



# Nitrogen supply influences photosynthetic acclimation of yellow birch (*Betula costata* Trautv.) to the combination of elevated CO<sub>2</sub> and warmer temperature

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## Abstract

Rising CO<sub>2</sub>, global warming, and N deposition create challenging environmental conditions to vegetation. Since elevated CO<sub>2</sub> 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 CO<sub>2</sub> and temperature) and the predicted future CO<sub>2</sub> and temperature conditions (fCT: elevated CO<sub>2</sub>, 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 CO<sub>2</sub> · Global warming · Yellow birch (*Betula costata* Trautv.) · Photosynthetic acclimation · Nitrogen use efficiency

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## Introduction

It is predicted that the atmospheric CO<sub>2</sub> 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 CO<sub>2</sub> 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). CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> and temperature (Ryan 2013). Furthermore, elevated CO<sub>2</sub> and warmer temperature can accelerate the mineralization rates of soil nutrients but reduce nutrient uptake by plants (Dai et al. 2020). However, elevated CO<sub>2</sub> can cause a reduction in leaf N concentration because elevated CO<sub>2</sub> increases biomass production and thus dilutes leaf N (Andrews et al. 2019). Furthermore, elevated CO<sub>2</sub> can reduce photorespiration and thus lead to reductions in N assimilation and NO<sub>3</sub><sup>-</sup> metabolism which are associated with photorespiration (Wujeska-Klaue et al. 2019). While warming alone or in combination with elevated CO<sub>2</sub> reduce N uptake, warming can lead to increases in leaf N concentration (Sharwood et al. 2017). However, the interactive effects of elevated CO<sub>2</sub> 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 (Delagrange 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 CO<sub>2</sub> and temperature under different N availabilities. We tested the hypotheses that birch trees growing under higher nitrogen supplies would benefit more from elevated CO<sub>2</sub> and warmer temperature and that trees would be more tolerant of low nitrogen stress when growing under elevated CO<sub>2</sub> 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 CO<sub>2</sub>-temperature combination treatment as the whole plot and nitrogen supply as the split plot. The current CO<sub>2</sub>-temperature treatment (cCT) was set at 400 μmol mol<sup>-1</sup> CO<sub>2</sub> and 25°C/16°C day/night temperatures while the future CO<sub>2</sub>-temperature treatment (fCT) was set at 750 μmol mol<sup>-1</sup> CO<sub>2</sub> 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 CO<sub>2</sub> elevation and the 4 °C warming in the fCT treatment were the predicted conditions at 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<sup>-1</sup> 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 (NH<sub>4</sub>NO<sub>3</sub>) 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<sup>-1</sup> P, 150 μmol mol<sup>-1</sup> K, 4 ml L<sup>-1</sup> 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, CO<sub>2</sub>, and humidity) were monitored and controlled with an Argus Titan Environment-Control System (Argus Control Systems Ltd, Vancouver, BC, Canada). The CO<sub>2</sub> elevation was achieved using CO<sub>2</sub> 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,  $\mu\text{mol mol}^{-1} \text{CO}_2$  ( $C_a$ ) 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<sup>-1</sup>) and a built-in CFM-3 Chlorophyll Fluorescence Module (PP Systems International, Inc. Amesbury, MA, USA). The photosynthetic rate at growth (i.e. treatment) CO<sub>2</sub> ( $A_{n-g}$ ) was measured at the beginning of the  $A/C_i$  curve measurement at 400  $\mu\text{mol mol}^{-1}$  and 750  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub>, 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 CO<sub>2</sub> compensation point ( $CCP_{aci}$ ). The transition point ( $C_{i-t}$ ,  $A_{n-t}$ ) 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 ( $N_{area}$ ,  $\text{g m}^{-2}$ ) was calculated by dividing the mass-based leaf N concentration ( $N_{mass}$ ,  $\text{mg g}^{-1}$ ) by the specific leaf area. The total leaf N

