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ORIGINAL ARTICLE

Nitrogen availability affects the ecophysiological responses of amur linden and white birch to CO_2 and temperature

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Abstract

Climate change, e.g., elevated CO_2, warmer temperature, and nitrogen (N) deposition, can have substantial effects on tree physiology and growth. This study explored the effects of future CO_2 and temperature (fCT, representative of future climate conditions) on the growth and photosynthetic traits of amur linden and white birch seedlings under different N availability. The results showed that fCT significantly increased the total seedling biomass and total seedling leaf area of amur linden and white birch synergistically with increasing nitrogen supply. However, the specific leaf area (leaf area to leaf mass ratio) of amur linden was significantly reduced by the two treatments. Increases in N availability alleviated the photosynthetic downregulation associated with fCT as indicated by the photosynthetic capacity parameters of V_{cmax} and J_{max}. Our results indicate that the primary limitation of photosynthesis under fCT in the two species will likely shift from Rubisco carboxylation to RuBP regeneration. However, the rate of photosynthesis was significantly higher under the fCT than control conditions in amur linden but was not significantly different between the two treatment conditions in white birch, indicating that the photosynthetic downregulation completely offset the positive effect of increased CO_2 on photosynthesis in white birch. Our results suggest that the relative performance and competitiveness of the two species may be very different in future, and more detailed studies are warranted on the responses of the two species to climate change.

Keywords Nitrogen availability · Elevated CO_2 · Global warming · Amur linden (*Tilia amurensis* Rupr.) · White birch (*Betula papyrifera* Marshall) · Photosynthetic acclimation

Introduction

Climate change continues to affect forests, especially temperate and boreal forests (Dusenge et al. 2020). Increases in atmospheric CO_2, temperature, and nitrogen (N) deposition will have great influence on future climate and environment conditions (Penuelas et al. 2020). The simultaneous elevations of atmospheric CO_2 and temperature may have broad effects on locate site conditions such as soil nitrogen availability (Maxwell et al. 2022). Soil nitrogen availability is also greatly affected by vegetations on the site (Qi et al. 2021). Climate change can even influence plant immunity via regulating defense signals (Kim et al. 2021). It is predicted that atmospheric CO_2 could rise to around 750 μmol mol^{-1} by 2100, while global temperature could increase by more than 4 ℃ (Kurepin et al. 2018). A good understanding how trees will perform under the predicted future climate conditions is critical for predicting the future growth and distribution of forests (Murphy and Way 2021; Wang et al. 2022a).

Increasing CO_2 can lead to photosynthetic downregulation in plants, as manifested by decreases in maximum rate of RuBP carboxylation (V_{cmax}), maximum photosynthetic electron transport rate (J_{max}), leaf nitrogen concentration (N_{mass}), and stomatal conductance (g_s) (Chavan et al. 2019; Kurepin et al. 2018). Elevated temperature can reduce the J_{max}/V_{camx} ratio (Dusenge et al. 2021; Smith and Keenan

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2020), indicating a shift of nitrogen allocation between the photochemical and biochemical machinery of photosynthesis. However, there are conflicting results on the interactive effects of elevated CO_2 and warmer temperature on plants (Abo Gamar et al. 2021; Su et al. 2023): some report positive interactions (Ainsworth and Long 2021), while others have found no interactions or negative interactions (Noyce et al. 2019). However, elevated CO_2 is inevitably coupled with higher air temperature. Therefore, it is most logical to investigate the effects of the combined or coupled effects of elevated CO_2 and higher temperature (Kizildeniz et al. 2021). Slot et al. (2021) report that the combination of elevated CO_2 and warmer temperature (800 µmol mol^{-1} CO_2, current + 4 ℃ temperature) reduces the photosynthetic capacity of *Tabebuia rosea*. Others have also found that the combination of elevated CO_2 and higher temperature induces photosynthetic downregulation (Cai et al. 2018; Ryan 2013), but there is generally a paucity of information of such effects on temperate and boreal tree species.

Nitrogen assimilation in leaves is closely related to photosynthetic rates, and low N availability limits the beneficial effects of elevated CO_2 (Ribeiro et al. 2021). Because elevated CO_2 increases substrate supply for photosynthesis and suppresses Rubisco oxygenase activity, plants will require less Rubisco for a given photosynthetic rate, leading to a downregulation of leaf N concentration and of photosynthetic capacity (Wujeska-Klause et al. 2019). Nitrogen availability affects photosynthetic responses to both CO_2 and temperature and photosynthetic downregulation under elevated CO_2 and warmer temperature generally occurs when N supply is insufficient (Ryan 2013), but the responses can vary with plant species (Zhang et al. 2022). N availability varies substantially with sites in the boreal forest and will likely influence tree's response to elevated CO_2 and higher temperature.

