RESEARCH ARTICLE

CO_2 elevation improves photosynthetic performance in progressive warming environment in white birch seedlings [v1; ref status: indexed, http://f1000r.es/up]

Shouren Zhang[1,2], Qing-Lai Dang[2]

[1]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing, China
[2]Faculty of Natural Resources Management, Lakehead University, Ontario, Canada

v1 **First published:** 15 Jan 2013, **2**:13 (doi: 10.12688/f1000research.2-13.v1)
Latest published: 15 Jan 2013, **2**:13 (doi: 10.12688/f1000research.2-13.v1)

Abstract

White birch (Betula paperifera Mash) seedlings were exposed to progressively warming in greenhouses under ambient and elevated CO_2 concentrations for 5 months to explore boreal tree species' potential capacity to acclimate to global climate warming and CO_2 elevation. In *situ* foliar gas exchange, in vivo carboxylation characteristics and chlorophyll fluorescence were measured at temperatures of 26°C and 37°C. Elevated CO_2 significantly increased net photosynthetic rate (Pn) at both measurement temperatures, and Pn at 37°C was higher than that at 26°C under elevated CO_2. Stomatal conductance (gs) was lower at 37°C than at 26°C, while transpiration rate (E) was higher at 37°C than that at 26°C. Elevated CO_2 significantly increased instantaneous water-use efficiency (WUE) at both 26°C and 37°C, but WUE was markedly enhanced at 37°C under elevated CO_2. The effect of temperature on maximal carboxylation rate (Vcmax), PAR-saturated electron transport rate (Jmax) and triose phosphate utilization (TPU) varied with CO_2, and the Vcmax and Jmax were significantly higher at 37°C than at 26°C under elevated CO_2. However, there were no significant interactive effects of CO_2 and temperature on TPU.

The actual photochemical efficiency of PSII ($\Delta F/ Fm'$), total photosynthetic linear electron transport rate through PSII (JT) and the partitioning of JT to carboxylation (Jc) were higher at 37°C than at 26°C under elevated CO_2. Elevated CO_2 significantly suppressed the partitioning of JT to oxygenation (Jo/JT). The data suggest that the CO_2 elevation and progressive warming greatly enhanced photosynthesis in white birch seedlings in an interactive fashion.

Article Status Summary

Referee Responses

Referees	**1**	**2**
v1 published 15 Jan 2013	☑ report	☑ report

1 **Xianzhong Wang**, Indiana University-Purdue USA

2 **Christian Koerner**, University of Basel Switzerland

Latest Comments

No Comments Yet

Corresponding author(s): Shouren Zhang (zsr@ibcas.ac.cn), Qing-Lai Dang (qdang@lakeheadu.ca)

How to cite this article: Zhang S, Dang QL (2013) CO2 elevation improves photosynthetic performance in progressive warming environment in white birch seedlings [v1; ref status: indexed, http://f1000r.es/up] *F1000Research* 2013, **2**:13 (doi: 10.12688/f1000research.2-13.v1)

Grant information: The author(s) declared that no grants were involved in supporting this work.

Competing Interests: No competing interests were disclosed.

First published: 15 Jan 2013, **2**:13 (doi: 10.12688/f1000research.2-13.v1)
First Approved: 08 Feb 2013, **2**:13 (doi: 10.12688/f1000research.2-13.v1)

Introduction

Global climate warming and increases in atmospheric CO_2 concentration are currently key topics for scientists, politicians and the general public alike[1]. Such changes have been observed in the past 150 years and supported by modeling results for the longer term, *e.g.*, warming ocean water, shrinking mountain glaciers, retreating snow cover, and CO_2 concentration dynamics in Arctic/Antarctic ice cores[2-6]. It is projected that the global temperatures will increase by an average of 3°C with a range of 2 to 4.5°C under the scenario of doubling atmospheric CO_2 concentration by the end of the century[2]. Global climate warming will likely have profound and diverse impacts on biological systems[2, 4–13].

