



Nitrogen supply influences photosynthetic acclimation of yellow birch (*Betula costata* Trautv.) to the combination of elevated CO₂ and warmer temperature

Gerong Wang^{1,2} · Jinping Zheng^{1,2} · Lei Wang^{1,3} · Qing-Lai Dang¹

Received: 7 October 2022 / Accepted: 26 September 2023
© The Author(s), under exclusive licence to Springer Nature B.V. 2023

Abstract

Rising CO₂, global warming, and N deposition create challenging environmental conditions to vegetation. Since elevated CO₂ and rising temperature are coupled with each other, it is important to understand their combined effects on plants. We investigated the growth and photosynthetic responses of yellow birch to five levels of nitrogen supply under the current (cCT: current CO₂ and temperature) and the predicted future CO₂ and temperature conditions (fCT: elevated CO₂, current+4°C temperature). The results show that fCT and high N supply increased seedling growth but fCT reduced photosynthetic capacity (e.g., maximum rate of Rubisco carboxylation- V_{cmax} , maximum rate of photosynthetic electron transport- J_{max}) and foliar N concentration. However, the magnitude of the fCT effect declined with increases in N supply. Furthermore, the fCT treatment significantly reduced the J_{max}/V_{cmax} ratio, indicating a possible shift of N allocation from J_{max} to V_{cmax} in the photosynthetic machinery. This result suggests that the photosynthesis of yellow birch may be more limited by electron transport under the predicted future climate condition. Both low N supply and fCT significantly increased photosynthetic N use efficiency (PNUE) and there was a negative relationship between PNUE and photosynthetic capacity. In general, yellow birch grew better under fCT than cCT, particularly above-ground growth.

Keywords Climate change · Elevated CO₂ · Global warming · Yellow birch (*Betula costata* Trautv.) · Photosynthetic acclimation · Nitrogen use efficiency

✉ Qing-Lai Dang
qdang@lakeheadu.ca

¹ Faculty of Natural Resources Management, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada

² Forestry College, Beihua University, 3999 Binjiang East Road, Jilin 132013, Jilin, China

³ Department of Biotechnology, College of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212003, Jiangsu, China

Published online: 16 October 2023

Springer

Introduction

It is predicted that the atmospheric CO₂ concentration and temperature will continue rise to until the end of the 21st century (Dai et al. 2020). Climate change will continue to have a escalating impact on forests (Newaz et al. 2021; Sperry et al. 2019). Elevated CO₂ and warming are major drivers of global change and may interact with increases in soil nutrient supply associated with increased nitrogen deposition (García P. et al. 2015; M. G. R. et al. 1998; Penuelas et al. 2020). CO₂ elevation and climate warming occur simultaneously and are closely related to each other (Jayawardena et al. 2019). It is therefore critical to better understand their combined effects.

Recent studies have found that elevated CO₂ and warmer temperature can increase or decrease the cycling (Dai et al. 2020; Maxwell et al. 2022) and availability of soil nutrients in boreal forests, depending on the specific site conditions (Karst et al. 2021). Elevated CO₂ and higher temperature can lead to the acclimation of photosynthesis and growth in plants (Jauregui et al. 2015; Wang et al. 2022b) and change the functional relationships between photosynthetic parameters and foliar N (Cai et al. 2018). Therefore, a good understanding of the combined effect of elevated CO₂ and warmer temperatures on plants may be more important than understanding their individual effects for predicting the overall impact of future climate change on vegetation (Dusenge et al. 2019; Noyce et al. 2019). Some studies found that elevated CO₂ and warmer temperature synergistically affect plant growth and stress resistance (Apgaua et al. 2019) while others have reported that they have opposite effects on plant growth and N metabolism, and that their individual effects can offset each other at least partially when the two treatments are applied simultaneously (Sharwood et al. 2017), making it difficult to use individual effects to predict their combined effects (Kim et al. 2019). Furthermore, it is generally not well understood how the combination of elevated CO₂ and warmer temperature will interact with other environmental factors such as N availability in affecting plants.

A good understanding of the relationship between photosynthetic acclimation to the combination of elevated CO₂ and temperature and N availability may be critical for predicting plants' growth and productivity under future climate conditions, particularly on sites with different nitrogen conditions (Tausz-Posch et al. 2020; Zhao et al. 2021). There are complicated relationships and interactions involved in carbon and nitrogen (N) metabolisms (Pastore et al. 2020). N supply can play a dominant role in photosynthetic responses to elevated CO₂ and temperature (Ryan 2013). Furthermore, elevated CO₂ and warmer temperature can accelerate the mineralization rates of soil nutrients but reduce nutrient uptake by plants (Dai et al. 2020). However, elevated CO₂ can cause a reduction in leaf N concentration because elevated CO₂ increases biomass production and thus dilutes leaf N (Andrews et al. 2019). Furthermore, elevated CO₂ can reduce photorespiration and thus lead to reductions in N assimilation and NO₃⁻ metabolism which are associated with photorespiration (Wujeska-Klaue et al. 2019). While warming alone or in combination with elevated CO₂ reduce N uptake, warming can lead to increases in leaf N concentration (Sharwood et al. 2017). However, the interactive effects of elevated CO₂ and temperature with other environmental factors on physiological processes and morphological traits are still poorly understood for most tree species (Shrestha et al. 2015). A good understanding of such interactions may be critically important for predicting the performance of plants under the predicted future climate conditions.

Yellow birch (*Betula costata* Trautv.) is a deciduous tree species in the northern temperate zone (Delagrangé et al. 2004). It is not only an important hardwood species for wood production but is also rich in bioactive substances. Its leaf extract has potential applications in food, pharmaceutical, and cosmetic industries (Lavoie and Stevanovic 2005). However, yellow birch may be highly vulnerable to global change because of its shallower root system and higher mortality rates in degraded stands (Cox and Zhu 2003), and its ecophysiological responses to climate change are not well understood. This study explored the mechanisms of growth and physiological acclimation of yellow birch to the predicted future CO₂ and temperature under different N availabilities. We tested the hypotheses that birch trees growing under higher nitrogen supplies would benefit more from elevated CO₂ and warmer temperature and that trees would be more tolerant of low nitrogen stress when growing under elevated CO₂ and warmer temperature.

Materials and methods

Plant materials and treatments

Yellow birch seeds were collected from 10 natural trees (healthy and about 15 cm in DBH) from a forest stand in the Changbai Mountain forest region (42.12°N, 127.51°E) of China and the trees were at least 100 m apart from each other. The seeds were sown in germination trays filled with vermiculite and peat moss mixture (1:1 volume ratio) at the Lakehead University Forest Ecology Research Complex (Thunder Bay campus, Ontario, Canada). Three-week-old seedlings were transplanted into 3.5 L pots (1 seedling per pot) filled with the same growing medium mixture as the one used for germination.