Amur linden (*Tilia amurensis* Rupr.) is a shade-tolerant tree species in temperate forests (Aasamaa and Aphalo 2017). Amur linden is a valuable tree species ecologically and economically and is often used for nectar and wood production as well as for landscaping (Kang et al. 2021). Elevated CO_2 can expedite leaf senescence, promote winter bud formation, and increase nitrogen absorption in linden species (Li et al. 2019). White birch (*Betula papyrifera* Marshall) also has great ecological and commercial values and is widely distributed in the boreal forest zone. Elevated CO_2 can affect the relationship between photosynthesis rate and leaf nitrogen concentration in white birch (Wang et al. 2022b). However, there is no information in the literature on how the combination of elevated CO_2 and temperature, which is representative of the future climate conditions, affects the growth and physiological traits of linden and birch species. This study investigated the responses of growth and photosynthetic traits of amur linden and white birch to the combination of the CO_2 and temperature predicted for the end of the current century and how soil nitrogen supply affects the responses.

Materials and methods

Plant materials

Amur linden and white birch seeds were collected from six trees in the Changbai Mountain Forest region of China (42.26°N, 127.38°E). The seeds from different trees were mixed and stratified for 2 months in moist sand at 4 ℃. They were then sown in a germination tray filled with a mixture of vermiculite and peat moss (1:1 v:v). Seed germination was carried out in the Lakehead University greenhouse facility (Thunder Bay, Ontario, Canada). The seedlings were transplanted individually into 4.5 L plastic pots when they reached about 5 cm tall. The greenhouse environmental conditions were 25/16 ºC day/night temperature, and 16 h photoperiod. High-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) were used to extend the natural photoperiod or to supplement the natural light when the flux density of photosynthetically active radiation (PAR) was below 500 µmol m^{-2} s^{-1}.

Experimental design and treatments

This was a split-plot design with the combination of CO_2 and temperature (CT) as the main plot (current CO_2 and current temperature [cCT: 400 µmol mol^{-1} (CO_2) and current temperature] vs. predicted future CO_2 and temperature [fCT: 750 µmol mol^{-1} (CO_2) and current + 4 ℃] and nitrogen N supply as split plot (10, 80, 150, 220, and 290 µmol mol^{-1} N). Each of the main plots (cCT and fCT) had two replicates (independent greenhouses). The physical arrangement of the five split plots within each main plot was randomized. There were 12 seedlings of each species per treatment combination. The seedlings were watered to the drip point every two days and were fertilized twice a week. The greenhouses' environmental conditions [photoperiod, air temperature, (CO_2), and humidity] were managed automatically using an Argus Titan Environment-control system (Argus Control Systems Ltd, Vancouver, BC, Canada). CO_2 generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA) were used to elevate CO_2.

Gas exchange measurements

After two months of treatments, three seedlings were randomly selected from each replicate of each treatment combination for gas exchange measurements using a PP Systems CIRAS-3 Portable Photosynthesis System (PLC3 Universal

Leaf Cuvette and built-in CFM-3 Chlorophyll Fluorescence Module, PP Systems International, Inc. Amesbury, MA, USA). The A/C_i response curve was measured on the first mature leaf from the top at 400, 300, 200, 150, 100, 50, 400, 600, 800, 1000, 1200, and 1500 μmol mol^{-1} CO$_2$ (C_a) and 800 μmol m^{-2} s^{-1} photosynthetically active radiation flux density (PAR). The gas exchange measurement was conducted at 25 °C temperature (±0.5 °C) and 50% RH.

The A/C_i data were fitted by the Plantecophys fitaci function of the R package (Duursma 2015) to estimate the maximum rate of electron transport (J_{max}, μmol m^{-2}) and maximum rate of RuBP carboxylation (V_{cmax}, μmol m^{-2} s^{-1}). The findCiTransition function (Duursma 2015) was used to determine the transition point ($C_{i\text{-}t}$, $A_{n\text{-}t}$) from Rubisco limitation to RuBP regeneration limitation of photosynthesis. The $C_{i\text{-}t}$ refers to the intercellular CO$_2$ concentration on the A/C_i curve at the transition point where the limitation of photosynthesis shifts from V_{cmax} limitation to J_{max} limitation, and $A_{n\text{-}t}$ is its corresponding A_n (net photosynthesis rate at 25 degree).