Increases in CO_2 and temperature to a certain extent should have positive impact on photosynthesis and growth, as the current atmospheric CO_2 concentration is below the saturation point for RuBisCO (Ribulose-1,5-bisphosphate carboxylase oxygenase)[14]. Furthermore, higher CO_2 concentrations suppress photorespiration and increase the partitioning of photosynthetic electron transport to carboxylation[14] . However, the situation will become complicated if the temperature goes beyond plants' ability to acclimate, or when the rate of temperature increase exceeds the pace of acclimation. In such cases, temperature and CO_2 will have opposite effects on photosynthesis, i.e., the higher temperature induced increase in photorespiration may exceed the beneficial effect of CO_2 elevation, resulting in a decline in net photosynthesis. Consequently, the direction and magnitude of change in net photosynthesis will be determined by the relative magnitudes of the two opposite effects[15]. Kirschbaum[16] has conducted a theoretical analysis on the dependence of photosynthesis on temperature and CO_2 concentration for C3 plants and found that at 35°C, photosynthesis at the ambient CO_2 concentration reaches only 50% of the rate at saturating CO_2 concentration, whereas the corresponding value at 5°C is 77%. Therefore, there is greater potential photosynthetic enhancement by CO_2 elevations at higher temperatures. This theory has been supported by the results of a number of studies[15, 17-19]. Long[20] has suggested that the increase in atmospheric CO_2 will not only increase photosynthetic rate, but also alter the photosynthetic response to temperature. Mooney *et al.*[21] indicate that the photosynthetic acclimation to elevated temperature and CO_2 mainly involves changes in the heat stability of the thylakoids and RuBisCO activity. Hence, high temperature and CO_2 elevations may have synergistic effect on photosynthesis and CO_2 elevations may lead to improved acclimation to high temperatures. However, such interactions may vary with plant species[22,23] and other environmental conditions. Variations in acclimation ability can change the interactions within and between species and the composition and functioning of plant communities under future climatic conditions. Furthermore, in most past studies, high temperature treatments are achieved in one step, which is in contrast with the gradual, progressive increases in temperature occurring in global climate changes.

The boreal forest is an important terrestrial ecosystem with a high carbon sequestration potential[24]. As the global climate change accelerates, the boreal forest has been experiencing progressive increases in temperatures and CO_2. The response of the boreal forests could have great impact on the global carbon balance[25]. White birch is one of the most widely distributed tree species in the boreal forest. The growth conditions of white birch in northwest Ontario are characterized by a long cold winter and short summer. For example, the annual mean temperature in Thunder Bay region is 2.4°C while the January and July average temperatures are −14.9°C and 17.6°C (based on Environment Canada's online weather records for the time period of January 1943 to December 2003). Nevertheless, based on our past experience in growing white birch seedlings in greenhouses, it appears that the species is capable of acclimating to continuous warming to more than 40°C in the early afternoon on sunny summer days (see Fig. 1). In this current study, we test the hypothesis that

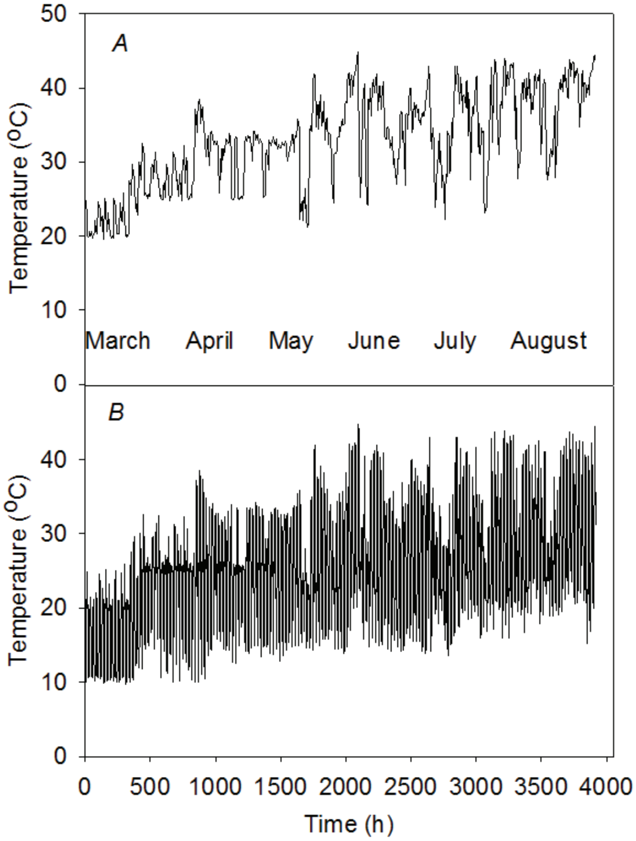

Figure 1 Time course of temperatures in the greenhouses during the experimental period (March 1 through August 15). (**A**) Postmeridian (hour) pattern between 13:00 and 16:00; (**B**) Diel (hour) pattern between 0:00 and 24:00.

CO_2 elevation will enhance the photosynthetic performance of white birch seedlings growing in a progressively warming environment.

Materials and methods

Plant materials

White birch (*Betula papyrifera* Mash.) seedlings were grown from seeds in the greenhouses at the Thunder Bay campus of Lakehead University. The growing medium was a mixture of peat moss and vermiculite (1:1 (v/v)).

