Nutrient supply has greater influence than sink strength on photosynthetic adaptation to CO_2 elevation in white birch seedlings

Shouren Zhang [a,b,*], Qing-Lai Dang [a], Bing Cao [a,c]

[a] *Faculty of Natural Resources Management, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 5E1, Canada*
[b] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China*
[c] *Department of Forestry and Horticulture, School of Agriculture, Ningxia University, Xixia District, Yinchuan, Ningxia 750021, China*

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ABSTRACT

To study the effects of source–sink ratio and nutrient supply on photosynthetic acclimation to CO_2 elevation, we subjected white birch seedlings to two levels of nutrient supply (high vs. low) and CO_2 concentrations (ambient vs. doubled $[CO_2]$) for two months and then shaded the lower canopy on half of the seedlings to reduce source/sink ratio for an additional month. The CO_2 elevation significantly increased P_n and IWUE at both nutrient levels but the increase was greater in the high than low nutrient treatment. The CO_2 elevation resulted in a down-regulation of V_{cmax} in the low nutrient treatment but up-regulation of J_{max}, TPU, $(F'_m - F)/F'_m$ and J_c in the high nutrient after 3 months of treatment. Both the CO_2 elevation and high nutrient supply increased the partition of total electron transport to carboxylation at the expense of oxidation. The seedlings responded to the shading of the lower canopy by reducing biomass allocation to roots rather than making physiological adjustments to unshaded leaves in the upper canopy. Our results suggest that the direction of photosynthetic acclimation to CO_2 elevation in white birch was nutrient-dependent and an increase in sink strength could reduce the feedback inhibition of photosynthesis.

1. Introduction

The global climate change associated with increasing atmospheric $[CO_2]$ has become progressively more significant in the past century [1]. Increases in atmospheric $[CO_2]$ are believed to favor photosynthesis because the current atmospheric $[CO_2]$ is below the saturation point for photosynthetic carboxylation and higher $[CO_2]$ will suppress the rate of photo-respiration catalyzed by Rubisco [2]. However, the projection of photosynthetic response to $[CO_2]$ elevation based on the above assumptions has been challenged since a negative acclimation of photosynthesis to CO_2 elevation (i.e., the down-regulation of photosynthetic capacity) was discovered. The down-regulation of photosynthetic capacity in response to CO_2 elevations is originally believed to be limited to growth chamber experiments where root growth is restricted by the container [3]. However, more recent FACE studies have shown that photosynthetic down-regulation also occurs in the field where roots grow freely [4–6]. The issue is further complicated by the finding of

photosynthetic up-regulation in some plants (i.e. increases in photosynthetic capacity) in response to CO_2 elevations [7–9].

Studies have shown that photosynthetic acclimation to elevated $[CO_2]$ is generally more pronounced when nitrogen supply is deficient, but may not occur at all when N supply is optimal [11–14]. These results suggest that photosynthetic acclimation to $[CO_2]$ may be controlled by the nutrient status of the plant, particularly nitrogen. However, Rogers et al. [15] and Ainsworth et al. [16] suggest that the down-regulation of photosynthesis at low nitrogen and elevated $[CO_2]$ is due to weakened sink demand for photosynthates rather than the direct effect of nitrogen supply on photosynthesis. Additionally, Stitt and Krapp [12] and Hymus et al. [17] argue that insufficient N supply can restrict the development of new sinks and therefore exacerbate the source–sink imbalance that tends to develop in plants grown under elevated $[CO_2]$. However, the occurrence of photosynthetic down-regulation in FACE experiments seems to contradict the sink limitation theory. Furthermore, the theory of source–sink imbalance under elevated $[CO_2]$ fails to explain photosynthetic up-regulation. Our previous study on $[CO_2]$–nutrient interactions has shown that the photosynthetic capacity of white birch seedlings down-regulated marginally after two months of treatment [9]. However, photosynthetic up-regulation occurred after an additional month of treatment, which is in contrast to the viewpoint that photosynthetic down-regulation occurs at the end of the growing season when sink strength declines

* Corresponding author at: State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China. Tel.: +86 10 62836946; fax: +86 10 82599518.

E-mail address: zsr@ibcas.ac.cn (S. Zhang).

