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#### **Research Article**

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# The combination of elevated CO<sub>2</sub> and warmer temperature reduces photosynthetic capacity without diluting leaf N concentration in Amur linden (*Tilia amurensis*)

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#### Abstract

Elevated CO<sub>2</sub> and warmer temperatures represent the future environmental conditions in the context of global change. A good understanding of plant response to their combined effects is, therefore, critically important for predicting future plant performance. This study investigated the photosynthetic acclimation of Amur linden (*Tilia amurensis*) seedlings (current year, about 60 cm tall), a shade-tolerant tree species in the temperate broadleaf deciduous forest, to the combination of current CO<sub>2</sub> concentration and temperature (CC) and the combination of the predicted future CO<sub>2</sub> concentration and temperature (FC). The results show that FC promoted aboveground growth, but reduced photosynthetic capacity ( $V_{cmax}$ : maximum rate of RuBP carboxylation and  $J_{max}$ : maximum photosynthetic electron transport rate). However, the photosynthetic rate measured under the corresponding growth CO<sub>2</sub> concentration was still higher under FC than under CC. FC depressed the photosynthetic limiting transition point ( $C_{ixt}$ ,  $A_{n,t}$ ) from Rubisco carboxylation to RuBP regeneration, i.e.  $A_{n,t}$  decreased without a change in  $C_{ixt}$ . FC did not change leaf N concentration but increased the total leaf N content per tree and photosynthetic nitrogen utilization efficiency. This suggests that N utilization, rather than photosynthetic capacity, may play an important role in the acclimation of the species to future climatic conditions. This study provides new insights into the photosynthetic acclimation of Amur linden and can be used to predict its possible performance under future climatic conditions.

*Keywords:* Amur linden (*Tilia amurensis*), photosynthetic acclimation, elevated CO<sub>2</sub> and temperature, temperate broadleaved tree, nitrogen concentration

#### CO,升高和增温没有稀释椴树叶片氮浓度却降低了叶片的光合能力

摘要:深入理解CO<sub>2</sub>升高和全球变暖对植物的影响是预测植物适应性的关键。本研究以温带阔叶耐荫 树种椴树(*Tilia amurensis*)幼苗为试验材料,研究了当前CO<sub>2</sub>浓度和气温组合处理(CC)与预测的未来气候 条件下CO<sub>2</sub>升高和增温组合处理(FC)对植物光合驯化的影响模式。结果发现,FC处理促进了椴树地上部 分的生长,降低了叶片的光合能力(最大羧化速率和最大电子传递速率),也降低了椴树光合作用生化限 制中从羧化限制向电子传递限制过渡点的光合速率。FC处理没有改变叶片氮浓度,但是提高了单株叶片

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关键词: 椴树(Tilia amurensis), 光合驯化, CO, 升高和增温, 温带阔叶树, 氮素浓度

#### INTRODUCTION

 $CO_2$  elevation and global warming will inevitably continue in the foreseeable future (Dong *et al.* 2023; Dusenge *et al.* 2020). The rising  $CO_2$ , warming temperature and nitrogen deposition are considered the most relevant environmental conditions for global change (Kim *et al.* 2021; Zheng *et al.* 2023). The combination of elevated  $CO_2$  and warmer temperature appears to be a continuous driver of environmental change (Maxwell *et al.* 2022) and controls the bioavailability of soil organic nitrogen and plant responses to global change (Hu *et al.* 2023; Liu *et al.* 2023).

There have been numerous reports in the literature on plant acclimation to increasing CO, and temperature (Ainsworth and Long 2021; Dusenge et al. 2019; Huang et al. 2021). It is generally believed that elevated CO<sub>2</sub> can reduce photosynthetic capacity, foliar nitrogen concentration and stomatal conductance (Chavan et al. 2019; Dang et al. 2021; Kurepin et al. 2018), while warmer temperature increases leaf-to-air vapor pressure deficit, specific leaf area, respiration rate and decreases  $J_{\text{max}}/V_{\text{cmax}}$ (Dusenge et al. 2021; Liang et al. 2013; Smith and Keenan 2020). However, there are inconsistent conclusions about the combined effects of elevated CO, and temperature on plants (Way et al. 2015). Some studies report synergistic effects (Ainsworth and Long 2021; Apgaua et al. 2019), while others find antagonistic effects (Dusenge et al. 2020; Noyce et al. 2019). While there are extensive studies on the effects of single factors, the ecophysiological significance of single-factor studies is limited under natural environmental conditions where multiple factors change simultaneously (Dong et al. 2023; Kizildeniz et al. 2021).