Experimental design and treatments

The experiment was a split-plot design with the CO₂-temperature combination treatment as the whole plot and nitrogen supply as the split plot. The current CO₂-temperature treatment (cCT) was set at 400 μmol mol⁻¹ CO₂ and 25°C/16°C day/night temperatures while the future CO₂-temperature treatment (fCT) was set at 750 μmol mol⁻¹ CO₂ and 29°C/21°C. The 25°C/16°C day/night temperature and 16 h photoperiod represent the optimal conditions for the species. The CO₂ elevation and the 4 °C warming in the fCT treatment were the predicted conditions at for the end of the 21st century (Dusenge et al. 2020). The cCT and fCT treatments were randomly assigned to four greenhouses (2 independent replicates for each treatment). The split plots consisted of five levels of nitrogen supply: 10, 80, 150, 220 and 290 μmol mol⁻¹ N (to be referred to as N1, N2, N3, N4, N5, respectively, hereafter). N1-N4 represent the range of soil N in the natural sites of the species (Cao et al. 2007). Ammonium nitrate (NH₄NO₃) was used as the nitrogen sources in all the nitrogen treatments (BioBasic Inc. 20 Konrad Crescent, Markham, ON, Canada). The concentrations of other nutrient elements were the same in all the nitrogen treatments (60 μmol mol⁻¹ P, 150 μmol mol⁻¹ K, 4 ml L⁻¹ pH-Perfect Micro micronutrients (Advanced Nutrients, 109 Wheel Avenue, Abbotsford, BC, Canada). There were 12 seedlings per treatment combination (per replicate). The seedlings within the same split-plot were spaced far enough from each other

to avoid mutual shading. The seedlings were fertilized twice a week (500 ml) and were watered to the drip point every two days.

The environmental conditions in all the greenhouses (i.e., temperature, light, photoperiod, CO₂, and humidity) were monitored and controlled with an Argus Titan Environment-Control System (Argus Control Systems Ltd, Vancouver, BC, Canada). The CO₂ elevation was achieved using CO₂ generators (model GEN-2E; Custom Automated Products Inc., Riverside, California, USA).

Gas exchange measurements and parameters

After two months of treatment, three seedlings were randomly selected from each replicate of each treatment (six seedlings per treatment combination) and A/C_i curves were measured on the first fully expanded leaf from the top at 400, 300, 200, 150, 100, 50, 400, 500, 750, 900, 1100, and 1300, $\mu\text{mol mol}^{-1}$ CO₂ (C_a) using a PP-Systems CIRAS-3 Portable Photosynthesis System equipped with a PLC3 Universal Leaf Cuvette with automatic climate control (flow rate: 300 cc min⁻¹) and a built-in CFM-3 Chlorophyll Fluorescence Module (PP Systems International, Inc. Amesbury, MA, USA). The photosynthetic rate at growth (i.e. treatment) CO₂ (A_{n-g}) was measured at the beginning of the A/C_i curve measurement at 400 $\mu\text{mol mol}^{-1}$ and 750 $\mu\text{mol mol}^{-1}$ CO₂, respectively, for the cCT and fCT treatment. Other conditions in the leaf cuvette were 25 °C temperature, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation flux density (the measured saturating PAR was about 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 50% RH.

The maximum rate of Rubisco carboxylation (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and maximum rate of photosynthetic electron transport (J_{max} , $\mu\text{mol m}^{-2}$) were estimated using the *Plantecophys fitaci* function of the R package from A/C_i data (Duursma 2015). The initial slope of the A/C_i curve was used as an estimate of the apparent carboxylation efficiency (ACE) and the X-axis intercept of the A/C_i curve was used as an estimate of the CO₂ compensation point (CCP_{aci}). The transition point (C_{i-p} , A_{n-p}) between the Rubisco limitation and RuBP regeneration limitation of photosynthesis was obtained from the A/C_i curve using *findCi-Transition* of the *plantecophys* R package (Duursma 2015).

Growth and biomass allocation

All the seedlings were harvested after four months of treatments and total leaf area per seedling was determined using a Regent WinFolia system (Regent Instruments Inc., Quebec City, QC, Canada). The plant materials were oven-dried at 75 °C for 48 h to obtain leaf biomass, leaf mass ratio (LMR=leaf mass/total biomass), stem mass ratio (SMR=stems mass/total biomass), root mass ratio (RMR=root mass/total biomass), and specific leaf area (SLA =leaf area/leaf mass).

Leaf nitrogen

Leaf N concentration was assayed using the dry combustion method using a CNS-2000 (LECO Corp., St. Joseph, MI, USA) by the Lakehead University Centre for Analytical Services. Leaf-area based N concentration (N_{area} , g m^{-2}) was calculated by dividing the mass-based leaf N concentration (N_{mass} , mg g^{-1}) by the specific leaf area. The total leaf N

content per plant (N_{leaf} , mg plant^{-1}) was calculated by multiplying N_{mass} (mg g^{-1}) by the total leaf mass of the seedling (g^{-1}). Photosynthetic N use efficiency ($PNUe$) was calculated as A_{n-g}/N_{area} .

Statistical analyses

The data were analyzed using two-way analysis of variance (ANOVA) for split-plot design. Tests show that all the variables met the two ANOVA assumptions: the normality of distribution (using probability plots for residuals) and the homogeneity of variance (using scatter plots). Tukey-HSD post hoc pairwise comparisons of means were conducted when the ANOVA showed a significant interaction ($p \leq 0.05$). Principal component analysis (PCA) was applied to all the physiological and biomass parameters using the PCA function of the FactoMineR package. All the analyses were performed using the R Package 4.0.6.

Results

Growth and biomass allocation

Seedling biomass and total leaf area both increased progressively with increasing soil N availability and the increases were much bigger under fCT than cCT (Table 1; Fig. 1a and b). The fCT treatment increased biomass and total leaf area and the increases were progressively bigger with increasing N supply but not statistically significant at the two lowest N levels (Fig. 1a and b). The fCT significantly increased SLA (Fig. 1c). SLA was significantly greater in the two highest than the two lowest N levels (Fig. 1d). In contrast, fCT and increasing N supply both reduced RMR (Fig. 1e and f). SMR generally showed an increasing trend with increases in N supply while fCT significantly increased SMR only at the intermediate and higher levels of N supply (Fig. 1g). LMR showed a general, but weak trend of increases with increasing N supply (Fig. 1h).

Photosynthesis and limiting transition point

The CT and N interactively affected V_{cmax} (Table 1): fCT generally decreased V_{cmax} and the decreases were greater at lower N supplies but not statistically significant at N4 and N5 (Fig. 2a); increasing N supply generally increased V_{cmax} only under the fCT treatment (Fig. 2a). The fCT treatment strongly reduced J_{max} and J_{max}/V_{cmax} ratio (Fig. 2b and d); J_{max} was significantly lower at the lowest N supply (N1) than at other N levels and there were no significant differences in J_{max} among other N supplies (Fig. 2c). The fCT significantly increased the photosynthetic rate at the growth CO_2 concentration at the two highest N supplies (Table 1; Fig. 2e). The fCT significantly decreased ACE at all N levels, and the response pattern were similar to that of V_{cmax} (Fig. 2a and f).