Experiment design

The seedlings were subject to a progressive warming in the greenhouses as the season progressed from March to August (Fig. 1). The temperatures in all the greenhouses were monitored and recorded using a computerized environment control system (*Argus*, Vancouver, Canada). The highest recorded temperature in the greenhouse was 44.8 °C in the later stages of the experiment (Fig. 1). The seedlings were grown under two CO_2 concentrations (i.e., the ambient (360 μmol mol^{-1}) and elevated (650 μmol mol^{-1})). The two CO_2 treatments were conducted simultaneously in separate greenhouses with identical design and dimensions. The CO_2 elevation was achieved using *Argus* CO_2 generators (*Argus*, Vancouver, Canada). A photoperiod of 16-hour was maintained (the natural light was supplemented by high-pressure sodium lamps on cloudy days, early mornings and late evenings).

The moisture content of the growing medium was maintained at around 50%, as measured using a *HH2 Moisture Meter* (*DELTA-T DEVICES*, Cambridge, UK). The seedlings were watered up to twice a day during the summer to maintain the soil moisture condition. The seedlings were fertilized weekly with a solution of 100 μmol mol^{-1}N, 35 μmol mol^{-1} P and 66 μmol mol^{-1} K.

Simultaneous measurements of *in situ* gas exchange and chlorophyll fluorescence

The foliage gas exchange was measured using a PP-Systems *CIRAS-1* open gas exchange system (*Hitchin*, Hertfordshire, UK). The environmental conditions in the broad-leaf chamber were controlled automatically. The environmental conditions for measuring the Pn-C_i (C_i = intercellular CO_2 concentration) curve were as follows: 26°C and 37°C air temperature, which were close to the highest temperatures in the early and late period of the experiment, 800 μmol m^{-2}s^{-1} PAR (PAR = photosynthetically-active radiation) and 50% relative humidity. The *in vivo* maximal carboxylation rate (V_{cmax}), PAR-saturated electron transport rate (Jmax), triose phosphate utilization (TPU) and other relevant parameters were calculated from the Pn-C_i curves according to Farquhar *et al.*[26], van Caemmerer and Farquhar[27], Sharkey[28], Harley and Sharkey[29] and Harley *et al.*[30]. The Pn-C_i curves were fit using the *Photosyn Assistant* software (*Dundee Scientific*, Scotland, UK) to estimate V_{cmax}, J_{max} and TPU. The parameters for the kinetics of RuBiscCO, i.e., Kc, Ko and τ, and their temperature dependencies were adopted from Harley *et al.*[30] and Wullschleger[31].

Three seedlings were selected randomly from each treatment combination for the measurement. The measurement was taken on the top 5th mature leaf. All the *in situ* measurements were made between 9:00 and 11:30 AM with the seedlings in their original positions and conditions of the treatments.

The chlorophyll fluorescence was measured using a *FMS-2* portable pulse-modulated fluorometer (*Hansatech Instruments Ltd*. Norfolk, UK). The probe was integrated in the leaf chambers of the gas exchange system and the control software for the two systems was also integrated to allow the simultaneous measurement of gas exchange and chlorophyll fluorescence. The following variables were obtained: fluorescence intensity at any time, F; the maximal fluorescence in light, F_m'; the actual photochemical efficiency of PSII in light, (F_m'-F)/F_m' or ΔF/F_m', which is the efficiency under the actual degree of reaction centre closure[32]. F_m' was obtained by illuminating the foliage with a pulse of strong light (around 14000 μmol photons m^{-2}s^{-1}) for 800 ms. The ΔF/ F_m' was measured simultaneously with each gas exchange measurement. Both gas exchange and chlorophyll fluorescence were measured after 5 months of the treatments.

The apparent rate of total electron transport (J_T) and its partitioning between carboxylation (J_c) and oxygenation (J_o) were calculated based on the methods of Farquhar *et al.*[26], Genty *et al.*[33] and Epron *et al.*[34].

Statistical analysis

All the data were examined graphically for the normality of distribution (probability plots for residual analysis) and the homogeneity of variance (scatter plots) using the Data Desk (version 6.01, Data Description, Inc. 1996)[35] before the Analysis of Variance (ANOVA) was carried out. Some of the data were log-transformed to meet the two assumptions for ANOVA. The data were analyzed using the two-way ANOVA procedure of the Data Desk. When the interaction between temperature and CO_2 was significant, Scheffe's F test for post hoc pairwise comparisons was conducted.