Climate change continues to impact forests, particularly boreal and temperate forests (Dusenge *et al.* 2020; Kurepin *et al.* 2018; Liu *et al.* 2023). Although temperate forests are one of the most well-studied forest types, it is still not well understood how well broadleaved deciduous tree species will be able to cope with the rapidly increasing CO<sub>2</sub> concentration and climate warming (Helcoski *et al.* 2019). Climate

change is altering fundamental physiological processes such as photosynthesis and respiration which affect tree growth and survival, and trees will need to acclimate to new climatic conditions physiologically and/or morphologically to persist at their current sites (Kramer et al. 2020). Furthermore, past studies on climate change are primarily focused on the responses of commercial tree species, such as Poplar, Betula, Quercus and Maple, to individual climate or environment factors (Dang et al. 2021; Gardner et al. 2021; Way et al. 2015). In reality, however, multiple factors change simultaneously, e.g., the elevation of CO, is coupled with warming. There are generally variations in response among different tree species and such variations will influence the responses of tree communities (Raizada et al. 2009; Smith et al. 2013). Therefore, a good understanding of the responses of different tree species, both commercial and noncommercial, to the combination of elevated CO<sub>2</sub> and warming may serve as a basis for assessing forest responses to climate change (Pastore et al. 2019).

Genus Tilia represents a major group of shadetolerant tree species in temperate broadleaved deciduous forests (Aasamaa and Aphalo 2017). These species provide not only an important source of nectar but also timber and ornamental trees (Baczewska-Dąbrowska et al. 2022; Kang et al. 2021). Tilia has very different ecophysiological traits than other temperate broadleaved deciduous tree species. For example, most of the photosynthates in Tilia are used in the synthesis of new tissues or lipids (triacylglycerols) stored in the wood (Keel et al. 2007) while the increased photosynthates in other broadleaved deciduous tree species are mainly used for the growth of new tissues in the aboveground organs (Wang et al. 2022). While the effects of elevated CO<sub>2</sub> on the physiological traits of Tilia species have been examined (Li et al. 2019), the photosynthetic responses of Tilia species to a combination of elevated CO<sub>2</sub> and higher temperature, a scenario of climate change, still have not been experimentally investigated. This study investigated the photosynthetic and growth responses of Amur

linden seedlings to a combination of elevated  $CO_2$ and warmer temperatures. Since the availability of light is the primary limit to trees growing under shade, the ability to expand leaf area is critical for the survival and growth of shade-tolerant tree species. We test the hypothesis that Amur linden will increase photosynthate production under elevated  $CO_2$  and warmer temperatures and that the increase in photosynthate production will lead to an increase in the total leaf area and biomass production.

#### MATERIALS AND METHODS

#### Plant materials and treatments

Amur linden (*Tilia amurensis* Rupr.) seeds were collected from 10 trees about 100 m apart from each other in a natural forest in the Changbai Mountain forest region, China (42.1° N, 127.5° E). Seeds were germinated in germination trays filled with a mixture of vermiculite and peat moss (1:1 in v:v) (Sun Gro<sup>®</sup> Peat Moss Grower Grade Green and Vermiculite Premium Grade, Sun Gro Horticulture, Agawam, USA) in a greenhouse at Lakehead University (Thunder Bay campus, ON, Canada). The environmental conditions during the 3-week germination period were 25°C/16°C day/night temperatures and 16-h photoperiod.