Both, fCT and the lowest N supply, significantly reduced A_{n-t} but there were no significant differences in A_{n-t} among other N supply levels (Table 1; Fig. 3a and b). The response patterns of A_{n-t} (Fig. 3a and b) were similar to those of J_{max} (Fig. 3a and b). C_{i-t} was interactively affected by CO_2 -temperature and N treatment, but no clear response pattern could be identified although it appeared to have decreased with increasing N supply (Fig. 3c).

Table 1 Two-way ANOVA *P*-values for the effects of nitrogen (N) and combined CO₂ and temperature treatments (CO₂&T) on the growth, photosynthesis, and whole-plant variables of yellow birch seedlings. The nitrogen treatments included 5 N levels and the CO₂&T comprised AET (ambient [CO₂] (400 μmol mol⁻¹) and temperature) and ECT (elevated [CO₂] (750 μmol mol⁻¹) and temperature (ambient + 4°C)) treatments. Significant effects (*P* ≤ 0.05) shown in bold and *, **, indicated interaction

Variables	CT	N	CT : N
Biomass	<0.001	<0.001	<0.001
Leaf area	<0.001	<0.001	<0.001
<i>SLA</i>	<0.001	<0.001	0.854
<i>Narea</i>	<0.001	<0.001	0.02
<i>Nleaf</i>	<0.001	<0.001	<0.001
<i>PNUE</i>	0.003	<0.001	0.519
<i>RMR</i>	<0.001	<0.001	0.769
<i>SMR</i>	<0.001	<0.001	<0.001
<i>LMR</i>	0.994	<0.001	0.001
<i>A_{n-g}</i>	<0.001	<0.001	<0.001
<i>V_{cm}max</i>	<0.001	<0.001	0.014
<i>Jmax</i>	<0.001	0.004	0.683
<i>Jmax/V_{cm}max</i>	<0.001	0.078	0.616
<i>Ci-t</i>	0.22	<0.001	0.012
<i>A_{n-t}</i>	<0.001	0.002	0.256
<i>ACE</i>	<0.001	<0.001	0.001
<i>CCPaci</i>	<0.001	0.047	0.313

Note: *SLA*: specific leaf area; *N_{area}*: leaf N per unit area; *PNUE*: photosynthetic N use efficiency; *RMR*: root mass ratio; *SMR*: stem mass ratio; *LMR*: leaf mass ratio; *A_{n-g}*: net photosynthesis rate at grow *C_a* which ECT treatment at 750 μmol mol⁻¹ (*A_{n-ECT750}*) and ACT treatment at 400 μmol mol⁻¹ (*A_{n-CT400}*); *V_{cm}max*: maximum rate of ribulose-1,5-bisphosphate carboxylation; *J_{max}*: maximum of photosynthetic electron transport rate; *C_{i-t}*: intercellular [CO₂] at the transition point (*C_{i-t}*, *A_{n-t}*) between Rubisco limitation and RuBP regeneration limitation based on *A/C_i* curve; *A_{n-t}*: net photosynthesis rate at *C_{i-t}*; *ACE*: apparent carboxylation efficiency; *CCP_{aci}*: *CO₂* compensation point from *A/C_i* curve

Leaf nitrogen and photosynthetic N use efficiency

Generally, fCT generally reduced N_{area} but the effect was only statistically significant at the intermediate N supply (Table 1; Fig. 4a). The interaction significantly affected N_{area} but no clear response patterns could be identified although N_{area} appeared to increase with increasing N supply under fCT (Fig. 4a). The total N content per seedling, in contrast, increased almost linearly with increasing N supply from the lowest to the highest level under fCT but from the lowest to the second highest N level under cCT while fCT significantly increased N_{area} only at the two highest N supply levels (Fig. 4b).

The fCT significantly increased $PNUE$ (Fig. 4c) and $PNUE$ decreased with increasing N supply from the lowest (N1) to the intermediate (N3) N supplies and did not change significantly as N supply increased from N3 to N5 (Fig. 4d).

Principal component analysis (PCA)

The PCA results revealed that seedlings grown under cCT were clustered in the upper left ellipse while those in the fCT were clustered in the lower right ellipse; the upper left cluster was characterized by parameters associated with high photosynthetic capacity and high leaf N; the lower right cluster was characterized by high seedling biomass, high total leaf area, high total foliar N content and high shoot mass ratio, and those variables were negatively correlated to $PNUE$ (180 degrees in arrow direction in Fig. 5a).

PCA results also show that high $PNUE$ was closely associated with the ellipse of seedlings in the N1 treatment and was negatively correlated with parameters related to high photosynthetic capacity and high total leaf N content (Fig. 5b). With increases in N supply level, seedlings tended to cluster in the ellipse characterized by high growth parameters which were negatively correlated to RMR (arrows pointing opposite directions in Fig. 5b).

Discussion

Growth stimulation by fCT and Nitrogen

Elevated CO_2 and warmer temperature predicted for the end of the 21st century substantially increased the seedling biomass and total leaf area of yellow birch seedlings and the effects were synergistically magnified by higher nitrogen supplies. These results are in general agreement with the literature for other plant species (Hu et al. 2021; Liang et al. 2020). Plant growth is generally interactively affected by multiple factors (Green and Keenan 2022). The magnitudes of increases in total seedling biomass and total leaf area by fCT increased dramatically with increasing N supply in yellow birch seedlings. Furthermore, the increases were primarily concentrated on aboveground organs, leading to increased shoot mass ratios in seedlings grown under fCT. Higher total leaf nutrient content in fCT than cCT implies higher rates of nutrient uptake under the elevated CO_2 and warmer temperature, which presumably contributed to the higher growth.

The increases in total leaf area and shoot mass ratio represent the expansion of the canopy and growth momentum, which should permit the trees to further increase CO_2 sequestration and carbohydrate production (Norby et al. 2022). This morphological acclimation presum-