Results

In situ photosynthetic gas exchange

There was a significant ($P<0.01$) interactive effect of temperature and CO_2 on Pn (Fig. 2. Pn was higher ($P<0.01$) at 37°C than at 26°C under elevated CO_2 (Fig. 2), but there was no significant ($P>0.05$) temperature effect on Pn under ambient CO_2. CO_2 elevation significantly increased Pn at both temperatures ($P<0.05$, $P<0.001$ at 26°C and 37°C, respectively). g_s significantly ($P<0.05$) decreased at 37°C under both ambient and elevated CO_2 (Fig. 2), and there was no significant ($P>0.05$) CO_2 effect on g_s. Meanwhile high temperature significantly ($P<0.05$) stimulated *E* under both ambient and elevated CO_2 (Fig. 2).

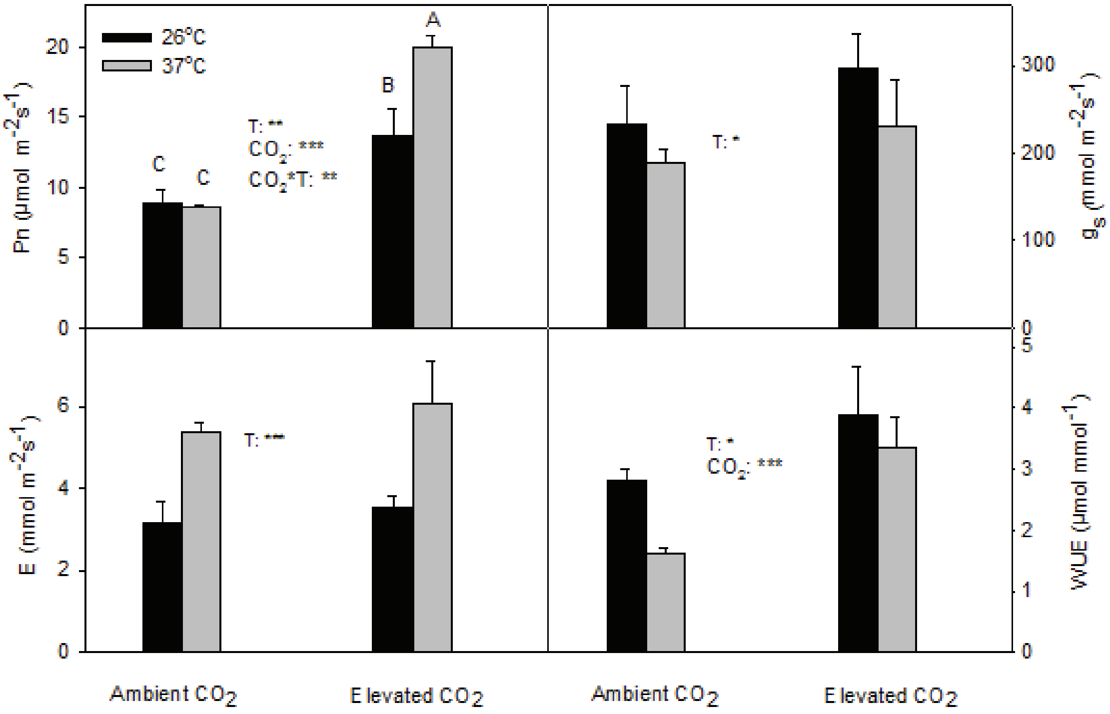

Figure 2 Pn, g_s, *E* and WUE (mean ± SD, n=3-4) for current year white birch seedlings after they were exposed to continuous warming under ambient CO_2 and elevated CO_2 concentrations for 5 months. The *in situ* measurements were taken at 26°C and 37°C under ambient CO_2 and elevated CO_2. The significance levels (*** = $P<0.001$, ** = $P<0.01$,* = $P<0.05$). If the interaction between measurement temperature and CO_2 was significant for a given parameter, Scheffe's F test for post hoc pairwise comparisons was conducted. Means sharing the same letter or letters are not significantly different.

Water-use efficiency (WUE) was significantly ($P<0.05$) higher at 26°C than that at 37°C under both CO_2 regimes. CO_2 elevation greatly ($P<0.001$) increased WUE at both temperatures.

High temperature significantly reduced C_i under both ambient and elevated CO_2 ($P<0.05$, $P<0.01$, respectively) and, also, elevated CO_2 significantly ($P<0.001$) increased C_i at both temperatures.

In vivo RuBisCO activity

V_{cmax}, J_{max} and TPU at 37°C were significantly ($P<0.001$) higher than those at 26°C (Fig. 3). The temperature dependencies of V_{cmax} and J_{max} were changed by CO_2, and those values at 37°C enhanced much more ($P<0.05$) under elevated CO_2 than under ambient CO_2.

Photosystem II efficiency and electron transport partitioning to carboxylation and oxygenation

There was a significant ($P<0.001$) interactive effect of CO_2 and temperature on $(F_m'-F)/F_m'$ and J_T (Fig. 4). $(F_m'-F)/F_m'$ and J_T greatly increased at 37°C as compared to at 26°C under elevated CO_2, and there was no significant temperature effect on $(F_m'-F)/F_m'$ and J_T under ambient CO_2.