Three weeks after germination, seedlings were transplanted into 3.5 L plastic pots filled with a vermiculite-peat moss mixture (1:1 in v:v). There were two combination treatments: the current climate condition (CC: 400 µmol mol<sup>-1</sup> CO<sub>2</sub> and current temperature) and the predicted future climate condition (FC: 750 µmol mol<sup>-1</sup> CO<sub>2</sub> and elevated temperature (current + 4°C)). Each of the two combination treatments was independently replicated twice. The treatments were randomly assigned to four independent greenhouses (2 treatments  $\times 2$  replicates per treatment = 4). There were 12 seedlings in each treatment replicate. Highpressure sodium lamps (Signify Canada Ltd, 281 Hillmount Road, Markham, ON L6C 2S3, Canada) were used to supplement the natural light when the flux density of natural light was below 500 µmol m<sup>-2</sup> s<sup>-1</sup>. The day/night temperatures for the CC treatment were 25°C/16°C while those for the FC were 29°C/20°C. The photoperiod was 16 h for both treatments. 25°C/16°C temperatures and 16-h period are optimal for the photosynthesis and growth of the species. The seedlings were irrigated every two days to the drip point (about 500 mL water) and fertilized twice a week using a fertilizer solution of 150–60–150 µmol mol<sup>-1</sup> N–P–K with 4 mL L<sup>-1</sup> Micro pH Perfect Fertilizer (Advanced Nutrients, 8687 Melrose Ave, Suite G320, West Hollywood, CA 90069, USA). Each greenhouse is equipped with a stirring fan to ensure uniformity in environmental conditions at different locations. Furthermore, the positions of seedlings were rotated weekly within the same greenhouse. The design and orientation of the four greenhouses are identical and the environmental conditions of each greenhouse were independently monitored and controlled using an Argus Titan Environment-control system (Argus Control Systems Ltd, Vancouver, BC, Canada). The elevated [CO<sub>2</sub>] was achieved by a CO, generator (model GEN-2E; Custom Automated Products Inc., Riverside, CA, USA).

#### **Biomass and leaf nitrogen measurements**

The height of all the seedlings was measured after 3-month treatment. The seedlings were then removed from the pot and separated into roots, stems and leaves. The total leaf area for each seedling was scanned and estimated using the Regent WinFolia system (Regent Instruments Inc., Quebec City, QC, Canada). The roots were washed off the growing media. All the materials were then oven-dried at 75°C for 48 h to determine the total biomass, leaf mass, shoot mass and root mass. The root/shoot ratio, leaf mass ratio (LMR = leaf mass/total biomass), shoot mass ratio (SMR = stems mass/total biomass) and root mass ratio (RMR = root mass/total biomass) were calculated.

Leaf N concentration was analyzed using the dry combustion method with a CNS-2000 (LECO Corp., St. Joseph, MI, USA) at the Lakehead University Centre for Analytical Services. The leaf-area-based N concentration ( $N_{\rm area}$ , g m<sup>-2</sup>) was calculated by dividing the mass-based leaf N concentration ( $N_{\rm mass'}$  mg g<sup>-1</sup>) by the specific leaf area. The total leaf nitrogen content per plant ( $N_{\rm leaf'}$  mg plant<sup>-1</sup>) was calculated as the product of  $N_{\rm mass}$  (mg g<sup>-1</sup>) and total leaf mass per tree. The photosynthetic nitrogen use efficiency (PNUE, µmol g<sup>-1</sup> N s<sup>-1</sup>) was the ratio of net photosynthesis rate ( $A_{\rm n}$ ) and  $N_{\rm area}$  ( $A_{\rm n}/N_{\rm area}$ ).

# $A/C_{i}$ curves measurement and calculations of $V_{cmax}$ and $J_{max}$

Three seedlings were randomly selected from each replicate of each treatment after 2 months of treatments for measuring photosynthetic  $CO_2$  response curves ( $A/C_i$  curves). The measurements were taken on the first fully expanded leaf from the

top at 400, 300, 200, 150, 100, 50, 400, 500, 750, 900, 1100 and 1300 µmol mol<sup>-1</sup> CO<sub>2</sub> ( $C_a$ ) using a PP-Systems CIRAS-3 Portable Photosynthesis System equipped with a PLC3 Universal Leaf Cuvette with automatic environment control and a built-in CFM-3 Chlorophyll Fluorescence Module (PP Systems International, Inc., Amesbury, MA, USA). The first measurement was taken at the treatment CO<sub>2</sub> for both treatments. The measurement conditions were set at 25°C air temperature, 50% relative humidity (RH) and 800 µmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation (PAR). It generally took 2–5 min to achieve a stable reading at each CO<sub>3</sub>.