content per plant ( $N_{leaf}$ ,  $\text{mg plant}^{-1}$ ) was calculated by multiplying  $N_{mass}$  ( $\text{mg g}^{-1}$ ) by the total leaf mass of the seedling ( $\text{g}^{-1}$ ). 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  $\text{CO}_2$  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  $\text{CO}_2$ -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 CO<sub>2</sub> and temperature treatments (CO<sub>2</sub>&T) on the growth, photosynthesis, and whole-plant variables of yellow birch seedlings. The nitrogen treatments included 5 N levels and the CO<sub>2</sub>&T comprised AET (ambient [CO<sub>2</sub>] (400 μmol mol<sup>-1</sup>) and temperature) and ECT (elevated [CO<sub>2</sub>] (750 μmol mol<sup>-1</sup>) 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
SLA	<0.001	<0.001	0.854
Narea	<0.001	<0.001	<b>0.02</b>
Nleaf	<0.001	<0.001	<0.001
PNUE	<b>0.003</b>	<0.001	0.519
RMR	<0.001	<0.001	0.769
SMR	<0.001	<0.001	<0.001
LMR	0.994	<0.001	<b>0.001</b>
A <sub>n-g</sub>	<0.001	<0.001	<0.001
V <sub>cmax</sub>	<0.001	<0.001	<b>0.014</b>
J <sub>max</sub>	<0.001	<b>0.004</b>	0.683
J <sub>max</sub> /V <sub>cmax</sub>	<0.001	0.078	0.616
Ci-I	0.22	<0.001	<b>0.012</b>
A <sub>n-I</sub>	<0.001	<b>0.002</b>	0.256
ACE	<0.001	<0.001	<b>0.001</b>
CCPact	<0.001	<b>0.047</b>	0.313

Note: SLA: specific leaf area; N<sub>area</sub>: leaf N per unit area; PNUE: photosynthetic N use efficiency; RMR: root mass ratio; SMR: stem mass ratio; A<sub>n-g</sub>: net photosynthesis rate at grow C<sub>d</sub> which ECT treatment at 750 μmol mol<sup>-1</sup> (A<sub>n-ECT750</sub>) and ACT treatment at 400 μmol mol<sup>-1</sup> (A<sub>n-ACT400</sub>); V<sub>cmax</sub>: maximum rate of ribulose-1,5-bisphosphate carboxylation; J<sub>max</sub>: maximum of photosynthetic electron transport rate; C<sub>i-I</sub>: intercellular [CO<sub>2</sub>] at the transition point (C<sub>i-r</sub>, A<sub>n-r</sub>) between Rubisco limitation and RuBP regeneration limitation based on A/C<sub>i</sub> curve; A<sub>n-r</sub>: net photosynthesis rate at C<sub>i-r</sub>; ACE: apparent carboxylation efficiency; CCPact: CO<sub>2</sub> compensation point from A/C<sub>i</sub> 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).

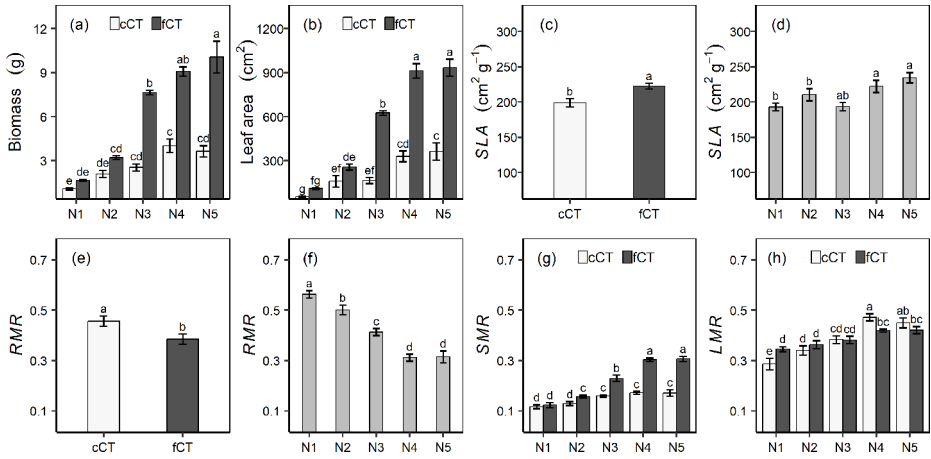
PCA results also show that high  $PNUE$  was closely associated with the ellipse of seedlings in the N1 treatment and was negatively correlated with parameters related to high photosynthetic capacity and high total leaf N content (Fig. 5b). With increases in N supply level, seedlings tended to cluster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