The pattern of CO_2 and temperature effects on J_c was almost the same as $(F_m'-F)/F_m'$ and J_T (Fig. 4), and J_c was greater ($P<0.001$) at 37°C than that at 26°C under elevated CO_2, and there was no significant temperature effect on J_c under ambient CO_2. Elevated CO_2 greatly suppressed J_o/J_T, and there was no significant ($P > 0.05$) effect of temperature on J_o/J_T (Fig. 4).

A-Ci curves from gas exchange at 26–37°C

1 Data File

http://dx.doi.org/10.6084/m9.figshare.105910

PSII efficiency curve, 26–37°C

1 Data File

http://dx.doi.org/10.6084/m9.figshare.105911

Temperature data

1 Data File

http://dx.doi.org/10.6084/m9.figshare.105912

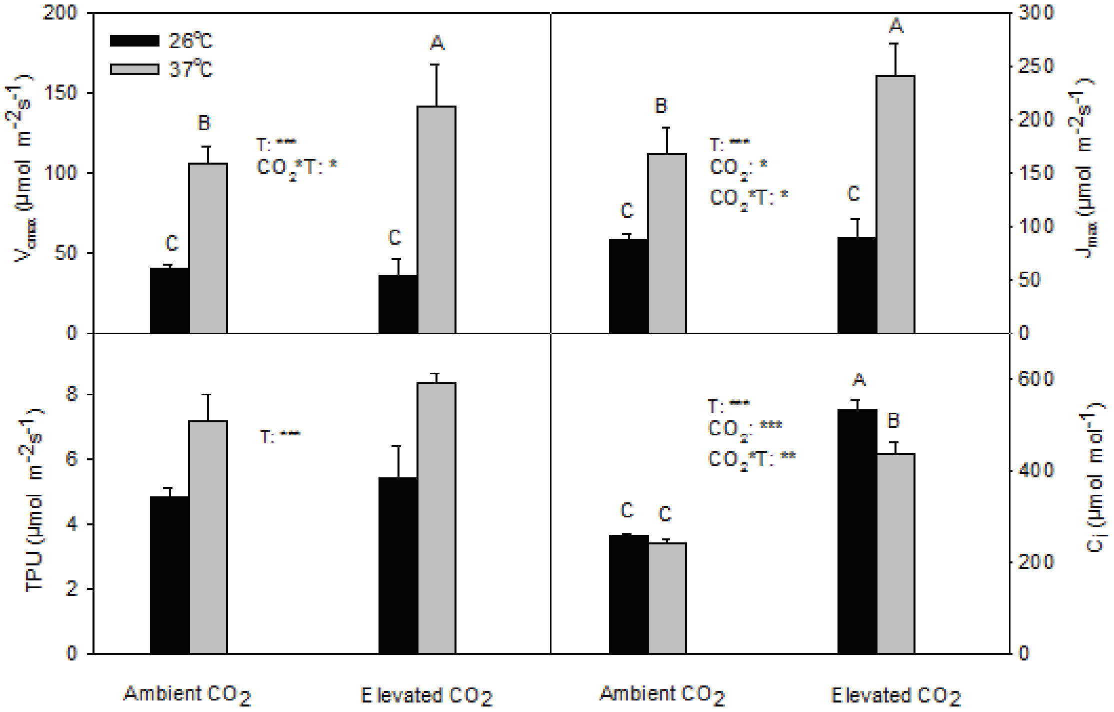

Figure 3 **V_{cmax}, J_{max}, TPU and C_i in current year white birch seedlings.** V_{cmax}, J_{max} and TPU were derived from A-Ci curves, which were measured at 26°C and 37°C under ambient CO$_2$ and elevated CO$_2$. See Fig. 2 for other explanations.