Biomass and biomass allocation

All the plants were harvested after four months of treatments. The total leaf area (fresh) for each seedling was determined using an EPSON EU-88 scanner (SEIKO EPSON CORP. JAPAN) and the Regent WinFOLIA program (Regent Instruments Inc., Quebec City, QC, Canada). All the organs of the seedlings were then oven-dried to constant weight at 80 °C and weighed. The specific leaf area was calculated by SLA = leaf area / leaf mass.

Leaf nitrogen concentration

Leaf N concentration was assayed using a CNS-2000 (LECO Corp., St. Joseph, MI, USA) at the Lakehead University Analytical Centre. Leaf area-based N concentration (N_{area}, g m^{-2}) was calculated by dividing the mass-based leaf N concentration by specific leaf area. Photosynthetic nitrogen use efficiency (PNUE) was determined by A_n/N_{area}.

Statistical analyses

The growth, gas exchange, and N-related data (split-plot design) were analyzed using a two-way analysis of variance (ANOVA). The normality distribution and the variance homogeneity of residuals were tested and confirmed before ANOVA. Principal component analysis (PCA) was applied to all the significant different gas exchange and biomass-related parameters using the PCA function from the FactoMineR package of R. All the analyses were performed using R 4.0.8.

Results

Biomass and leaf morphology

Both seedling biomass and total leaf area per seedling of the two species increased with increasing nitrogen supply and the increases were greater under fCT than under the cCT (Tables 1 and 2, Fig. 1). The combination of elevated CO$_2$ and warmer temperature (fCT) significantly increased both seedling biomass and total leaf area at the two highest nitrogen levels but had no significant effects on them at lower N supplies (Tables 1 and 2, Fig. 1).

The SLA of amur linden was mainly affected by the CT treatments and the fCT significantly reduced SLA (Table 1), while SLA showed a slight downward trend with the increase of nitrogen supply level under fCT treatment where there was no significant difference in SLA under cCT treatment across different N levels (Table 1). In white birch, SLA increased with the increase in nitrogen supply (Table 2).

Photosynthetic characteristics

The fCT treatment generally increased the photosynthetic rate measured under the treatment CO$_2$ in amur linden but had no significant effect in white birch (Tables 1 and 2). $A_{n\text{-}g}$ generally increased slightly with increasing N supply under cCT in amur linden, but under fCT only $A_{n\text{-}g}$ at highest N level was significantly greater than those at other N levels (Table 1). In contrast, $A_{n\text{-}g}$ in white birch was significantly smaller at the lowest N level than those at other N levels under both cCT and fCT (Table 2). The fCT treatment significantly reduced the V_{cmax} and J_{max} of the two tested tree species, while they both increased slightly with increases in N supply (Tables 1 and 2).

There was no significant difference in $C_{i\text{-}t}$ across N levels in amur linden (Fig. 2a), while there was a general decreasing trend in $C_{i\text{-}t}$ with increasing N supply in white birch grown under fCT treatment (Fig. 2b). fCT significantly increased $C_{i\text{-}t}$ in white birch under the two lowest N levels but had no significantly effect at other N levels or in amur linden (Fig. 2a, b). The fCT treatment generally decreased $A_{n\text{-}t}$ of two tree species at all N levels, but the effect was not statistically significantly at intermediate N levels in amur linden (Fig. 2c, d).

Leaf nitrogen concentration and PNUE

N_{area} increased with increasing N levels, but there was no significant difference in N_{area} between cCT and fCT treatments at the same N level in amur linden (Fig. 3a). In white birch, in contrast, N_{area} increased with increasing

Table 1 Leaf area, *SLA,* and photosynthesis-related traits of amur linden growth at ambient or elevated CO_2 in response to different N supply