The maximum electron transport rate  $(J_{max'} \mu mol m^{-2} s^{-1})$  and maximum Rubisco carboxylation rate  $(V_{cmax'} \mu mol m^{-2} s^{-1})$  of each sample leaf were estimated using the Plantecophys fitaci function of the R package (Duursma 2015). The initial slope of the  $A/C_i$  curve (linear part) was taken as the apparent carboxylation efficiency (ACE), and the CO<sub>2</sub> compensation point (CCP<sub>aci</sub>) was estimated from the *x*-axis intercept of the  $A/C_i$  curve. The transition point  $(C_{i\cdotl}, A_{n\cdot l})$  of the photosynthesis limitation from Rubisco carboxylation to RuBP regeneration was obtained using the findCiTransition function of plantecophys R (Duursma 2015).

The Laisk dataset was measured at 200, 150, 100 and 50 µmol mol<sup>-1</sup> [CO<sub>2</sub>] and 300, 150 and 75 µmol m<sup>-2</sup> s<sup>-1</sup> PAR for estimating intercellular CO<sub>2</sub> compensation point ( $C_i^*$ ) and daytime respiration ( $R_d$ ) (Farquhar *et al.* 1980). The rate of electron transport (*J*) was estimated from the chlorophyll fluorescence measurement. The variable *J* method was employed to calculate the mesophyll conductance  $g_m$  (Peter *et al.* 1992), which assumed that  $C_i^*$  (intercellular CO<sub>2</sub> compensation point) was equal to  $\Gamma^*$  (CO<sub>2</sub> compensation point in the chloroplast) according to Momayyezi's protocol (Momayyezi and Guy 2017).

#### **Statistical analyses**

The effects of  $CO_2$  and temperature combined treatment were analyzed using ANOVA. The treatment was considered as fixed effects while replicates were considered as random effects in the analysis, and the sampling unit (tree seedling) was nested within each treatment replicate. Before the ANOVA, the normality distribution and homogeneity of the data were examined using probability plots for residuals. Log transformation was used when the distribution was not normal. The physiological and biomass parameters with a significant difference were subjected to principal component analysis (PCA) using the PCA function from the FactoMineR package. All the analyses were performed using the R Package 4.2.1.

#### RESULTS

#### Growth and photosynthetic traits

Seeding biomass, height, total leaf area per tree and SMR were greater under FC (Table 1; Fig. 1).  $V_{cmax'}$   $J_{max'}$   $g_m$  and  $R_d$  were lower in FC than CC (Fig. 2d and e), whereas iWUE and  $C_i^*$  were higher (Fig. 2c and f). PCA revealed that the seedlings exposed to FC were primarily grouped to growth and CO<sub>2</sub> compensation point (dominated by biomass,  $N_{leaf'}$  CCP<sub>aci</sub> and  $C_i^*$ ) on the right ellipse of PC1 (Fig. 3), while the seedlings in the CC treatment were mainly clustered in the left ellipse dominated by  $A_{n-t}$  and  $g_m$  (Fig. 3).

#### Features of $A/C_{i}$

The transition point  $(C_{i-t'} A_{n-t})$  was lowered by the FC treatment (Fig. 4).  $A_{n-t}$  was lower in FC than in CC but the difference in  $C_{i-t}$  was not statistically significant (Table 1; Fig. 4b). Photosynthetic rate measured at growth CO<sub>2</sub>  $(A_{n-g})$  was higher in FC than in CC (Fig. 4). The FC treatment increased the CO<sub>2</sub> compensation point estimated from  $A/C_i$  curves (CCP<sub>aci</sub>) but reduced the ACE (Table 1; Fig. 4).