## Discussion

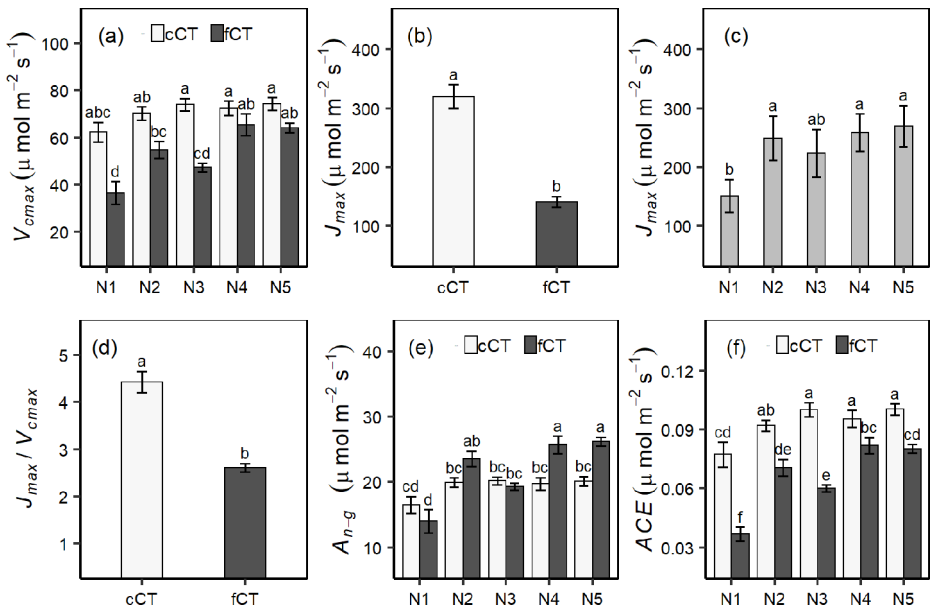
### Growth stimulation by fCT and Nitrogen

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 presu-

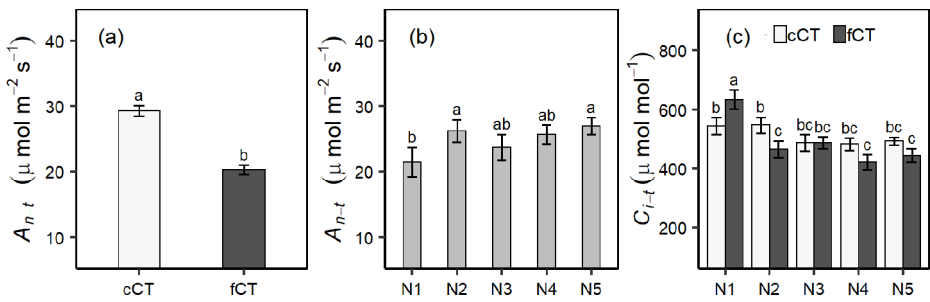


**Fig. 1** Effects of CO<sub>2</sub> & temperature combination (CT) and nitrogen supply (N) on growth and biomass allocation of yellow birch. Biomass (a), Leaf area (b), SLA (c, d), RMR (e, f), SMR (g), LMR (h). Means (±SE, n=6 for panels a, b, g, h; n=30 for panel c, e; n=12 for panel d, f) with different 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 CO<sub>2</sub> & 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





