVA. All the figures were produced by OriginPro 9.0.

5. Conclusions

The responses of S. superba to eCO₂ are mediated by N fertilization. Although N fertilization increased leaf N of S. superba, leaf N was decreased by eCO₂ in both low N and high N, and N fertilization accelerated this reduction with larger slope of eCO₂ and leaf N relationship. High N decreased leaf C:N compared to low N, and the slope and intercept of leaf C:N and eCO₂ relationship were both decreased, indicating N fertilization mitigated the negative effects of eCO₂ on the imbalance of leaf C and N. N fertilization also modified the responses of Pn and gs to eCO₂. In low N treatments, Pn was improved with CO₂ concentration increasing in July, while, in August and September, Pn was increased and then decreased by eCO₂ with a threshold of 733 µmol·mol⁻¹ CO₂, and, in high N
treatments, Pn was consistently increased by eCO₂ 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 eCO₂ and N deposition, could have stronger effects on growth of woody plants, which should be paid continuous attention.

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