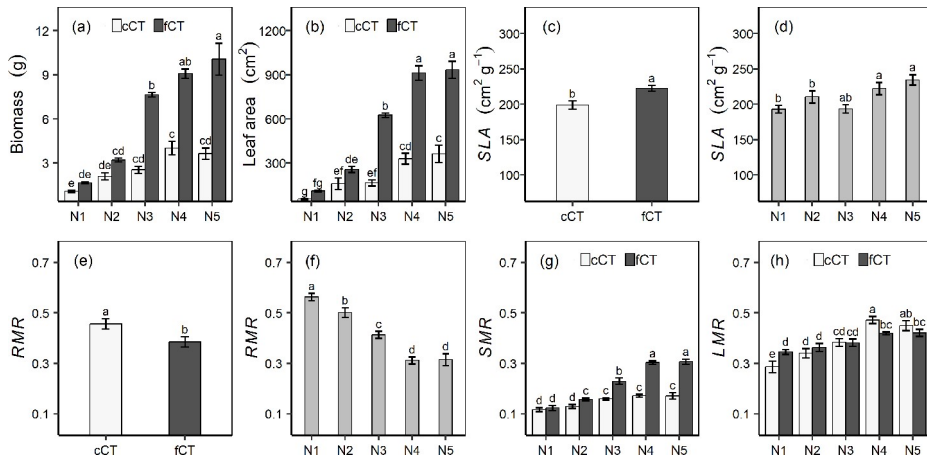


Fig. 1 Effects of CO₂ & temperature combination (CT) and nitrogen supply (N) on growth and biomass allocation of yellow birch. Biomass (a), Leaf area (b), SLA (c, d), RMR (e, f), SMR (g), LMR (h). Means (±SE, n=6 for panels a, b, g, h; n=30 for panel c, e; n=12 for panel d, f) with different letters is significantly different from each other (p≤0.05). According to the P values in Table 1, only the values with significant differences are presented

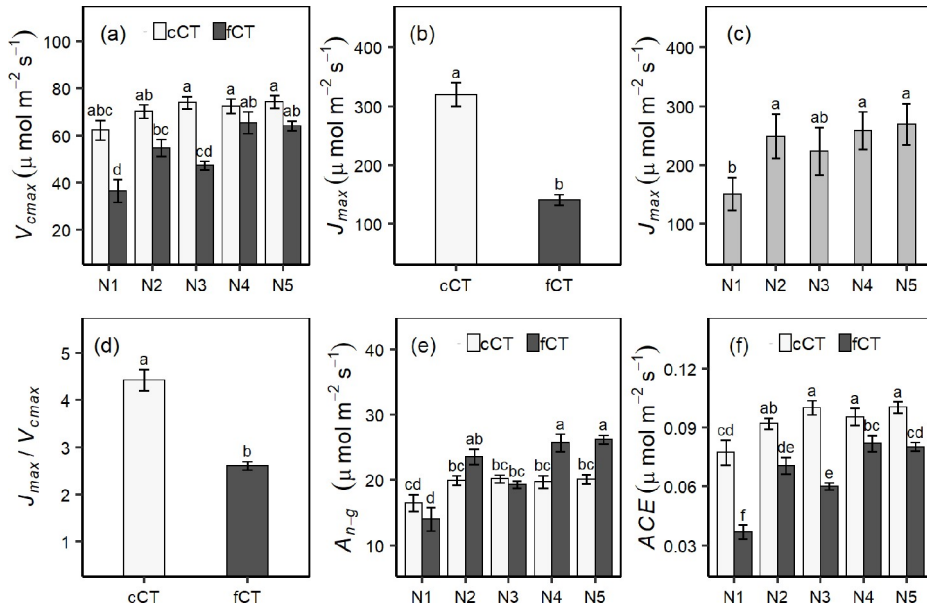


Fig. 2 Effects of CO₂ & Temperature combination (CT) and nitrogen supply (N) on V_{max} (a), J_{max} (b, d), J_{max}/V_{max} (d), A_{n-g} (e), and ACE (f) in yellow birch. Means (±SE, n=6 for panels a, e, f; n=30 for panels b, d; n=12 for panel c) with different letters are significantly different from each other (p≤0.05). When the interaction was significant (p<0.05 in Table 1), the values of each treatment combination were presented

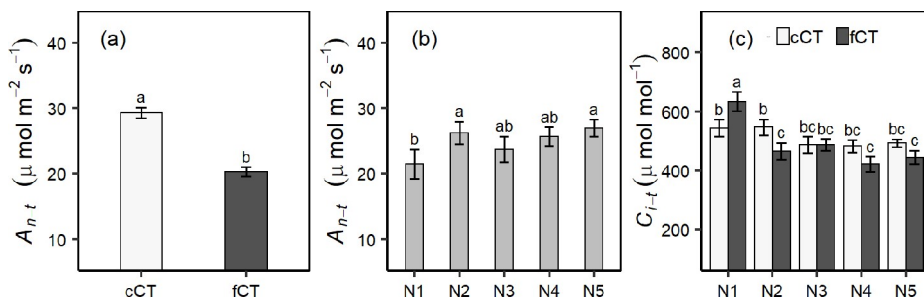


Fig. 3 Effects of CO₂ & Temperature combination (CT) and nitrogen supply (N) on transition point (C_{i-t} , A_{n-t}) between RuBP carboxylation to regeneration limitation from A/C_i of yellow birch. A_{n-t} (a, b), C_{i-t} (c). Means (\pm SE, $n=30$ for panel a; $n=12$ for panel b; $n=6$ for panel c) with different letters are significantly different from each other ($p \leq 0.05$). According to the P values in Table 1, only the values with significant differences are presented

ably compensated for the downregulation of photosynthetic capacity, leading to higher biomass production under the fCT treatment and thus more C sequestration (Cabon et al. 2022). This acclimation strategy, i.e., coordinated physiological and morphological acclimations, may represent an optimal carbon acquisition under future climate conditions (Smith and Keenan 2020). Our results indicate that this acclimation strategy may be further magnified by increases in soil nutrient availability. The PCA results show that yellow birch seedlings grown under higher nutrient supplies clustered in a direction that is more favorable for growth and photosynthesis. Furthermore, our results indicate that sufficiently high soil N supplies could stimulate the growth and photosynthesis of yellow birch to a much greater extent under the predicted future CO₂ and temperature than the current conditions. Such synergistical effects may increase the competitiveness of the species on nutrient rich sites in the future. In contrast, the result that fCT and N deficiency both increase *NUE* suggests that the species may be more tolerant of low N supplies in the future. The contrast effects of fCT and N on *NUE*, photosynthesis and growth are further demonstrated by the PCA results. These results suggest that increases in soil nutrient availability will likely synergistically increase the growth of yellow birch under the predicted future CO₂ and temperature.

Photosynthetic acclimation was affected by leaf nitrogen concentration

Our data show that the combination of elevated CO₂ and warmer temperature led to the down-regulation of photosynthetic capacity in yellow birch, which is consistent with the effect of elevated CO₂ alone reported by other studies (Kanno et al. 2017; Ruiz-Vera et al. 2017). The decline in the photosynthetic capacity of yellow birch seemed to be more attributable to the downregulation of J_{max} than to that of V_{cmax} , because there was no significant difference in V_{cmax} between fCT and cCT treatments under N limited conditions (i.e., N1) and there were sharp declines in J_{max} in seedlings grown under fCT (relative to those grown under cCT). Our results lend support to the theory that photosynthetic downregulation tends to occur when N supply is insufficient (Vicente et al. 2016).