CO_2&T	N	Leaf area (cm^{-2})	*SLA* (cm^2 g^{-1})	$A_{n\text{-}g}$ (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})
cCT	N1	223.6 ± 47.1 de	244 ± 11.8 a	8.7 ± 0.4 c	42.1 ± 0.7 ab	117.7 ± 3.5 bc
	N2	181.2 ± 9 de	230.6 ± 10 ab	8.7 ± 0.5 c	35.6 ± 2.5 cd	97.6 ± 9.9 cd
	N3	404.6 ± 47.2 bc	230.6 ± 8.9 ab	11.3 ± 0.8 bc	47.7 ± 2.8 a	136.4 ± 10.6 ab
	N4	314.2 ± 24.7 cd	229.5 ± 7.6 ab	9.4 ± 0.8 bc	50.6 ± 2 a	152.7 ± 9.3 a
	N5	535.3 ± 69.6 b	225.3 ± 8 bc	12.9 ± 1 bc	48.6 ± 3.5 a	160.5 ± 16.8 a
fCT	N1	103.5 ± 10.1 e	222.9 ± 8.8 bc	11.8 ± 0.6 bc	28 ± 1.5 d	75 ± 5.3 d
	N2	221.3 ± 21.4 de	218.4 ± 11.8 bc	13.4 ± 1.3 ab	33.4 ± 3.1 cd	86.8 ± 9.1 cd
	N3	383.3 ± 37.1 c	203.4 ± 7.7 bc	10.5 ± 1.2 bc	45.4 ± 2.7 ab	144.7 ± 10.6 ab
	N4	682.7 ± 52.4 a	194.6 ± 24.4 c	12.2 ± 1.9 bc	40.1 ± 2.3 ab	130.3 ± 16 ab
	N5	717.5 ± 103.5 a	192.5 ± 6.9 c	17.7 ± 0.3 a	42.8 ± 0.7 ab	125.5 ± 4.3 ab
P-value	CT	**0.007**	**0.004**	**< 0.001**	**< 0.001**	**0.003**
	N	**< 0.001**	0.145	**< 0.001**	**< 0.001**	**< 0.001**
	CT:N	**< 0.001**	0.644	**0.042**	0.058	0.134

Each value represents mean ± SE ($n = 6$). Two-way ANOVA was performed to analyze CO_2 and nitrogen (N) as well as their interactive effects (CT**:**N). Significant effects ($P \leq 0.05$) are marked in bold and "**:**" indicates interaction. Different letters within the same column indicate statistically significant differences between treatments (Tukey post hoc test, $P < 0.05$). cCO_2: current CO_2 (400 μmol mol^{-1}) and temperature; fCO_2: elevated CO_2 (750 μmol mol^{-1}) and current + 4 ℃; N1–N5 means the lowest to highest N supply. *SLA*: specific leaf area. $A_{n\text{-}g}$: net photosynthesis rate measured at growth Ca (750 μmol mol^{-1} for fCT; 400 μmol mol^{-1} for cCT); V_{cmax} maximum rate of Rubisco carboxylation; J_{max} maximum of photosynthetic electron transport rate

Table 2 Leaf area, *SLA,* and photosynthesis-related traits of white birch growth at ambient or elevated CO_2 in response to different N supply

CO_2 and T	N	Leaf area (cm^{-2})	*SLA* (cm^2 g^{-1})	$A_{n\text{-}g}$ (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})
cCT	N1	88.8 ± 6.3 f	206.5 ± 7.3 c	13.6 ± 1.2 b	50.8 ± 3.4 c	124.3 ± 11.5 c
	N2	348.8 ± 45 e	242.5 ± 11 bc	17.5 ± 0.4 a	69.6 ± 3 b	174.8 ± 10.5 b
	N3	432.2 ± 60.4 e	244.1 ± 17.6 bc	20.1 ± 0.2 a	75.8 ± 2 ab	278.7 ± 24.8 a
	N4	700.7 ± 43.6 cd	265 ± 12.6 ab	19.7 ± 0.7 a	83 ± 1.8 a	307.6 ± 27.8 a
	N5	1072.2 ± 83.1 b	284.9 ± 9.5 a	19.5 ± 0.5 a	78.6 ± 2.1 ab	279.7 ± 17 a
fCT	N1	115.8 ± 2.2 f	230.1 ± 5.4 bc	11.3 ± 0.6 b	25.2 ± 1.7 d	65.9 ± 3.9 d
	N2	306.3 ± 32.4 ef	243.3 ± 10.6 bc	19.5 ± 1.4 a	45.3 ± 3.1 c	114.6 ± 9.9 c
	N3	668.4 ± 49.2 d	258.2 ± 11.6 ab	20.2 ± 0.8 a	49 ± 2.2 c	122.6 ± 6.3 c
	N4	889.4 ± 42.8 bc	272.7 ± 7.6 ab	20.6 ± 0.9 a	51.5 ± 2 c	126.8 ± 5.8 c
	N5	1400.4 ± 41.1 a	273.8 ± 5.9 ab	21.2 ± 0.5 a	56.1 ± 2.6 c	126.8 ± 5.5 c
P-value	CT	**< 0.001**	0.291	0.353	**< 0.001**	**< 0.001**
	N	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	CT:N	**0.001**	0.528	0.078	0.448	**< 0.001**