Nomenclature

PAR	photosynthetically active radiation (μmol m^{-2} s^{-1})
P_n	net photosynthetic rate (μmol m^{-2} s^{-1})
g_s	stomatal conductance (mmol m^{-2} s^{-1})
E	transpiration rate (mmol m^{-2} s^{-1})
IWUE	instantaneous photosynthetic water use efficiency (μmol CO_2 mmol^{-1} H_2O)
PNUE	photosynthetic nitrogen use efficiency (μmol CO_2 s^{-1} g^{-1} N)
C_i	intercellular CO_2 concentration (μmol mol^{-1})
V_{cmax}	maximal carboxylation rate (μmol m^{-2} s^{-1})
J_{max}	PAR-saturated electron transport rate contributing to RuBP regeneration (μmol m^{-2} s^{-1})
TPU	rate of triosephosphate utilization (μmol m^{-2} s^{-1})
F	intensity of chlorophyll florescence at any time
F'_m	maximal intensity of chlorophyll florescence in light
$(F'_m - F)/F'_m$ or $\Delta F/F'_m$	actual photochemical efficiency of PSII in light
J_T	the total rate of photosynthetic linear electron transport through PSII (μmol m^{-2} s^{-1})
J_c	the partition of photosynthetic linear electron transport to carboxylation (μmol m^{-2} s^{-1})
J_o	the partition of photosynthetic linear electron transport to oxygenation (μmol m^{-2} s^{-1})

and other environmental factors become more limiting [18]. Apparently it is difficult to predict trees' response to [CO_2] elevations without a good understanding of photosynthetic acclimation to [CO_2]. In this study, we subjected white birch seedlings to two [CO_2] (ambient vs. doubled) and two nutrient levels (high vs. low N–P–K) for two months and then reduced the source strength (thus the source/sink ratio) on 50% of the seedlings by shading the lower canopy. The shading reduced the flux density of photosynthetically active radiation by 65%. This design has allowed us to investigate how a change in source/sink ratio interacts with nutrient supply in affecting the acclimation of photosynthesis to CO_2 elevations. Based on the theory that CO_2-elevation-induced photosynthetic down-regulation is a result of photosynthetic carbohydrate production exceeding sink capacity for its utilization and that nutrient deficiency reduces sink strength [8,11–14], we hypothesize that shading the lower part of the canopy will reduce the degree of photosynthetic down-regulation under elevated CO_2 particularly in the low nutrient treatment. Further, we investigate whether the up-regulation of photosynthesis under elevated [CO_2] and high nutrient supply [as reported by 7,8,9] is reproducible.

2. Materials and methods

2.1. Plant materials

White birch (*Betula papyrifera* Mash.) seedlings were grown from seeds in germination trays. Seedlings of relatively uniform size (about 3 cm tall) were selected, transplanted into containers (21 cm tall, 20.8 cm top diameter) and subjected to two atmospheric CO_2 concentrations (360 versus 720 μmol mol^{-1}) and two nutrient levels (low and high, see the next section for details). The growing medium was a mixture of peat moss and vermiculite (1:1 (v/v)).

2.2. Experimental design

At the beginning of the experiment, the treatments consisted of two CO_2 concentrations (360 versus 720 μmol mol^{-1}) and two nutrient levels (low and high). For the high nutrient treatment,

the seedlings were fertilized twice a week with a solution of 100 mg N L^{-1}, 44 mg P L^{-1} and 83 mg K L^{-1} using a dripping irrigation system. The high nutrient level was similar to the level used in a previous study [9], which represents the optimal level for seedlings [10]. Soil nutrient supply can also be evaluated by the foliar nutrient concentrations of the plant. The foliar nutrient concentrations in our high nutrient treatment were about 50–100% higher than those of trees in the natural boreal forest. The seedlings in the low nutrient treatment were fertilized at the same time as those in the high nutrient treatment, but the nutrient concentrations were only 1/10 of those in the high nutrient treatment, i.e., 10 mg N L^{-1}, 4.4 mg P L^{-1} and 8.3 mg K L^{-1}. The experiment was conducted in four independent greenhouses with identical dimensions (6.4 m wide, 6.4 m long and 2.13 m high with ridge roof), design and environmental control devices. Each CO_2 treatment had two independent replications (greenhouses). The nutrient treatments were nested within CO_2 treatments, i.e. each replicate of the CO_2 treatments contained both high- and low-nutrient treatments. There were 12 potted seedlings in each treatment combination (i.e., each replicate of CO_2 × nutrient combination × shading, giving a total of 192 seedlings). The spacing between adjacent pots was 30 cm. The locations of individual seedlings within a treatment combination were initially randomized and then periodically rotated to remove possible position effects. Other environmental conditions were set as follows for all the treatments: day/night temperatures 25–26 °C/16–17 °C, and photoperiod 16-h (high-pressure sodium lamps were used to lengthen the natural photoperiod).

After two months of treatments, the lower half of the canopy was shaded on 50% of the seedlings in each treatment combination. The shading was achieved using neutral density shading mesh (black). The shading reduced the flux density of photosynthetically active radiation by 65%. All the environmental conditions in the greenhouses were monitored and controlled continuously using an Argus61® controlling system (Argus, Vancouver, Canada). The volumetric moist content of the growing medium was maintained around 50% as measured using a HH2 Moisture Meter and ML2X ThetaProbe (DELTA-T DEVICES, Cambridge, UK). The seedlings were watered up to twice a day to maintain the soil moisture condition. The soil moisture was measured prior to photosynthesis measurements each day to ensure that all the measurements were taken under similar soil moisture conditions. The experiment lasted 14 weeks.