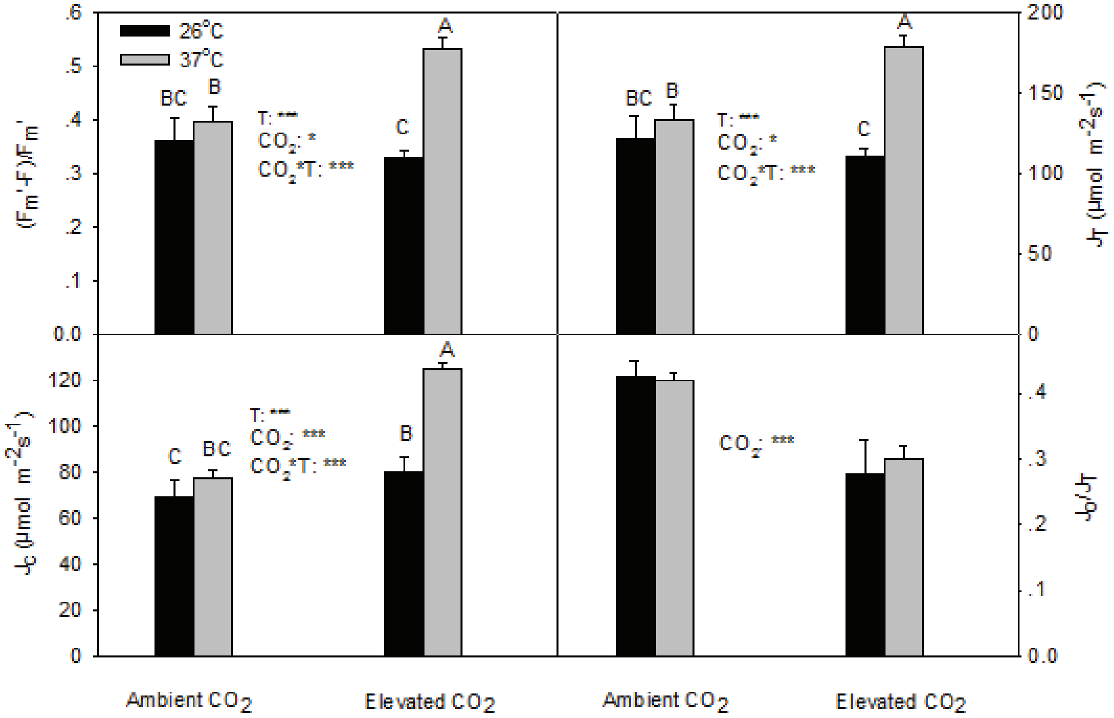

Figure 4 **(F$_m$'-F)/F$_m$', J_T, J_c and Jo/J_T in the current year white birch seedlings (F$_m$'-F)/ F$_m$' and J_T were derived from chlorophyll fluorescence measurements, and J_c and J_o were derived from both chlorophyll fluorescence and gas exchange measurements.** See Fig. 2 for other explanations.

Discussion

Our results suggest that the photosynthetic mechanisms of white birch seedlings have high capacity to acclimate to a progressively warming environment, particularly under elevated CO_2. This result is in contrast to the results of most studies with a single step warming treatment. Larcher[36] has suggested that plants' optimal temperature is closely related to the climate in which they grow. The measurement temperatures of 26°C and 37°C used in this study are believed to be the normal (or optimal) and stressful temperature, respectively, for most boreal forest tree species growing at their natural environments. Zhang *et al.*[37] have found that the Pn of mature oak even in warm-temperate zones decline greatly at temperatures over 30°C, as compared to measurements at temperatures of 20–30°C, which occurs naturally north of temperate zones or even warm-temperate zones. However, in this experiment the Pn of white birch didn't decline at 37°C under ambient CO_2, as compared to that at 26°C; furthermore, Pn increased substantially at 37°C under elevated CO_2. These results indicate that the photosynthetic mechanisms of white birch acclimated to the progressive warming environment, and this high temperature acclimation was greatly strengthened by elevated CO_2. Long[20] argued that CO_2 elevation could change the photosynthesis dependence of temperature.

The activity of RuBisCO is highly temperature-dependent. According to Jordan and Ogren[38], the Rubisco's specificity for CO_2/O_2 decreases as increasing temperatures over the optimal range, but the increase in RuBisCO oxygenation will exceed that of carboxylation because the solubility of CO_2 declines faster than that of O_2 at even higher temperatures, resulting in a decline in net photosynthetic rate. White birch's acclimation to warming was also evidenced by V_{cmax} measured at the two different temperatures and two CO_2 regimes. V_{cmax} at 37°C was much higher than at 26°C under both ambient and elevated CO_2, indicating a shift in the temperature dependency of RuBisCO. Furthermore, the partitioning of total electron transport to oxygenation was not significantly different between the two temperatures under either ambient CO_2 or elevated CO_2, suggesting that the higher temperature did not change the RUBisCO specificity for CO_2/O_2 which could be a contributing factor for the enhanced acclimation of photosynthesis to the progressive warming. Overdieck *et al.*[15] have also found that both the temperature treatment alone and the combination of elevated CO_2 and temperature depressed V_{cmax} in Scots pine at temperatures below the optimum range, but increased V_{cmax} when the temperature was above the optimum. Additionally, the magnitude of the change in V_{cmax} increased as temperature increased.