Photosynthetic downregulation generally occurs when increases in growth stimulated by a factor lead to dilutions in leaf N concentration (Wujeska-Klaue et al. 2019). Therefore, photosynthetic downregulation should be prevented or reduced if N supply is increased as

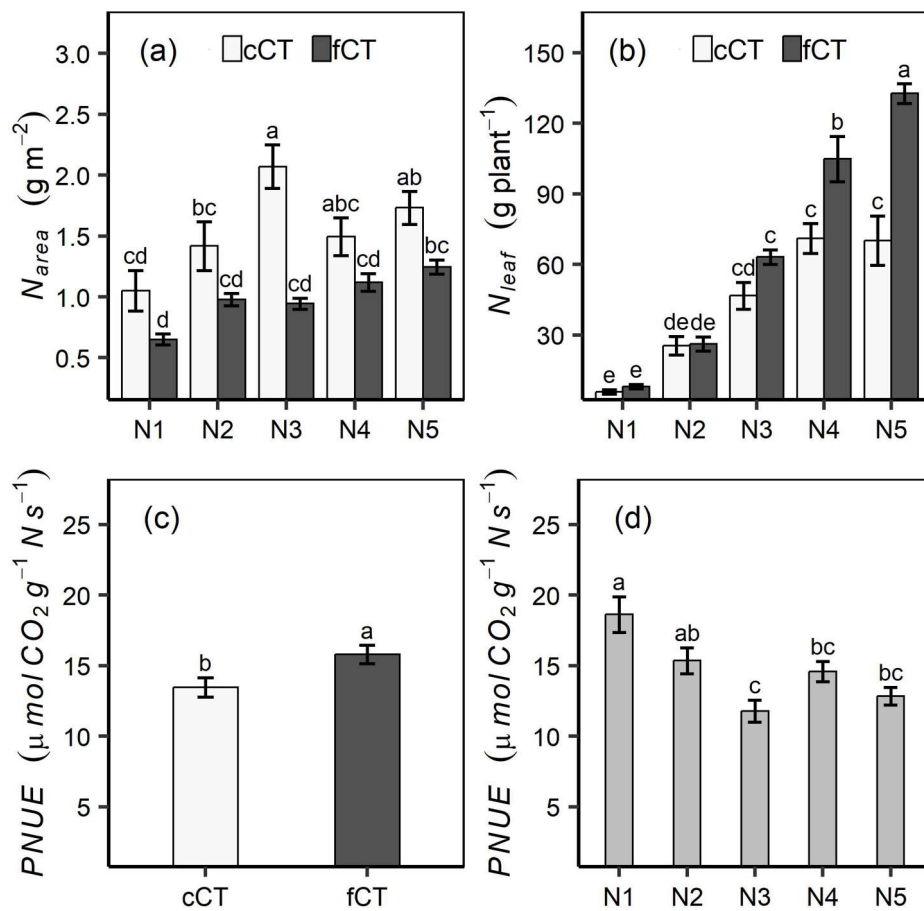


Fig. 4 Effects of CO₂ & Temperature combination (CT) and nitrogen supply (N) on leaf N concentration (N_{area} (a)), total plant leaf N content (N_{leaf} (b)) and photosynthetic nitrogen use efficacy (PNUE, (c) and (d)). (Means \pm SE, n=6 for panel a and b; n=30 for panel c; n=12 for panel d) with different letters are significantly different from each other (p \leq 0.05). According to the P values in Table 1, only the values with significant differences are presented

growth increases to prevent or reduce the dilution of leaf N as demonstrated in this study. The fact that a large portion of leaf N is associated with the photosynthetic enzyme Rubisco explains the positive correlation between leaf N concentration and photosynthetic capacity in this and other studies (Luo et al. 2021). However, the increases in N supply in this study were not high to eliminate the diluting effect of increased seedling growth under fCT, leading to decreases in both leaf N concentration and photosynthetic capacity even in seedlings exposed to the highest N supply in this study.

The photosynthetic acclimation and changes in leaf N concentration also reflect the interaction between CO₂ fixation and N assimilation in plants. On the one hand, photosynthesis requires proteins provided by N assimilation. On the other hand, N assimilation requires the C skeleton, energy, and reductants produced by photosynthesis (Zhao et al. 2021). This mutual supply-demand relationship complicates the relationship between elevated CO₂ and