2.3. Simultaneous measurements of in situ photosynthetic gas exchange and chlorophyll fluorescence

Three seedlings were selected randomly from each replication of each treatment combination for foliar gas exchange measurement (a total of 48 seedlings). The A/C_i measurement is time consuming and the relative small sample size is to reduce errors related to possible changes in physiological conditions between different sample seedlings. Photosynthetic gas exchange was measured using a PP-Systems CIRAS-1 open gas exchange system and a Parkinson leaf chamber with automatic environmental control (Hitchin, Hertfordshire, UK) on a new fully expanded leaf (4th or 5th leaf from the top). The gas exchange measurement was taken prior to and one month after the start of the shading treatment (referred to as 2-month and 3-month respectively hereafter). The photosynthetic response to CO_2 concentration (A–C_i curves) was measured at 50, 120, 180, 350, 500, 700, 1000 and 1200 μmol mol^{-1} CO_2 (in ascending order). The 3-month measurements were taken on unshaded leaves the selection of which was as described previously. The environmental conditions for the measurements were as follows: 25 °C leaf temperature, 800 μmol m^{-2} s^{-1} PAR and 50% RH. All the in situ measurements were made between 9:00 and 11:30 AM (when gas exchange was stable, eliminating the influence of diurnal changes

in physiological traits) with the seedlings in their original positions and conditions of the treatments. Furthermore, the sequence of measurement among sample seedlings was randomized. The in vivo maximal carboxylation rate (V_{cmax}), PAR-saturated electron transport rate (J_{max}), TPU and other relevant parameters were calculated from the A–C_i curve according to Farquhar et al. [19], van Caemmerer and Farquhar [20], Sharkey [21], Harley and Sharkey [22] and Harley et al. [23]. The A–C_i curves were fit using the Mechanistic Model of the Photosyn Assistant software (Dundee Scientific, Scotland, UK) to estimate V_{cmax}, J_{max} and TPU. The parameters for Rubisco kinetics, i.e., K_c, K_o, τ and their temperature dependencies were adopted from Harley et al. [23] and Wullschleger [24]. P_n, g_s and E were measured at the corresponding growth [CO_2] for each CO_2 treatment.

The chlorophyll fluorescence was measured using a FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk, UK) simultaneously with the post-shading gas exchange measurement. The probe is integrated in the leaf chamber of the gas exchange system and the MFS-2 and CIRAS-1 are also integrated electronically to allow the simultaneous measurement of both 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 $\Delta F/F'_m$, which is the efficiency under the actual degree of reaction center closure [25]. F'_m was obtained by illuminating the foliage with a pulse of strong light (around 14,000 μmol photons m^{-2} s^{-1}) for 800 ms.

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 method of Farquhar et al. [19], Genty et al. [26] and Epron et al. [27]. The details of the calculation are given in Zhang and Dang [9].

2.4. Measurements of biomass and allocation

The seedlings used in the above measurements were harvested at the completion of all the measurements and the project area of the foliage was measured using the Regent WinFolia System (Regent Instruments Inc., Quebec, Canada) for determining the specific leaf area. All the materials were then oven-dried at 70 °C for 48 h and dry mass was measured separately for leaves, roots and stem on an analytic balance. The mass ratio for leaf to total (LMR) and root to total (RMR) were used as indicators of biomass allocations.

2.5. Foliar nitrogen assay

The top 4th–6th fully expanded leaves from each sample tree were used for nutrient analysis. Total leaf N concentration (%) was assayed using the "Dumas Method" [28]. Leaf area based N concentration (g m^{-2}) was calculated from specific leaf area (m^2 kg^{-1}) and the mass-based N concentration (%).

2.6. Statistical analysis

All the data were examined graphically for the normality of distribution (probability plots of residuals) and the homogeneity of variance (scatter plots) using Data Desk 6.01 [29] before the Analysis of Variance (ANOVA) was carried out. The tests showed that all the data except V_{cmax} and J_{max} satisfied the above ANOVA assumptions. The V_{cmax} and J_{max} were log-transformed to satisfy the ANOVA assumptions. The effects of nutrient, [CO_2], shading (3-month measurement) and their interactions were tested using the ANOVA procedure of Data Desk 6.01. When an interaction was significant, Scheffe's F test for post hoc pairwise comparison was conducted.

3. Results

3.1. In situ photosynthetic gas exchange and leaf nutrient concentration

Nutrient and [CO_2] had significant interactive effects on P_n in both measurements ($P < 0.05$ & 0.001, respectively). After two months of treatment, the CO_2 elevation stimulated P_n only in seedlings grown under high nutrient supply, but the high nutrient supply increased P_n in both [CO_2] and the magnitude of increase was greater under the elevated [CO_2] (Fig. 1). After three months of treatment, however, the CO_2 elevation increased P_n at both nutrient treatments, but the increase was greater at the high than low nutrient. While the nutrient effect remained significant at both [CO_2], the increase was still greater under the elevated than ambient [CO_2] (Fig. 1). Neither shading nor its interactions with CO_2 or N had any significant effect on P_n ($P > 0.05$).