The decrease in Ci at the high temperature could be attributable to either enhanced RuBisCO activity or declines in stomatal conductance or both. Not only V_{cmax}, but J_{max} and TPU were also higher at 37°C than at 26°C, suggesting that the CO_2 assimilation process, including carboxylation, electron transport for RuBP regeneration, ATP supply and the translocation of the primary photosynthates, all maintained at high levels in the warm environment. In this study, there was no down-regulation of RuBisCO activity in association with the CO_2 elevation, to the contrary, CO_2 elevation greatly increased V_{cmax} and J_{max} at 37°C, as well as Pn at both 26°C and 37°C.

While high temperature enhanced V_{cmax} under both ambient and elevated CO_2, the increases in actual PSII efficiency ($\Delta F'/\ F_m'$) and J_c associated with the high temperature only occurred under elevated CO_2, suggesting that the high temperature did not significantly affect the total electron transport, and its partitioning to carboxylation, t under the ambient CO_2. Conversely, the partitioning of total electron flow to oxygenation increased more than 40% in response to the high temperature under elevated CO_2. The reduced electron transport partitioning to carboxylation and low Ci might explain why Pn was relatively low at 37°C under the ambient CO_2, even though the corresponding V_{cmax} was quite high, implying that the slow electron transport to carboxylation and CO_2 supply at high temperature under ambient CO_2 didn't match the high activity of RuBisCO. This again confirms Kirschbaum's theoretical analysis that photosynthesis has a higher potential to be stimulated by CO_2 elevation at high temperatures than at low temperatures[16].

Author contributions

QLD and SZ conceived and designed the experiment. SZ conducted the measurements and analyzed the data. SZ and QLD wrote and revised the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

This study was supported by Lakehead University, Canada Foundation for Innovation, Ontario Innovation Trust and NSERC Discovery Grants to Q.L. Dang (Project # 203198-2008).

Acknowledgements

The authors thank Dr. J. Wang at Lakehead University for providing white birch seeds, and Dr. K. Brown for statistical advice.