Each value represents mean ± SE ($n = 6$). Two-way ANOVA was performed to analyze CO_2 and nitrogen (N) as well as their interactive effects (CT**:**N). Significant effects ($P \leq 0.05$) are marked in bold and "**:**" indicates interaction. Different letters within the same column indicate statistically significant differences between treatments (Tukey post hoc test, $P < 0.05$). cCO_2: current CO_2 (400 μmol mol^{-1}) and temperature; fCO_2: elevated CO_2 (750 μmol mol^{-1}) and current + 4 ℃; N1–N5 means the lowest to highest N supply. *SLA* specific leaf area. $A_{n\text{-}g}$ net photosynthesis rate measured at growth C_a (750 μmol mol^{-1} for fCT; 400 μmol mol^{-1} for cCT); V_{cmax} maximum rate of Rubisco carboxylation; J_{max} maximum of photosynthetic electron transport rate

N supply only the N supply was low (i.e., N1 to N2) and was significantly lower in fCT than in cCT (Fig. 3b). The responses of PNUE to variation in N supply were very different between the two tree species: in amur linden, PNUE initially declined with increasing N supply, but then the trend was the opposite with further increase in N supply (Fig. 3c); in white birch, N supply generally did not affect PNUE (Fig. 3d). The fCT treatment generally increased PNUE in both species, but the effect was not statistically significant at all the N levels (Fig. 3c, d).

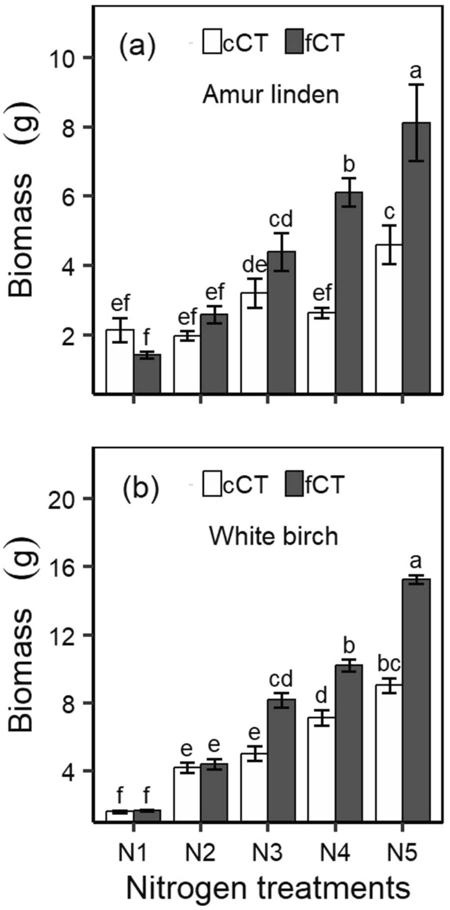

Fig. 1 Effects of CO_2 and Temperature (CT) and nitrogen supply (N) on biomass in amur linden (**a**) and in white birch (**b**). Means (±SE, $n=6$) with different letters are significantly different from each other ($p \leq 0.05$)

Principal component analysis (PCA)

The seedlings grown under fCT clustered in the upper ellipse that was characterized by high PUNE for both species (Figs. 4a, 5a), while those grown under cCT clustered in the lower ellipse that was characterized by high *SLA* for amur linden (Fig. 4a) and by photosynthetic capacity for white birch (Fig. 5a). *SLA* was closed and positively related to biomass in white birch (Fig. 5a) but not in amur linden. The $A_{n\text{-}g}$ was positively related to biomass and total leaf area in amur linden grown under fCT, but no correlation with photosynthetic capacity was observed in amur linden (the directions of arrows almost perpendicular to each other in Fig. 4a). The $A_{n\text{-}g}$ was closely related to N levels (Fig. 5b) but showed no correlation with CT treatment in white birch (Fig. 5a).

Seedlings exposed to different N treatments were more distinguishable on PC1 and those exposed to high N supplies were clustered in ellipses on the right-hand side