The CO_2 elevation suppressed g_s and E in the 2-month measurement while nutrient supply did not have any significant effect in either 2-month or 3-month measurement (Fig. 1). However, the CO_2 effect became statistically insignificant in the 3-month measurement and the shading did not significantly affect g_s or E ($P > 0.05$).

Nutrient supply and [CO_2] had significant interactive effects on IWUE in both measurements ($P < 0.01$ & 0.001, respectively). The CO_2 elevation significantly increased IWUE in both nutrient treatments but the increase was greater at the high than low nutrient supply (Fig. 1). The high nutrient stimulation of IWUE was greater in the elevated than ambient [CO_2] (Fig. 1). However, shading the lower canopy did not significantly affect IWUE in any [CO_2]–nutrient treatment combination ($P > 0.05$).

There were significant ($P < 0.05$) interactive effects of nutrient and [CO_2] on mass-based leaf N concentration after 3 months' treatment (Fig. 2). The high nutrient supply resulted in a greater increase in leaf N in the ambient than doubled [CO_2] (Fig. 2). The CO_2 elevation decreased leaf N in the high but not the low nutrient treatment (Fig. 2). The nutrient–CO_2 interaction did not significantly affect the area-based leaf N ($P > 0.05$) but the high N treatment did result in significantly higher leaf-area-based N concentration ($P < 0.001$, Fig. 2).

The CO_2 elevation increased while high nutrient supply decreased photosynthetic N use efficiency (Fig. 2). The CO_2 elevation decreased while the high nutrient supply increased specific leaf area (Fig. 2).

3.2. In vivo carboxylation activity

The low nutrient and CO_2 elevation significantly decreased V_{cmax} after 2 months of treatment ($P < 0.001$ & 0.05, respectively) (Fig. 3). While the effects of nutrient and [CO_2] remained significant after one additional month of treatment, their interactions became significant ($P < 0.01$): the CO_2 elevation significantly decreased V_{cmax} only in the low nutrient treatment ($P < 0.01$) and the nutrient effect was greater under the elevated than ambient [CO_2] (Fig. 3).

The low nutrient supply significantly reduced J_{max} ($P < 0.001$) while CO_2 elevation had no significant effect on J_{max} ($P > 0.05$) in the 2-month measurement (Fig. 3). After an additional month of treatment, the CO_2 elevation significantly increased J_{max} in the high nutrient treatment while its effect in the low nutrient treatment remained to be statistically insignificant (Fig. 3).