References

1. **Kerr RA** (2007) Scientists tell policymakers we are all warming the world. *Science* **315**: 754–757.
2. **IPCC**. (2007) Climate Change 2007: Impacts, Adaptation, and Vulnerability. Cambridge Univ. Press.
3. **Keeling CD, Whorf TD** (2002) Atmospheric CO_2 records from sites in the SIO air sampling network. In: Trends: a compendium of data on global change. Oak Ridge National Laboratory, US Department of Energy.
4. **Parmesan C** (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Glob. Change Biol.* **13**: 1860–1872.
5. **Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, et al.** (2003) Fingerprints of global warming on wild animals and plants. *Nature* **421**: 57–60.
6. **Rosenzweig C, Karoly D, Vicarelli M, Neofotis P, Wu Q, et al.** (2008) Attributing physical and biological impacts to anthropogenic climate change. *Nature* **453**: 353–357.
7. **Both C, van Asch M, Bijlsma R, van den Burg A, Visser M** (2009) Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? *J. Anim. Ecol.* **78**: 73–83.
8. **Bradshaw W, Holzapfel C** (2001) Genetic shift in photoperiodic response correlated with global warming. *Proc. Natl Acad. Sci. USA* **98**: 14509–14511.
9. **Colwell R, Brehm G, Cardelus C, Gilman AC, Longino JT** (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **322**: 258–261.
10. **Parmesan C, Yohe G** (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42.
11. **Sinervo, B. Méndez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, et al.** (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**: 894–899.
12. **Umina PA, Weeks AR, Kearney MR, McKechnie SW, Hoffmann AA** (2005) A rapid shift in a classic clinal pattern in Drosophila reflecting climate change. *Science* **308**: 691–693.
13. **Walther, G.-R.** et al. (2002) Ecological responses to recent climate change. *Nature* **416**: 389–395.
14. **Amthor JS** (1991) Respiration in a future, higher-CO2 world. *Plant. Cell and Environment* **14**: 13–20.
15. **Overdieck D, Kellomäki S, Wang K-Y** (1998) Do the effects of temperature and CO_2 interact? In: European Forests and Global Change: the likely impacts of rising CO_2 and temperature (Eds. Jarvis PG). Cambridge University Press, pp 236–273.
16. **Kirschbaum MUF** (1994) The sensitivity of C3 photosynthesis to increasing CO_2 concentration: a theoretical analysis of its dependence on temperature and background CO_2 concentration. *Plant. Cell Environ.* **17**: 747–54.
17. **Idso KE, Idso SB** (1994) Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past ten years' research. *Agricultural and Forest Meteorology* **69**: 153–203.
18. **Idso SB, Idso KE, Garcia RL, Kimball BA, Hoober JK** (1995) Effects of atmospheric CO_2 enrichment and foliar methanol application on net photosynthesis of sour orange treee (Citrus aurantium; Rutaceae) leaves. *American Journal of Botany* **82**: 26–30.
19. **Tissue DT, Thomas RB, Strain BR** (1996) Growth and photosynthesis of loblolly pine (***Pinus taeda***) after exposure to elevated CO_2 for 19 months in the field. *Tree Physiol* **16**: 49–59.
20. **Long SP** (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentration, has its importance been underestimated? *Plant. Cell Environ.* **14**: 729–739
21. **Mooney HA, Bjorkman O, Collatz GJ** (1978) Photosynthetic acclimation to temperature in the desert shrub, Larrea divaricata I. Carbon dioxide exchange characteristics of intact leaves. *Plant. Physiology:* **61**: 406–10.
22. **Stanghellini C, Bunce JA** (1993) Response of photosynthesis and conductance to light, CO_2, temperature and humidity in tomato plants acclimated to ambient and elevated CO_2. *Photosynthetica* **29**: 487–97.
23. **Teskey RO** (1997) Combined effects of elevated CO_2 and air temperature on carbon assimilation of Pinus taeda trees. *Plant. Cell Environ.* **20**: 373–380.
24. **Apps MJ, Kurz WA, Luxmoor RJ, Nilsson LO, Sedjo RA, et al.** (1993) Boreal forest and tundra. *Water, Air, & Soil Pollution* **70**: 39–53.
25. **Peng CH, Apps MJ** (1998) Simulating carbon dynamics along the Boreal Forest Transect Case Study (BFTCS) in central Canada. 2. Sensitivity to climate change. *Global Biogeochem. Cycles* **12**: 393–402.
26. **Farquhar GD, von Caemmerer S, Berry JA** (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta* **149**: 78–90.
27. **von Caemmerer S, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**: 376–87.
28. **Sharkey TD** (1985) Photosynthesis in intact leaves of C3 plants: physics, physiology and limitations. *Botanical Review* **51**: 53–105.
29. **Harley PC, Sharkey TD** (1991) An improved model of C3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynthesis Research* **27**:169–178.
30. **Harley PC, Thomas RB, Reynolds JF, Strain BR** (1992) Modelling photosynthesis of cotton grown in elevated CO_2. *Plant. Cell Environ.* **15**: 271–82.
31. **Wullschleger SD** (1993) Biochemical limitations to carbon assimilation in C3 plants – a retrospective analysis of the A/Ci curves from 109 species. *Journal of Experimental Botany* **44**: 907–920.
32. **Bilger W, Björkman O** (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthsis Research* **25**: 173–185.
33. **Genty B, Briantais JM, Baker NR** (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim et Biophysica* **900**: 87–92.
34. **Epron D, Godard D, Cornic G, Genty B** (1995) Limitation of net CO_2 assimilation rate by internal resistances to CO_2 transfer in the leaves of two tree species (Facus sylavatica L. & Castanea sativa Mill.). *Plant. Cell Environ.* **18**: 43–51.
35. Data Desk. 1996. Version 6.01, Data Description, Inc., Ithaca, New York, USA.
36. **Larcher W** (2003) Physiological plant ecology: ecophysiology and stress physiology of functional groups. Springer-Verlag, Berlin Heidelberg New York.
37. **Zhang S, Li Q, Ma K, Chen L** (2001) Temperature-dependent gas exchange and stomatal/non-stomatal limitation to CO_2 assimilation of *Quercus liaotungensis* under midday high irradiance. *Photosynthetica*, **39 (3)**: 383–388.
38. **Jordan DB, Ogren WL** (1984) The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. *Planta* **161**: 308–313.

Current Referee Status: ☑ ☑

Referee Responses for Version 1

☑ **Christian Koerner**, Institute of Botany, University of Basel, Basel, CH -4056, Switzerland
Approved: 01 July 2013

Ref Report: 01 July 2013
Having stated that the work makes a very sound impression to me and reflects state of the art methodology, I still have concerns about the implied meaning. The authors carefully avoid making any statement with regard to growth or productivity implications. However, why would one make such measurements if not for the implied meaning for growth and overall performance? From all what we know to date, such a link has not been shown outside horticultural conditions. It will be most unlikely that a birch seedling in the wild is carbon limited. Whatever the photosynthetic performance, such a seedling would only incorporate structural carbon to the extent nutrients permit, and these are finite per unit land area (except for N) and are competitively foraged for.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

☑ **Xianzhong Wang**, Department of Biology, Indiana University-Purdue, Indianapolis, IN, USA
Approved: 08 February 2013

Ref Report: 08 February 2013
This is certainly a valuable manuscript.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.