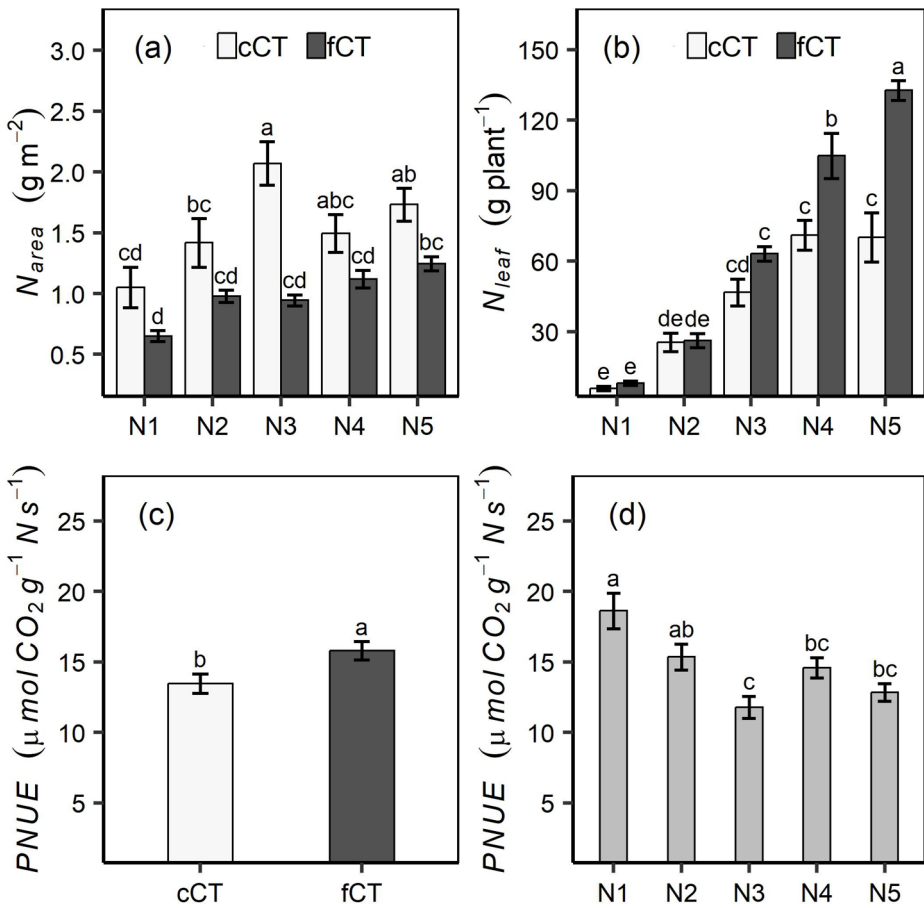
**Fig. 3** Effects of CO<sub>2</sub> & Temperature combination (CT) and nitrogen supply (N) on transition point ( $C_{i-t}$ ,  $A_{n-t}$ ) between RuBP carboxylation to regeneration limitation from  $A/C_i$  of yellow birch.  $A_{n-t}$  (a, b),  $C_{i-t}$  (c). Means ( $\pm$ SE,  $n=30$  for panel a;  $n=12$  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 CO<sub>2</sub> 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 CO<sub>2</sub> and temperature.

### Photosynthetic acclimation was affected by leaf nitrogen concentration

Our data show that the combination of elevated CO<sub>2</sub> and warmer temperature led to the down-regulation of photosynthetic capacity in yellow birch, which is consistent with the effect of elevated CO<sub>2</sub> 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).