The low nutrient supply suppressed TPU in both measurements ($P < 0.001$, Fig. 3). However, [CO_2] interacted with nutrient supply ($P < 0.05$) and shading ($P < 0.01$) in affecting TPU in the 3-month measurement (Fig. 3). The CO_2 elevation enhanced TPU in the high nutrient but not in the low nutrient treatment (Fig. 3). The CO_2
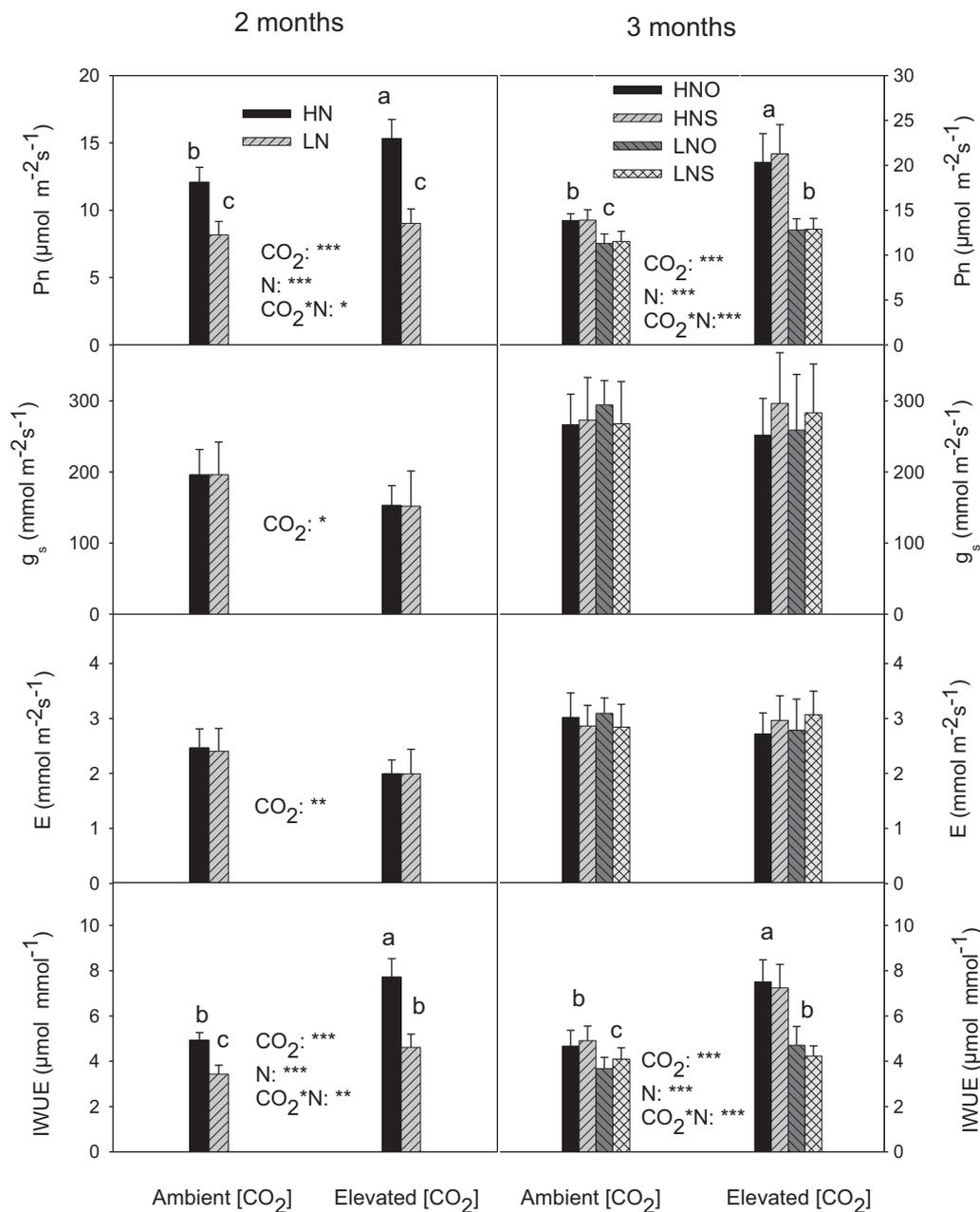
Fig. 1. P_n, g_s, E and IWUE (mean ± SE, $n = 3$–4) of current year white birch seedlings grown under ambient/doubled (360 vs. 720 μmol mol^{-1}) [CO_2] and high/low nutrient. The lower half part of canopy was shaded using neutral density mesh on 50% of the seedlings after two months of CO_2 and nutrient treatment. The shading reduced the PAR flux density by 65%. Measurements were taken immediately before and one month after the start of shading. Abbreviations: HN = high nutrient supply, LN = low nutrient, HNO = high nutrient with no shading, HNS = high nutrient with shading, LNO = low nutrient with no shading, LNS = low nutrient with shading. The significance levels are: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. When an interaction was significant, Scheffe's F test for post hoc pairwise comparisons was conducted. Means with different letters are significantly different from each other.

elevation increased TPU in the shaded but not in unshaded seedlings (Fig. 3).

3.3. PS-II efficiency and electron transport partitioning between carboxylation and oxygenation

There was a significant interactive effect of CO_2 and nutrient on $(F_m' - F)/F_m'$ after 3 months of treatment: the high nutrient significantly ($P < 0.01$) enhanced $(F_m' - F)/F_m'$ under the elevated but not ambient [CO_2] and the CO_2 elevation significantly increased $(F_m' - F)/F_m'$ only in the high nutrient treatment (Fig. 4).

[CO_2] and nutrient supply had significant interactive effects on J_c after 3 months of treatment ($P < 0.001$, Fig. 4). The high nutrient

supply resulted in a greater increase in J_c in the elevated than ambient [CO_2] (Fig. 4). The CO_2 elevation significantly increased J_c under the high nutrient but not under low nutrient treatment (Fig. 4). In contrast to J_c, the CO_2 elevation significantly suppressed J_o in both low and high nutrient treatment ($P < 0.01$). Both the CO_2 elevation and high nutrient supply significantly ($P < 0.001$) reduced J_o/J_T after 3 months' treatments (Fig. 4).