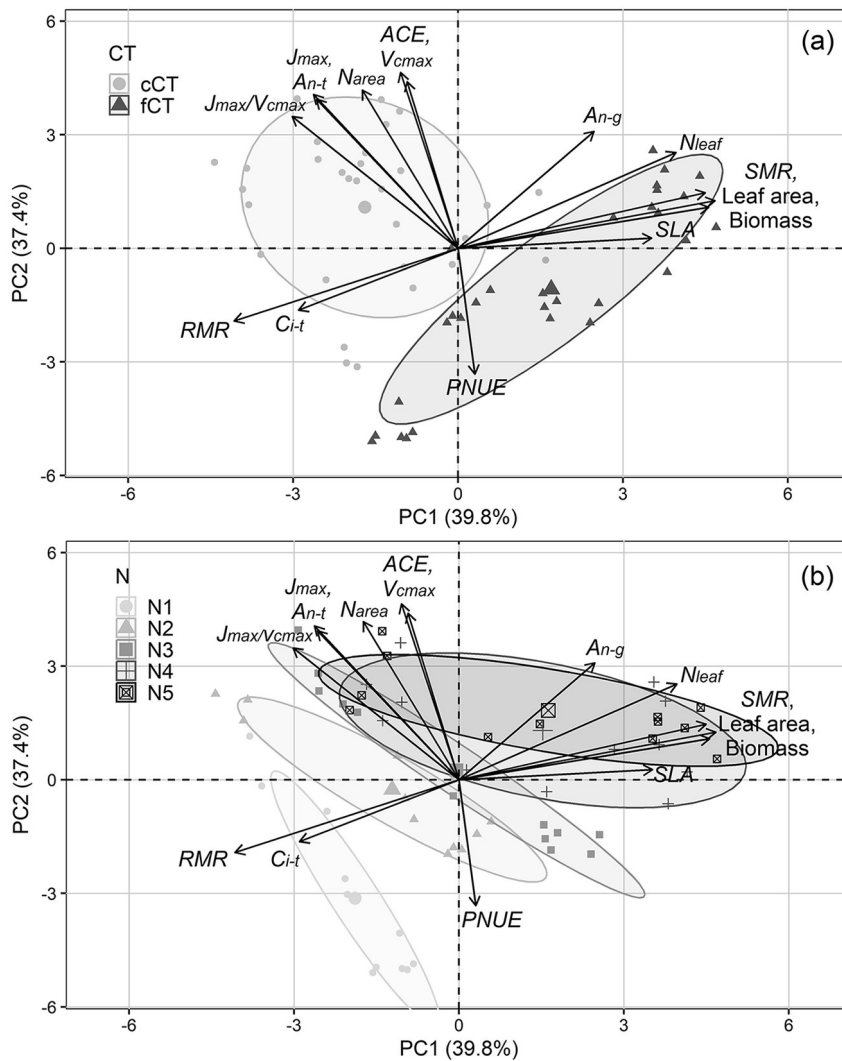


Fig. 5 Principal Component Analysis (PCA, using FactoMineR package in R) of growth and photosynthetic parameters. The biaxial diagrams were generated by the factoextra package in R. The ellipses indicate CO₂ & Temperature combination treatment (CT) clustering, cCT and fCT represented current and future CT conditions (a), while the ellipses indicate nitrogen (N) treatments clustering, N1 to N5 represents from lower to higher levels (in degree of gray) of nitrogen supply (b). The parameters within the same ellipse were closely correlated with each other; parameters with arrows pointing to similar directions were positively correlated while those with arrows pointing to opposite directions were inversely correlated to each other; a smaller angle between two arrow lines indicates a closer correlation between the two parameters. Abbreviations: *RMR*: root mass ratio; *SMR*: stem mass ratio; *LMR*: leaf mass ratio; *N_{area}*: leaf N per unit area; *N_{leaf}*: total N of the whole-plant leaf; *PNUE*: photosynthetic nitrogen use efficiency; *A_{n-g}*: net photosynthesis rate at grow C_a which fCT treatment at 750 μmol mol⁻¹ (*A_{n-gCT750}*) and cCT treatment at 400 μmol mol⁻¹ (*A_{n-gCT400}*); *V_{cmax}*: maximum rate of ribulose-1,5-bisphosphate carboxylation; *J_{max}*: maximum of photosynthetic electron transport rate; *A_{n-t}*: net photosynthesis rate at transition point (*C_{i-t}*, *A_{n-t}*) between Rubisco limitation and RuBP regeneration limitation based on *A/C_i* curve; *ACE*: apparent carboxylation efficiency

N assimilation (Rubio-Asensio and Bloom 2017). This relationship may have contributed to the opposite effects of increased N supply and elevated CO₂ on *PNUE* as well as the different directions of ovals in the PCA results in this study.

Electron transport was the primary contributor to photosynthetic limitation under fCT

The precipitous descent of J_{max} (55.9%) by fCT treatment resulted in a significant decrease in J_{max}/V_{cmax} . This result suggests that under the combination of elevated CO₂ and warmer temperature, photosynthesis of yellow birch might have been primarily limited by electron transport rather than Rubisco carboxylation, as suggested by (Smith and Keenan 2020). J_{max}/V_{cmax} reflects the partitioning of N between electron transport and carbon fixation (Zhou et al. 2015) and the shift of primary photosynthetic limitation (Walker et al. 2014). It is found that elevated CO₂ tends to increase J_{max}/V_{cmax} (Smith and Keenan 2020) while warming generally decreases J_{max}/V_{cmax} (Fernández-Marín et al. 2020; Stefanski et al. 2020). The fact that the J_{max}/V_{cmax} ratio declined under fCT in this study suggests that thermal acclimation was the predominant response mechanism to fCT in yellow birch. The results suggest that climate warming may weaken the effects of elevated CO₂ on plant acclimation and may even totally offset the effect of elevated CO₂ (Sharwood et al. 2017). Since elevated CO₂ and warming are coupled to each other under the predicted future climate conditions, the photosynthesis of yellow birch may be more limited by electron transport as suggested by the result of this study and those of some other studies (Slot et al. 2021).

J_{max} and A_{n-t} were closely related to each other and showed similar response patterns to all the treatments in this study. A_{n-t} represents the photosynthetic rate at the transition point of the primary limitation to photosynthesis between Rubisco carboxylation and RuBP regeneration. Its close relationship with J_{max} implies the dominant role of J_{max} in the transition process under fCT treatment (Stefanski et al. 2020). For a given tree species and under certain environmental conditions, the transition point of photosynthetic limitation is generally a function of V_{cmax} , J_{max} , J_{max}/V_{cmax} , g_m , and R_d (Miao et al. 2009). Therefore, our results appear to suggest that the fCT-induced shift of photosynthetic limitation in yellow birch was primarily a result of the decline in J_{max} .

Conclusions

This study investigated the growth and photosynthetic characteristics of yellow birch exposed to simulated future CO₂ and temperature (fCT) under different levels of N supply. The results show that fCT and higher nitrogen supply had synergistical effects on seedling biomass production and growth momentum as indicated by the total leaf area, suggesting that yellow birch trees growing on nutrient rich sites will likely benefit more from the elevated CO₂ and warmer temperature in the future than those growing on nutrient poor sites. The results also suggest that fertilization may be more financially viable in the future for growing yellow birch. The synergistical effects are attributable to both morphological and physiological acclimations. Morphologically, trees grown under fCT and higher nitrogen had greater total leaf area per tree, greater specific leaf area, and greater total amount of leaf nitrogen. Physiologically, the trees grown in fCT and higher nitrogen supplies had smaller

downregulation of photosynthetic capacity and thus benefited more from the stimulation of photosynthesis by the elevated CO₂. Furthermore, fCT significantly increased the nitrogen use efficiency of photosynthesis, making leaf nitrogen more productive in carbohydrate production. Lastly, fCT shifted the primary limitation of photosynthesis from Rubisco carboxylation to RuBP regeneration and thermal acclimation was the primary mechanism for this shift.

Acknowledgements We want to thank Ms. Keri Pidgen, the Greenhouse Manager, for her logistic support and other operational assistance during the experiments. The study was supported by an NSERC Discovery Development Grant to QL Dang (Project No. DDG-2020-00008).

Author contributions QL Dang initiated, funded the project, and supervised the others during the entire process; GR Wand and JP Zheng planned and executed the experiment and carried out all the measurements; L Wang authored the manuscript, including all the figures.

Declarations

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

Competing interests The authors declare no competing interests.