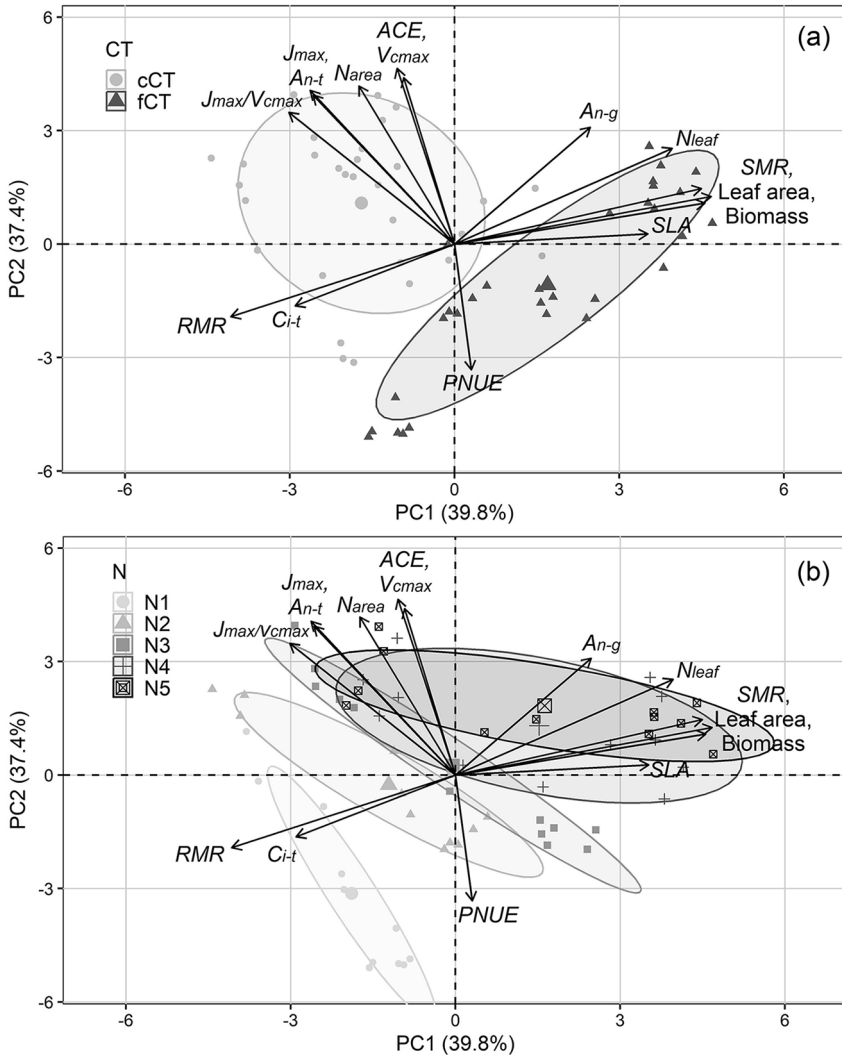
Photosynthetic downregulation generally occurs when increases in growth stimulated by a factor lead to dilutions in leaf N concentration (Wujeska-Klaue et al. 2019). Therefore, photosynthetic downregulation should be prevented or reduced if N supply is increased as



**Fig. 4** Effects of CO<sub>2</sub> & 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> & 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<sub>area</sub>*: leaf N per unit area; *N<sub>leaf</sub>*: total N of the whole-plant leaf; *PNUE*: photosynthetic nitrogen use efficiency; *A<sub>n-g</sub>*: net photosynthesis rate at grow *C<sub>a</sub>* which fCT treatment at 750 μmol mol<sup>-1</sup> (*A<sub>n-gCT750</sub>*) and cCT treatment at 400 μmol mol<sup>-1</sup> (*A<sub>n-gCT400</sub>*); *V<sub>cmax</sub>*: maximum rate of ribulose-1,5-bisphosphate carboxylation; *J<sub>max</sub>*: maximum of photosynthetic electron transport rate; *A<sub>n-t</sub>*: net photosynthesis rate at transition point (*C<sub>i-t</sub>*, *A<sub>n-t</sub>*) between Rubisco limitation and RuBP regeneration limitation based on *A/C<sub>i</sub>* 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> on plant acclimation and may even totally offset the effect of elevated CO<sub>2</sub> (Sharwood et al. 2017). Since elevated CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub>. 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.

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## Declarations

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

**Competing interests** The authors declare no competing interests.

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