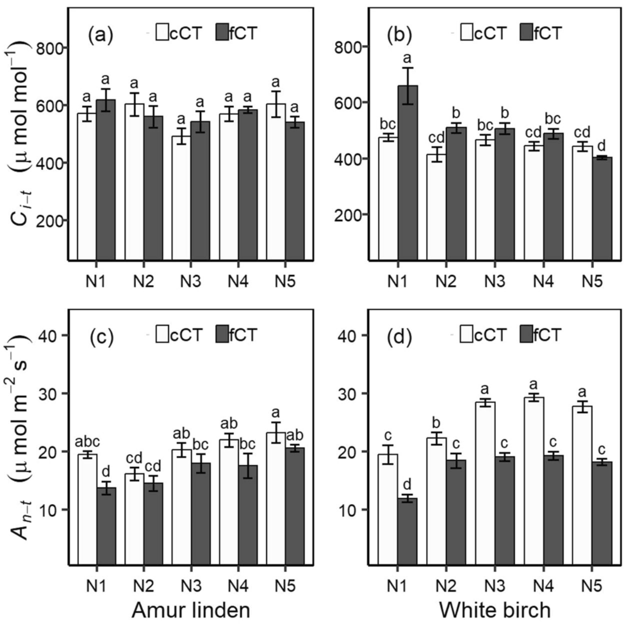

Fig. 2 Effects of CO_2 and Temperature (CT) and nitrogen supply (N) on photosynthesis transition point ($C_{i\text{-}t}$, $A_{n\text{-}t}$) between Rubisco limitation and RuBP regeneration limitation based on A/C_i curve. Lift plots (**a**, **c**) are amur linden and right plots (**b**, **d**) are white birch. Means (±SE, $n=6$) with different letters are significantly different from each other ($p \leq 0.05$)

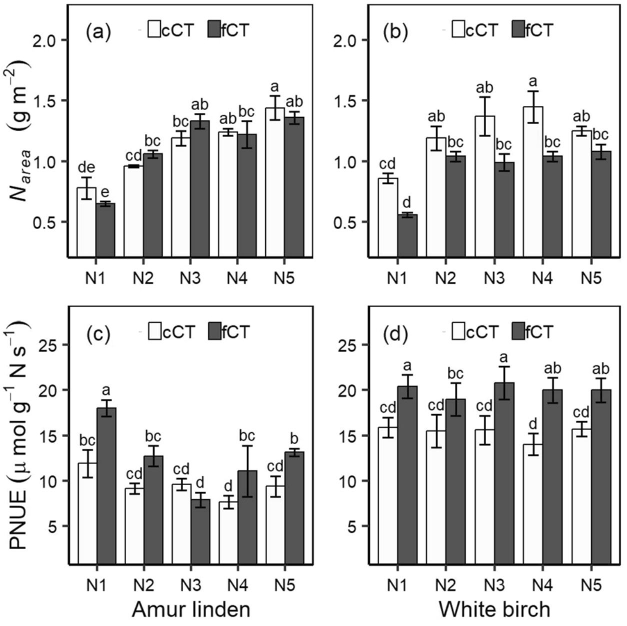

Fig. 3 Fig. 2: Effects of CO_2 and Temperature (CT) and nitrogen supply (N) on area-based leaf N concentration (N_{area}) and photosynthetic nitrogen use efficiency (PNUE) in amur linden (**a**, **c**) and white birch (**b**, **d**). Means (±SE, $n=6$) with different letters are significantly different from each other ($p \leq 0.05$)