#### Leaf N content and concentration

Neither mass-based  $(N_{\text{mass}})$  nor area-based  $(N_{\text{area}})$  leaf N concentration was significantly affected by the FC treatment (Fig. 5). However, the total leaf N content per seedling  $(N_{\text{leaf}})$  and photosynthetic N use efficiency (PNUE =  $A_{\text{n-g}}/N_{\text{area}}$ ) were increased in FC (Fig. 5d).

#### DISCUSSION

#### Growth and photosynthesis acclimation

In general, a higher carboxylation capacity is a physiological mechanism to compensate for the deficient  $CO_2$  supply under current  $CO_2$  conditions in C3 plants and the photosynthetic capacity generally declines in response to elevated  $CO_2$ . Amur linden seedlings in this study demonstrated both morphological (e.g. total leaf area) and physiological (e.g.  $CCP_{aci}$ ) acclimation to the FC treatment. The reduction in leaf-area-based photosynthetic capacity in FC was accompanied by an increase in total leaf area per tree, resulting in greater seedling

Variables	F	Р	Variables	F	Р
Biomass	56.632	<0.001	$V_{ m cmax}$	18.156	0.002
RMR	0.539	0.482	$J_{\max}$	6.672	0.03
SMR	16.787	0.003	$J_{\rm max}/V_{\rm cmax}$	0.002	0.964
LMR	0.112	0.746	$C_{\rm i-t}$	0.754	0.408
Leaf area	35.677	<0.001	$A_{n-t}$	27.195	<0.001
Height	28.965	<0.001	${\mathcal{G}}_{\mathrm{m}}$	35.404	<0.001
$A_{\text{n-g}}$	6.569	0.028	$R_{\rm d}$	7.498	0.023
$N_{\rm area}$	2.181	0.174	$C_{i}^{*}$	49.68	<0.001
$N_{ m mass}$	0.014	0.907	ACE	5.903	0.038
$N_{ m leaf}$	53.273	<0.001	CCP <sub>aci</sub>	29.082	<0.001
PNUE	10.6	0.01	iWUE	113.1	<0.001

**Table 1:** ANOVA *P* values for the effects of CO<sub>2</sub> and temperature combination (CC *vs.* FC) on the growth, and photosynthesis of Amur linden seedlings

Abbreviations:  $A_{n-g}$  = net photosynthesis rate at corresponding growth  $C_a$ ,  $A_{n-1}$  = net photosynthesis rate at  $C_{i-t}$ ,  $CCP_{aci} = CO_2$  compensation point from  $A/C_i$  curve,  $C_i^*$  = intercellular  $CO_2$  compensation point,  $C_{i-t}$  = intercellular  $[CO_2]$  at the transition point ( $C_{i-t}$ ,  $A_{n-1}$ ) between Rubisco limitation and RuBP regeneration limitation based on  $A/C_i$  curve,  $N_{area}$  = leaf N per unit area,  $N_{leaf}$  = total N of the whole-plant leaf,  $N_{mass}$  = leaf N concentration,  $R_d$  = daytime respiration. Significant effects ( $P \le 0.05$ ) are shown in bold.



**Figure 1:** Effects of CO<sub>2</sub> and temperature combination treatment (CC *vs.* FC) on biomass (a), height (b), leaf area (c) and stem mass ratio (d). Means ( $\pm$ SE, *n* = 6) with different letters were different from each other (*P* ≤ 0.05).

growth and higher nitrogen use efficiency in the FC treatment. While temperate broad-leaf tree species, e.g. *Fagus* and *Quercus*, tend to retain a significant portion of new photosynthates in the leaf,

Tilia species generally translocate nearly all newly synthesized photosynthates to sinks to produce new tissues and storage lipid (triacylglycerol) in wood (Keel et al. 2007), which helps to explain the positive relationship between seedling biomass, leaf area and photosynthetic rates in response to the FC treatment. The conclusion was further corroborated by the results of the PCA that seedling biomass was positively associated with  $N_{\text{leaf'}}$   $C_i^*$  and  $\text{CCP}_{\text{aci}}$ . These findings support our hypothesis that Amur linden will increase photosynthate production under elevated CO<sub>2</sub> and warmer temperatures and that the increase in photosynthate production will lead to an increase in the total leaf area and biomass. Our results are consistent with the conclusion of Smith and Keenan (2020) that elevated CO<sub>2</sub> increases the proportion of photosynthates used for the synthesis of new tissues and growth.