3.4. Biomass formation and allocation to leaves and roots

Both the CO_2 elevation and high nutrient supply significantly ($P < 0.001$) increased both leaf and root biomass (Fig. 5). The CO_2 elevation significantly reduced the leaf mass ratio only in the high
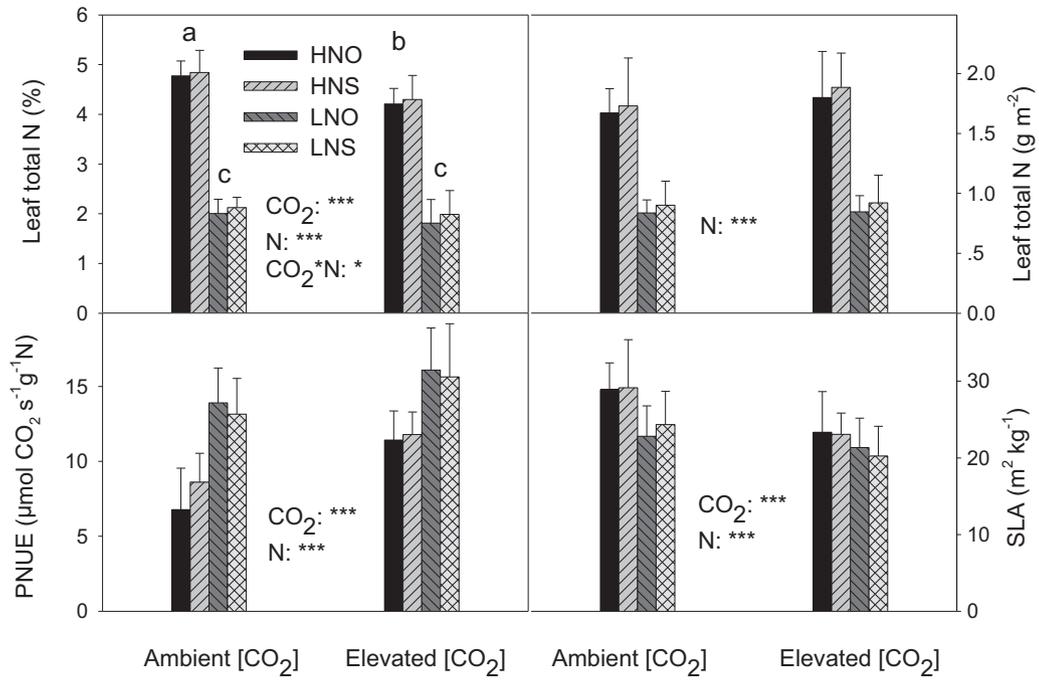
Fig. 2. Effects of [CO_2], nutrient supply and shading on total foliar nitrogen concentration (% and leaf area-based), photosynthetic nitrogen use efficiency (PNUE) and specific leaf area (SLA) (mean ± SE, n = 3–4). Measurements were taken after two months of [CO_2] and nutrient treatment and again after one month of shading. See Fig. 1 for other explanations.