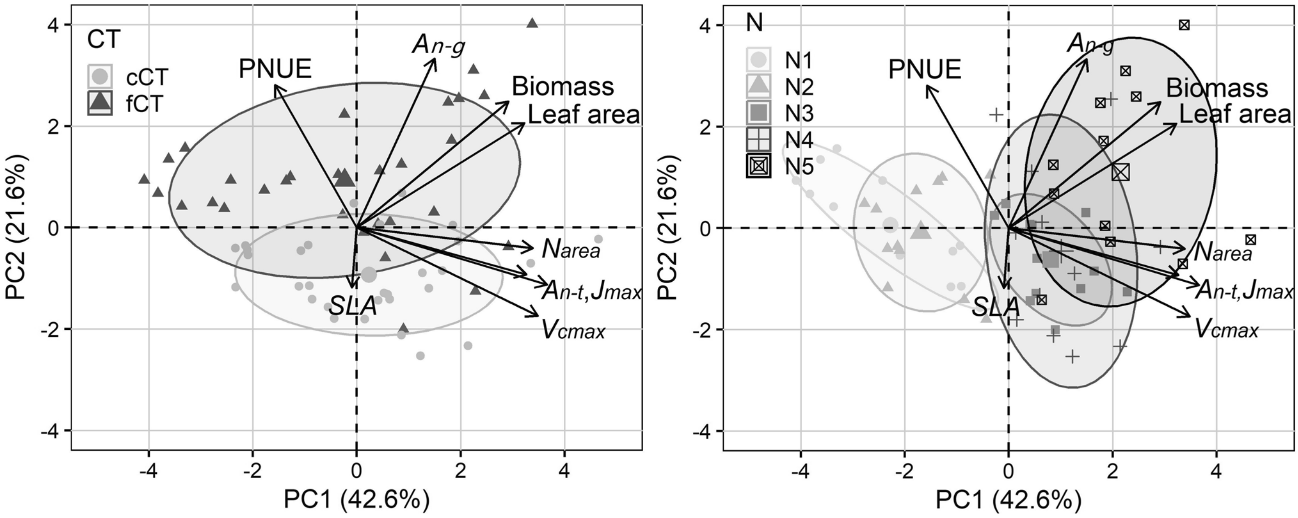

Fig. 4 The ellipses in Principal Component Analysis indicate the clustering of different parameters in amur linden from the two CO_2 and Temperature (CT) treatments (current cCT vs. predicted future fCT) and from different N supply. See previous tables and figures for other explanations

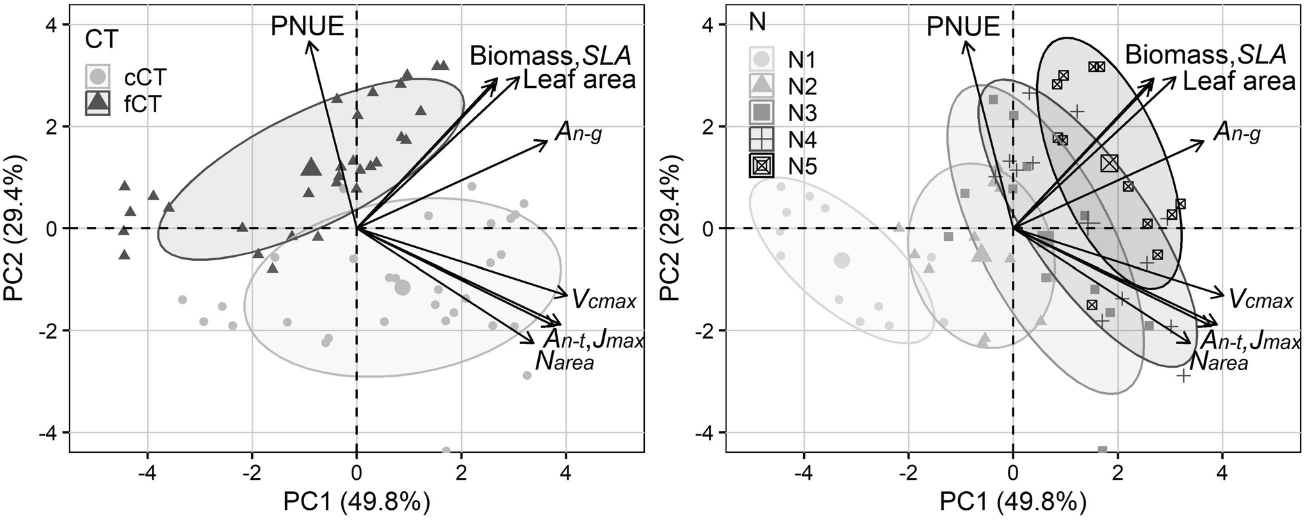

Fig. 5 The ellipses indicate clustering of white birch subjected to two CO_2 and Temperature (CT) treatments and different nitrogen treatments (N1 (lowest) to N5 (highest) N supply). Please see previous tables and figures for the definitions of abbreviations

characterized by high biomass, high leaf area, and high photosynthetic parameters (Figs. 4b, 5b).

Discussion

Biomass and leaf morphology

Elevated CO_2, warmer temperature, and higher N supply synergistically increased the biomass production of amur linden and white birch. While both elevated CO_2 and increased N supply can increase plant growth (Hu et al. 2021; Unnikrishnan et al. 2021), the synergy is probably a reflection of increases in nitrogen use efficiency under elevated CO_2 (Kanno et al. 2017). When the availability of CO_2 and N changes, plants will achieve a new balance between source and sink by adjusting the distribution of resources and biomass among different organs to maximize the total carbon acquisition of the plant (Piñero et al. 2016; Wang et al. 2022c). The increase in leaf area can serve as a transient sink to expedite the translocation and utilization of carbohydrates produced by existing leaves and serve as new sources to provide carbohydrates to other sinks (Kauwe et al. 2021; Perchlik and Tegeder

2018). The result that fCT significantly increased leaf area and total biomass in both tree species in this study provided further confirmation for the close positive relationship between leaf area and biomass and the synergistic effect of increasing nitrogen availability on that relationship (Wang et al. 2022b).