References

- Andrews M, Condrón LM, Kemp PD, Topping JF, Lindsey K, Hodge S, Raven JA (2019) Elevated CO₂ effects on nitrogen assimilation and growth of C₃ vascular plants are similar regardless of N-form assimilated. *J Exp Bot* 70:683–690. <https://doi.org/10.1093/jxb/ery371>
- Apgaua DMG, Tng DYP, Forbes SJ, Ishida YF, Vogado NO, Cernusak LA, Laurance SGW (2019) Elevated temperature and CO₂ cause differential growth stimulation and drought survival responses in eucalypt species from contrasting habitats. *Tree Physiol* 39:1806–1820. <https://doi.org/10.1093/treephys/tpz095>
- C MGR, T JHM, MA DC (1998) UK conifer forests may be growing faster in response to increased N deposition, atmospheric CO₂ and temperature. *Forestry* 71
- Cabon A, Kannenberg SA, Arain A, Babst F, Baldocchi D, Belmecheri S, Delpierre N, Guerrieri R, Maxwell JT, McKenzie S, Meinzer FC, Moore DJP, Pappas C, Rocha AV, Szejner P, Ueyama M, Ulrich D, Vincke C, Voelker SL, Wei J, Woodruff D, Anderegg WRL (2022) Cross-biome synthesis of source versus sink limits to tree growth. *Science* 376:758–761. <https://doi.org/10.1126/science.abm4875>
- Cai C, Li G, Yang H, Yang J, Liu H, Struik PC, Luo W, Yin X, Di L, Guo X, Jiang W, Si C, Pan G, Zhu J (2018) Do all leaf photosynthesis parameters of rice acclimate to elevated CO₂, elevated temperature, and their combination, in FACE environments? *Glob Chang Biol* 24:1685–1707. <https://doi.org/10.1111/gcb.13961>
- Cao B, Dang Q, Zhang S (2007) Relationship between photosynthesis and leaf nitrogen concentration in ambient and elevated [CO₂] in white birch seedlings. *Tree Physiol* 27:891–899. <https://doi.org/10.1093/treephys/27.6.891>
- Cox RM, Zhu XB (2003) Effects of simulated thaw on xylem cavitation, residual embolism, spring dieback and shoot growth in yellow birch. *Tree Physiol* 23:615–624. <https://doi.org/10.1093/treephys/23.9.615>
- Dai Z, Yu M, Chen H, Zhao H, Huang Y, Su W, Xia F, Chang SX, Brookes PC, Dahlgren RA, Xu J (2020) Elevated temperature shifts soil N cycling from microbial immobilization to enhanced mineralization, nitrification and denitrification across global terrestrial ecosystems. *Glob Chang Biol* 26:5267–5276. <https://doi.org/10.1111/gcb.15211>
- Delagrèze S, Messier C, Lechowicz MJ, Dizengremel P (2004) Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. *Tree Physiol* 24:775–784. <https://doi.org/10.1093/treephys/24.7.775>
- Dusenge ME, Duarte AG, Way DA (2019) Plant carbon metabolism and climate change: elevated CO₂ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytol* 221:32–49. <https://doi.org/10.1111/nph.15283>

- Dusenge ME, Madhavji S, Way DA (2020) Contrasting acclimation responses to elevated CO₂ and warming between an evergreen and a deciduous boreal conifer. *Glob Chang Biol* 26:3639–3657. <https://doi.org/10.1111/gcb.15084>
- Duursma RA (2015) *Plantecophys—An R Package for Analysing and Modelling Leaf Gas Exchange Data*. PLoS ONE 10:e0143346. <https://doi.org/10.1371/journal.pone.0143346>
- Fernández-Marín B, Gullás J, Figueroa CM, Iñiguez C, Clemente-Moreno MJ, Nunes-Nesi A, Fernie AR, Cavieres LA, Bravo LA, García-Plazaola JI, Gago J (2020) How do vascular plants perform photosynthesis in extreme environments? An integrative ecophysiological and biochemical story. *Plant J* 101:979–1000. <https://doi.org/10.1111/tpj.14694>
- García-Palacios P, Vandegehuchte ML, Shaw EA, Dam M, Post KH, Ramirez KS, Sylvain ZA, de Tomasel CM, Wall DH (2015) Are there links between responses of soil microbes and ecosystem functioning to elevated CO₂, N deposition and warming? A global perspective. *Glob Chang Biol* 21:1590–1600. <https://doi.org/10.1111/gcb.12788>
- Green JK, Keenan TF (2022) The limits of forest carbon sequestration. *Science* 376:692–693. <https://doi.org/10.1126/science.abc6547>
- Hu S, Wang Y, Yang L (2021) Response of rice yield traits to elevated atmospheric CO₂ concentration and its interaction with cultivar, nitrogen application rate and temperature: a meta-analysis of 20 years FACE studies. *Sci Total Environ* 764:142797. <https://doi.org/10.1016/j.scitotenv.2020.142797>
- Jauregui I, Aroca R, Garnica M, Zamarreño AM, García-Mina JM, Serret MD, Parry M, Irigoyen JJ, Aranjuelo I (2015) Nitrogen assimilation and transpiration: key processes conditioning responsiveness of wheat to elevated CO₂ and temperature. *Physiol Plant* 155:338–354. <https://doi.org/10.1111/ppl.12345>
- Jayawardena DM, Heckathorn SA, Bista DR, Boldt JK (2019) Elevated carbon dioxide plus chronic warming causes dramatic increases in leaf angle in tomato, which correlates with reduced plant growth. *Plant Cell Environ* 42:1247–1256. <https://doi.org/10.1111/pce.13489>
- Kanno K, Suzuki Y, Makino A (2017) A small decrease in Rubisco Content by Individual suppression of RBCS genes leads to Improvement of Photosynthesis and Greater Biomass production in Rice under Conditions of elevated CO₂. *Plant Cell Physiol* 58:635–642. <https://doi.org/10.1093/pcp/pcx018>
- Karst J, Wasyliw J, Birch JD, Franklin J, Chang SX, Erbilgin N (2021) Long-term nitrogen addition does not sustain host tree stem radial growth but doubles the abundance of high-biomass ectomycorrhizal fungi. *Glob Chang Biol* 27:4125–4138. <https://doi.org/10.1111/gcb.15713>
- Kim D, Medvigy D, Maier CA, Johnsen K, Palmroth S (2019) Biomass increases attributed to both faster tree growth and altered allometric relationships under long-term carbon dioxide enrichment at a temperate forest. *Glob Chang Biol*. <https://doi.org/10.1111/gcb.14971>
- Lavoie J-M, Stevanovic T (2005) Variation of chemical composition of the lipophilic extracts from yellow birch (*Betula alleghaniensis*) foliage. *J Agric Food Chem* 53:4747–4756. <https://doi.org/10.1021/jf050301y>
- Liang X, Zhang T, Lu X, Ellsworth DS, BassiriRad H, You C, Wang D, He P, Deng Q, Liu H, Mo J, Ye Q (2020) Global response patterns of plant photosynthesis to nitrogen addition: a meta-analysis. *Glob Chang Biol* 26:3585–3600. <https://doi.org/10.1111/gcb.15071>
- Luo X, Keenan TF, Chen JM, Croft H, Colin Prentice I, Smith NG, Walker AP, Wang H, Wang R, Xu C, Zhang Y (2021) Global variation in the fraction of leaf nitrogen allocated to photosynthesis. *Nat Commun* 12:4866. <https://doi.org/10.1038/s41467-021-25163-9>
- Maxwell TL, Canarini A, Bogdanovic I, Böckle T, Martin V, Noll L, Prommer J, Séneca J, Simon E, Piepho H-P, Herndl M, Pötsch EM, Kaiser C, Richter A, Bahn M, Wanek W (2022) Contrasting drivers of belowground nitrogen cycling in a montane grassland exposed to a multifactorial global change experiment with elevated CO₂, warming, and drought. *Glob Chang Biol* 28:2425–2441. <https://doi.org/10.1111/gcb.16035>
- Miao Z, Xu M, Lathrop RG, Wang Y (2009) Comparison of the A-Cc curve fitting methods in determining maximum ribulose 1.5-bisphosphate carboxylase/oxygenase carboxylation rate, potential light saturated electron transport rate and leaf dark respiration. *Plant Cell Environ* 32:109–122. <https://doi.org/10.1111/j.1365-3040.2008.01900.x>
- Newaz SM, Dang Q-L, Man R (2021) CO₂ elevation and soil warming reduce cold hardiness of jack pine under photoperiods of seed origin and latitudes of potential migration. *New Forest* 52:777–790. <https://doi.org/10.1007/s11056-020-09831-0>
- Norby RJ, Warren JM, Iversen CM, Childs J, Jawdy SS, Walker AP (2022) Forest stand and canopy development unaltered by 12 years of CO₂ enrichment. *Tree Physiol* 42:428–440. <https://doi.org/10.1093/treephys/tpab107>
- Noyce GL, Kirwan ML, Rich RL, Megonigal JP (2019) Asynchronous nitrogen supply and demand produce nonlinear plant allocation responses to warming and elevated CO₂. *Proc Natl Acad Sci U S A* 116:21623–21628. <https://doi.org/10.1073/pnas.1904990116>