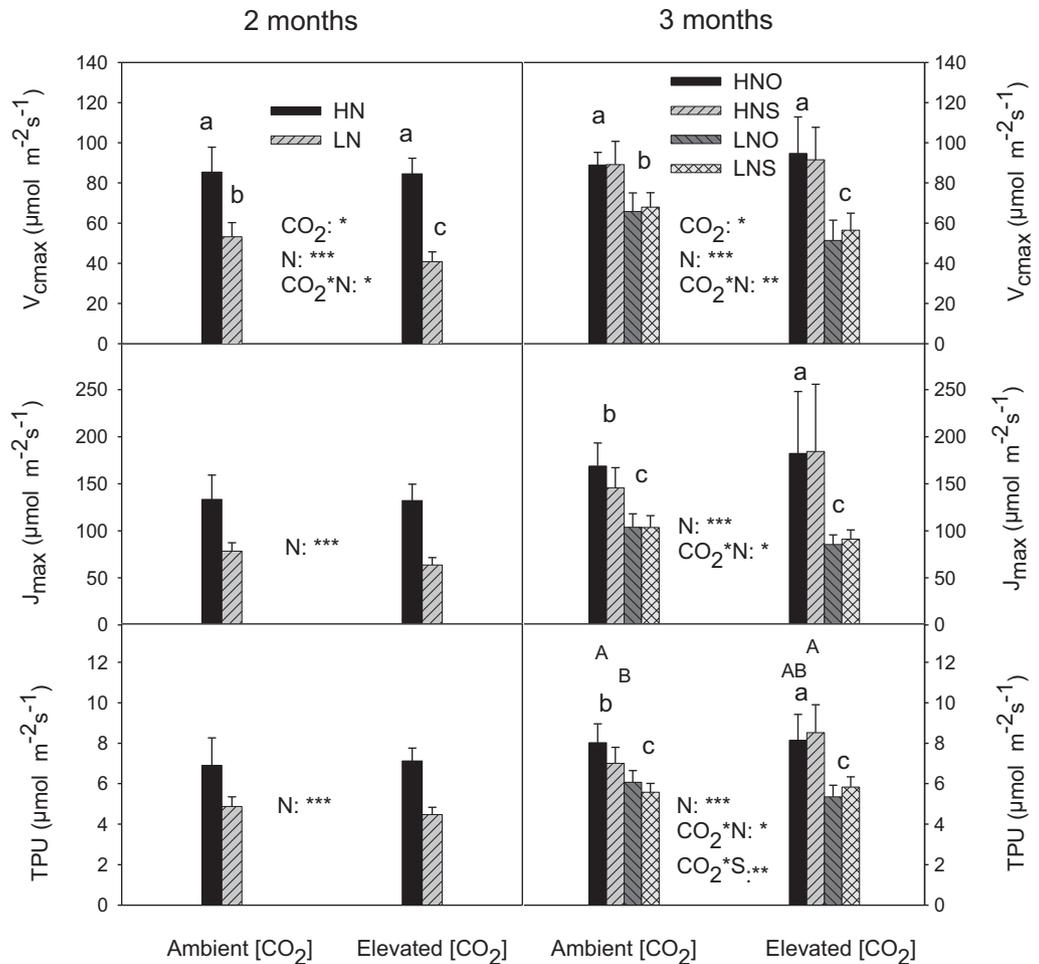
Fig. 3. Effects of [CO_2], nutrient supply and shading on V_{cmax}, J_{max} and TPU (mean ± SE, n = 3–4). Means with different letters are significantly different from each other. Capital letters represent interactions between CO_2 and shading while lower case letters describe interactions between CO_2 and nutrient. See Figs. 1 and 2 for other explanations.
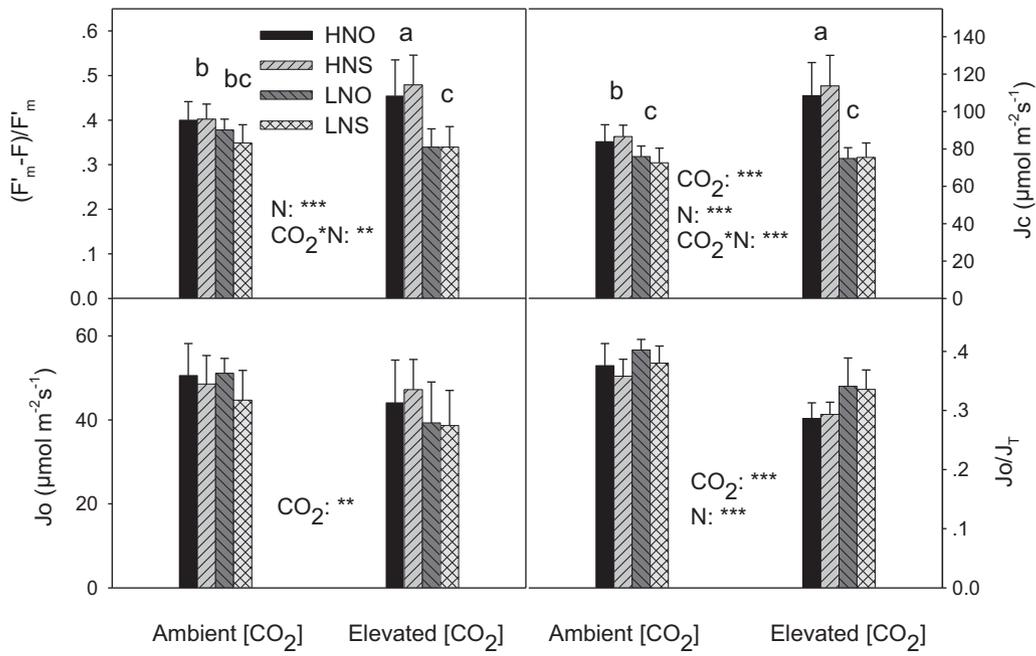
Fig. 4. Effects of [CO_2], nutrient supply and shading on $(F'_m - F)/F'_m$, J_c, J_o and J_o/J_T (mean ± SE, $n = 3$–4). See Fig. 1 for other explanations.