#### A/C<sub>i</sub> curve patterns

 $A/C_{\rm i}$  curves can be used to calculate  $V_{\rm cmax}$  and  $J_{\rm max}$ , and to obtain the transition point  $(C_{\rm i-t}, A_{\rm n-t})$  of photosynthetic limitation from the Rubisco carboxylation to RuBP regeneration, CCP<sub>aci</sub> and ACE (Fernández-Marín *et al.* 2020). It should be noted that photosynthetic capacity  $(V_{\rm cmax} \text{ and } J_{\rm max})$  is the potential maximum



**Figure 2:** Effects of CO<sub>2</sub> and temperature combination treatment on  $V_{cmax}$ : maximum rate of Rubisco carboxylation (a),  $J_{max}$ : maximum rate of photosynthetic electron transport rate (b), iWUE: instantaneous water use efficiency with instantaneous photosynthetic (c),  $g_m$ : mesophyll conductance (d),  $R_d$ : daytime respiration (e), and  $C_i^*$ : intercellular CO<sub>2</sub> compensation point (f). Means (±SE, n = 6) with different letters are different from each other ( $P \le 0.05$ ).



**Figure 3:** PCA (using FactoMineR package in R) of growth and photosynthetic parameters with significant treatment effects (CC vs. FC). The polygons indicate treatment clustering. Abbreviations:  $A_{n-i}$  = net photosynthesis rate at the transition point between Rubisco limitation and RuBP regeneration limitation based on  $A/C_i$  curve,  $CCP_{aci} = C_i$  compensation point from  $A/C_i$  curve,  $C_i^*$  = intercellular CO<sub>2</sub> compensation point,  $g_m$  = mesophyll conductance,  $J_{max}$  = maximum of photosynthetic electron transport rate,  $N_{leaf}$  = total N of the whole-plant leaf,  $R_d$  = daytime respiration,  $V_{cmax}$  = maximum rate of ribulose-1,5-bisphosphate carboxylation. See Table 1 for other explanations.

rate while the actual rate of photosynthesis is the portion of the photosynthetic capacity i.e. realized under specific physiological and environmental conditions, such as the availability of substrates, the activation state of enzymes activation, light and temperature (Stefanski *et al.* 2020). Consequently,



**Figure 4:**  $A/C_i$  curves of *Tilia* seedlings grown either under CC (a) or FC (b). Each point in the plots represents the average of six sample seedlings. The measurements of all the sample seedlings (3 seedlings per replicate, 2 replicates per treatment) were used to generate the curves to better represent the average performance of all the seedlings in the treatment and the variations among seedlings were represented by error bars in the figure. The open circle at the crossing point in each plot denotes the transition point ( $C_{i-t}$ ,  $A_{n-t}$ ) from the Rubisco limitation to the RuBP limitation.  $C_{i-g}$  and  $A_{n-g}$  represent values measured at growth CO<sub>2</sub> concentration. Abbreviations: ACE = apparent carboxylation efficiency derived from the initial slope of the  $A/C_i$  curve, CCP<sub>aci</sub> = CO<sub>2</sub> compensation point derived from  $A/C_i$  curve, i.e.  $C_i$  when  $A_n$  was zero. Both ACE and CCP<sub>aci</sub> were different between C and F ( $P \le 0.05$ , Table 1).  $A_c = V_{cmax}$  limited photosynthetic rate,  $A_i = J_{max}$  limited rate.



**Figure 5:** Effects of CO<sub>2</sub> and temperature combination (CC *vs.* FC) on leaf mass ( $N_{\text{mass'}}$  a), leaf area ( $N_{\text{area'}}$  b), leaf of the whole plant ( $N_{\text{leaf'}}$  c) of N concentration and photosynthetic nitrogen use efficiency (PNUE, d). Means (±SEM, n = 6) with different letters are different from each other ( $P \le 0.05$ ). See Table 1 for other explanations.

the qualities of photosynthetic acclimation may be evaluated by analyzing the varying  $A/C_i$  patterns of plants exposed to FC.