Photosynthetic acclimation

Elevated CO_2 can lead to photosynthetic downregulation, as reflected in the reduction of V_{cmax} and J_{max} (Zhang et al. 2013). Similar results were observed in this study, and the decline in photosynthetic capacity in white birch exposed to fCT was greater than that of amur linden. In fact, the downregulation of photosynthetic capacity in white birch was to such an extent that it completely offset the positive effect of increased CO_2 supply that the photosynthetic rate was similar in the two CO_2 and temperature treatments. Photosynthetic capacity is typically positively correlated with leaf nitrogen concentration (Jauregui et al. 2015). Therefore, it is believed that increasing N supply can at least reduce the magnitude of elevated CO_2-induced photosynthetic downregulation (Ruiz-Vera et al. 2017) or even eliminate photosynthetic downregulation (Jauregui et al. 2015). The combination of elevated CO_2 and warmer temperature in this study resulted in reductions in V_{cmax} and J_{max} in amur linden and white birch. Some studies suggest that photosynthetic downregulation is caused by the dilution of leaf nitrogen due to increased carbohydrate production under elevated CO_2 (Byeon et al. 2021), but most researchers believe that photosynthetic downregulation is triggered by insufficient N supply (Wujeska-Klause et al. 2019). We found a close correlation between V_{cmax}, J_{max}, and N_{area} in the two tree species, suggesting that leaf N concentration may have indeed played an important role in the regulation of photosynthetic capacity and affected tree acclimation to future climate conditions (Wang et al. 2022c). $C_{i\text{-}t}$ was found to be insensitive to CT and N treatments in our study, but fCT caused a significant reduction in $A_{n\text{-}t}$, indicating the transition point of photosynthetic limitation from carboxylation limitation to RuBP regeneration limitation. In other words, J_{max} may play a greater role than V_{cmax} in limiting the positive effects of the future elevated CO_2 condition on photosynthetic rates (Wang et al. 2022a). $A_{n\text{-}t}$ reflects the transition of photosynthetic limitation from V_{cmax} to J_{max}. A notable positive correlation was identified in the PCA analysis between J_{max} and $A_{n\text{-}t}$ in the two tested tree species, indicating a potential restriction in photosynthesis by electron transfer capacity under increased CO_2 levels. These findings corroborate the results by Wang et al. in white birch (Wang et al. 2022b).

PNUE vs. Nitrogen concentration

The photosynthetic Nitrogen use efficiency (PNUE) is strongly affected by N uptake, photosynthesis, and N metabolism (Cohen et al. 2019). Some studies have suggested that *OsNLP3* (NIN-like protein 3) (Zhang et al. 2022) and the GATA transcription factor gene *PdGNC* (Shen et al. 2022) may be involved in the molecular mechanisms regulating PNUE. Our results show that PNUE of both tree species was promoted by fCT. The increase in PNUE may be attributable to both the increase of Rubisco carboxylation and the suppression of Rubisco oxygenation by elevated CO_2 (Bonsu et al. 2021). However, the genetic and physiological mechanisms regulating PNUE are highly complex (Mahboob et al. 2023), and the molecular mechanisms of the regulation are still elusive (Zhang et al. 2022). No significant correlation was observed between PNUE and growth and photosynthetic traits in this study (Figs. 4, 5), further indicating the complicated nature of PNUE regulation. The principal component analysis showed that linden PNUE pointed to the ellipse of elevated CO_2 and warming treatments. This may support the theory that plants grown under elevated CO_2 tend to remobilize nitrogen from Rubisco to support canopy expansion and PNUE (Kanno et al. 2017).

Conclusions

This study shows that the combination of elevated CO_2 and warmer temperature, and higher N availability increased the biomass and leaf area of amur linden and white birch. The predicted future CO_2 and temperature treatment fCT significantly reduced V_{cmax} and J_{max} in both species; however, this effect was alleviated by higher N supplies. The two species appeared to have different acclimation strategies to the future CO_2 and temperature conditions: The fCT treatment resulted in significantly thicker leaves, but increased PNUE and $A_{n\text{-}g}$ in amur linden; fCT significantly increased PNUE while maintaining a relatively constant *SLA* and $A_{n\text{-}g}$ in white birch. As N supply increased, the *SLA* decreased in amur linden and increased in white birch. Changes in nitrogen availability influenced the acclimation of both species to future CO_2 and temperature conditions.

Author's contribution J. Zheng and QL Dang conceived and designed the experiment. J Zheng and G Wang performed experiments and all the measurements. L Wang analyzed the data and produced all the figures. All the authors contributed to data interpretation and the production of the manuscript.

Data availability The data support the findings of this study is available at Lakehead University Dataverse.

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