- Pastore MA, Lee TD, Hobbie SE, Reich PB (2020) Interactive effects of elevated CO₂, warming, reduced rainfall, and nitrogen on leaf gas exchange in five perennial grassland species. *Plant Cell Environ* 43:1862–1878. <https://doi.org/10.1111/pce.13783>
- Penuelas J, Fernández-Martínez M, Vallicrosa H, Maspons J, Zuccarini P, Carnicer J, Sanders TGM, Krüger I, Obersteiner M, Janssens IA, Ciais P, Sardans J (2020) Increasing atmospheric CO₂ concentrations correlate with declining nutritional status of European forests. *Commun Biol* 3:125. <https://doi.org/10.1038/s42003-020-0839-y>
- Rubio-Asensio JS, Bloom AJ (2017) Inorganic nitrogen form: a major player in wheat and Arabidopsis responses to elevated CO₂. *J Exp Bot* 68:2611–2625. <https://doi.org/10.1093/jxb/erw465>
- Ruiz-Vera UM, de Souza AP, Long SP, Ort DR (2017) The role of Sink Strength and Nitrogen availability in the down-regulation of photosynthetic capacity in field-grown *Nicotiana tabacum* L. at elevated CO₂ concentration. *Front Plant Sci* 8:998. <https://doi.org/10.3389/fpls.2017.00998>
- Ryan MG (2013) Three decades of research at Flakaliden advancing whole-tree physiology, forest ecosystem and global change research. *Tree Physiol* 33:1123–1131. <https://doi.org/10.1093/treephys/tp1100>
- Sharwood RE, Crous KY, Whitney SM, Ellsworth DS, Ghannoum O (2017) Linking photosynthesis and leaf N allocation under future elevated CO₂ and climate warming in *Eucalyptus globulus*. *J Exp Bot* 68:1157–1167. <https://doi.org/10.1093/jxb/erw484>
- Shrestha RK, Strahm BD, Sucre EB (2015) Greenhouse gas emissions in response to nitrogen fertilization in managed forest ecosystems. *New Forest* 46:167–193. <https://doi.org/10.1007/s11056-014-9454-4>
- Slot M, Rifai SW, Winter K (2021) Photosynthetic plasticity of a tropical tree species, *Tabebuia rosea*, in response to elevated temperature and CO₂. *Plant Cell Environ* 44:2347–2364. <https://doi.org/10.1111/pce.14049>
- Smith NG, Keenan TF (2020) Mechanisms underlying leaf photosynthetic acclimation to warming and elevated CO₂ as inferred from least-cost optimality theory. *Glob Chang Biol* 26:5202–5216. <https://doi.org/10.1111/gcb.15212>
- Sperry SJ, Venturas DM, Todd NH, Trugman TA, Anderegg RLW, Wang Y, Tai X (2019) The impact of rising CO₂ and acclimation on the response of US forests to global warming. *Proc Natl Acad Sci U S A* 116:25734–25744
- Stefanski A, Bermudez R, Sendall KM, Montgomery RA, Reich PB (2020) Surprising lack of sensitivity of biochemical limitation of photosynthesis of nine tree species to open-air experimental warming and reduced rainfall in a southern boreal forest. *Glob Chang Biol* 26:746–759. <https://doi.org/10.1111/gcb.14805>
- Tausz-Posch S, Tausz M, Bourgault M (2020) Elevated CO₂ effects on crops: advances in understanding acclimation, nitrogen dynamics and interactions with drought and other organisms. *Plant Biol (Stuttg)* 22(Suppl 1):38–51. <https://doi.org/10.1111/plb.12994>
- Vicente R, Pérez P, Martínez-Carrasco R, Feil R, Lunn JE, Watanabe M, Arrivault S, Stitt M, Hoefgen R, Morcuende R (2016) Metabolic and transcriptional analysis of Durum Wheat responses to elevated CO₂ at low and high nitrate supply. *Plant Cell Physiol* 57:2133–2146. <https://doi.org/10.1093/pcp/pcw131>
- Walker AP, Beckerman AP, Gu L, Kattge J, Cernusak LA, Domingues TF, Scales JC, Wohlfahrt G, Wulfschleger SD, Woodward FI (2014) The relationship of leaf photosynthetic traits - V_{cmax} and J_{max} - to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. *Ecol Evol* 4:3218–3235. <https://doi.org/10.1002/ece3.1173>
- Wang L, Zheng J, Wang G, Dang Q-L (2022a) Combined effects of elevated CO₂ and warmer temperature on limitations to photosynthesis and carbon sequestration in yellow birch. *Tree Physiol*. <https://doi.org/10.1093/treephys/tpac128>
- Wang L, Zheng J, Wang G, Dang Q-L (2022b) Increased leaf area compensated photosynthetic downregulation in response to elevated CO₂ and warming in white birch. *Can J Res* 52:1176–1185. <https://doi.org/10.1139/cjfr-2022-0076>
- Wujeska-Klaue A, Crous KY, Ghannoum O, Ellsworth DS (2019) Lower photorespiration in elevated CO₂ reduces leaf N concentrations in mature *Eucalyptus* trees in the field. *Glob Chang Biol*. <https://doi.org/10.1111/gcb.14555>
- Zhao H-L, Chang T-G, Xiao Y, Zhu X-G (2021) Potential metabolic mechanisms for inhibited chloroplast nitrogen assimilation under high CO₂. *Plant Physiol* 187:1812–1833. <https://doi.org/10.1093/plphys/kiab345>
- Zhou H, Xu M, Pan H, Yu X (2015) Leaf-age effects on temperature responses of photosynthesis and respiration of an alpine oak, *Quercus aquifolioides*, in southwestern China. *Tree Physiol* 35:1236–1248. <https://doi.org/10.1093/treephys/tpv101>

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.