It is interesting to note that there was no significant difference in  $C_{i-t}$  between the two treatments, and both were greater than the corresponding  $C_i$  ( $C_{i,t}$  >  $C_{i,a}$ , indicating that photosynthesis was still limited by Rubisco carboxylation under the future elevated CO<sub>2</sub> of 750 µmol mol<sup>-1</sup> (Fig. 4b). This result is in contrast to the findings of some other studies that photosynthesis typically operates at the RuBP regeneration restriction stage  $(C_{i-t} < C_{i-p})$  under elevated CO<sub>2</sub> (Suzuki et al. 2021). Our results suggest that the photosynthesis of Amur linden may continue to be limited by  $V_{\rm cmax}$  under elevated  $\rm CO_2$  levels in the foreseeable future (Kumarathunge et al. 2019). The  $J_{\rm max}/V_{\rm cmax}$  ratio was not altered by the FC treatment. The  $J_{\text{max}}/V_{\text{cmax}}$  ratio indicates the balance between electron transport and Rubisco capacity (Silva-Pérez et al. 2020).

The  $A/C_i$  data indicate that the FC treatment reduced ACE but increased  $A_n$  and  $CCP_{aci}$  in Amur linden. This combination of changes is generally considered a typical manifestation of a decrease in leaf-area-based photosynthetic capacity (Bohley *et al.* 2019). The effects of FC treatment on  $A/C_i$  curves can be summarized as reduced initial slope (related to  $V_{\rm cmax}$  and ACE), and increased the actual  $A_n$  (related to  $J_{\rm max}$ ), but FC did not affect the relatively high transition point of photosynthetic limitation from Rubisco limitation to RuBP regeneration limitation ( $C_{i-1}$ ).

It is interesting to note that the principle component analysis showed close positive relationships among the  $CO_2$  compensation point  $(CCP_{aci} \text{ or } C_i^*)$  of photosynthesis, seedling biomass, height, total leaf area and leaf nitrogen concentration in Amur linden seedlings. The CO<sub>2</sub> compensation point reflects the balance in resource allocation between photosynthetic and respiratory apparatuses (Walker and Cousins 2013). There are two  $CO_2$  compensation points along the CO<sub>2</sub> diffusion pathway to the carboxylation site in the chloroplast that are commonly estimated: the CO<sub>2</sub> compensation point in the chloroplast  $(C_c^*)$ and that in the intercellular space  $(C_i^*)$ . Walker and Cousins (2013) suggest that  $C_c^*$  quantifies the photorespiration production of CO<sub>2</sub> and the kinetic properties of Rubisco, which should be fundamental terms in photosynthetic models (Walker and Cousins 2013). The difference between  $C_i^*$  and  $C_a^*$  is affected by the mesophyll conductance  $g_m$ . Therefore, the CO<sub>2</sub> compensation point estimated from the  $A/C_i$  curve may reflect the effects of multiple limitations of the photosynthetic processes (Niessen et al. 2012).

#### CONCLUSIONS

This study suggests that the combination of elevated CO<sub>2</sub> and warmer temperature as predicted for the end of the century could increase Amur linden growth, especially height growth and total leaf area. Such increases can potentially further increase the shade tolerance of the species. Although photosynthetic capacity-related parameters were downregulated in the FC treatment, the photosynthetic rate was higher in FC than in the CC treatment. Our results suggest that the photosynthesis of Amur linden was still primarily limited by Rubisco carboxylation in seedings grown under the FC rather than shifting to RuBP regeneration limitation. RuBP regeneration limitation is generally related to a reduction in resource allocation to light harvesting and electron transport apparatus. The maintenance of this capacity in combination with an increase in total leaf area would likely boost the shade tolerance of the species in the future, particularly under dynamic light conditions under a forest canopy. The relatively constant  $C_{i-t}$  may have played an important role in the photosynthetic acclimation of Amur linden seedlings.

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*Conflict of interest statement.* The authors declare that they have no conflict of interest.

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