nutrient treatment while the low nutrient treatment significantly reduced the LMR in both CO_2 treatments although the magnitude was greater in the ambient and elevated [CO_2] ($P < 0.001$, Fig. 5). While the high nutrient treatment significantly reduced the root mass ratio in both CO_2 treatments, the CO_2 elevation significantly increased the RMR only in the high nutrient treatment ($P < 0.05$, Fig. 5). Shading the lower canopy significantly reduced leaf ($P < 0.01$) and root ($P < 0.001$) biomass (Fig. 5). The shading significantly ($P < 0.05$) reduced the RMR but had no significant effect on LMR (Fig. 5).

4. Discussion

The results from this study suggest that the photosynthetic acclimation to CO_2 elevation in white birch is dependent on nutrient supply and the effect of nutrient supply can be modified by CO_2 elevations. A down-regulation of photosynthetic capacity (i.e. V_{cmax}) did occur under the elevated [CO_2], but it occurred only in the low nutrient supply. The result is consistent with the findings in similar studies conducted in the greenhouse, OTC (open top chamber) and FACE [5–7,12,13]. Furthermore, the CO_2 elevation
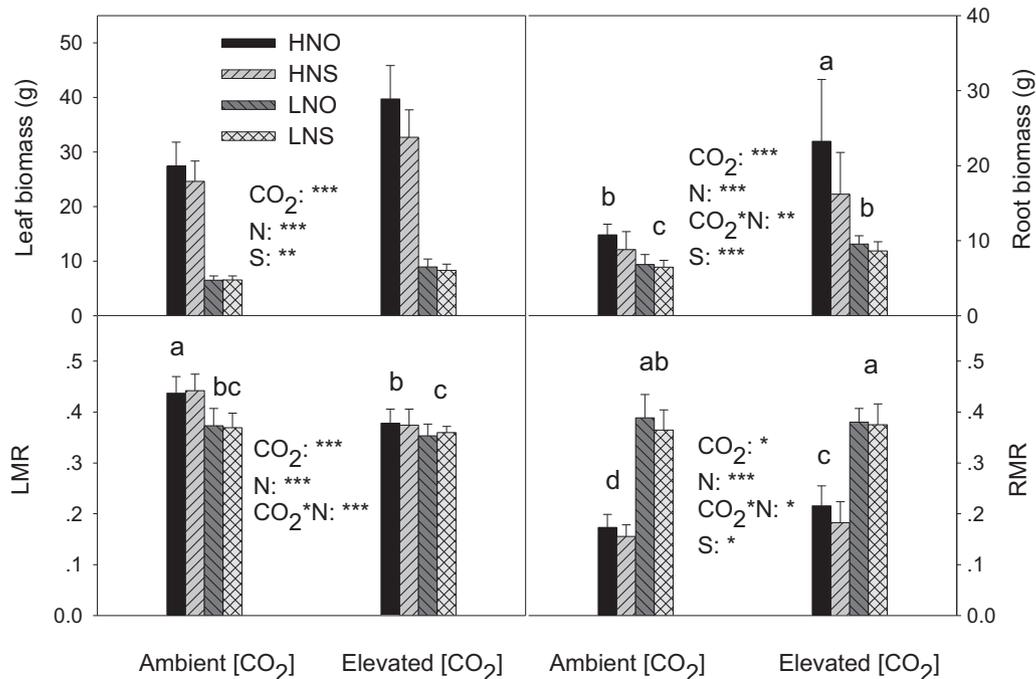
Fig. 5. Effects of [CO_2], nutrient supply and shading on biomass and biomass allocation to leaves (LMR) and Roots (RMR) (mean ± SE, $n = 4$). See Fig. 1 for other explanations.

increased the utilization of photosynthates in the high nutrient but not in the low nutrient treatment and the positive effect of high nutrient on J_{max} was much greater under the elevated than ambient $[CO_2]$. Both the CO_2 elevation and high nutrient increased the partitioning of total electron flow toward carboxylation. However, the hypothesis that shading the lower canopy would ameliorate the magnitude of the down-regulation of photosynthetic capacity under elevated $[CO_2]$ and low nutrient by reducing source strength (equivalent to increasing sink strength) was not supported. It appears that nutrient supply had a greater impact on the photosynthetic acclimation to $[CO_2]$ than did the change in sink/source ratio in white birch seedlings. The research of Ainsworth et al. [16] using soybean mutants suggests that the magnitude of photosynthetic acclimation to elevated $[CO_2]$ is dependent on growth habit (i.e., determinate versus indeterminate). Their results have confirmed the significance of sink strength but could not rule out nutrient effect as soybean is not sensitive to nutrient supply, particularly nitrogen, due to its capability of symbiotic N-fixation. The indeterminate growth habit of white birch, like most broadleaved pioneer deciduous tree species, probably contributed to its relatively greater response to the $[CO_2]$ elevation and the higher dependency of the response on nutrient regimes as compared to the responses of most climax and/or coniferous tree species that have determinate growth habit [16].

The hypothesis that high nutrient supply will lead to an up-regulation of photosynthesis in white birch was partially supported. While there was no up-regulation in V_{cmax}, the CO_2 elevation under the high nutrient supply did lead to significant increases in in vivo photosynthetic electron transport (J_{max}), photosynthate utilization (TPU), the actual quantum efficiency of PS-II ($\Delta F/F'_m$), and photosynthetic electron flow to carboxylation (J_c) with one more month of the CO_2 elevation, which implies that the up-regulation of photosynthetic functions developed gradually over time. The delayed up-regulation indicates that the timing of measurement is critically important for a more accurate assessment of physiological responses and also raises the question how the responses may change over time as trees get older. Thus the results from short term studies on young trees should be interpreted with caution. Furthermore, the CO_2 elevation induced increase of photosynthesis was greater in the high nutrient than low nutrient treatment. These results are in a general agreement with the findings of Ceulemans et al. [7], Curtis et al. [8] and Zhang and Dang [9]. The duplication of photosynthetic up-regulation in white birch seedlings under elevated $[CO_2]$ and high nutrient supply in this study suggests that the stimulation of photosynthetic carbohydrate production in white birch by CO_2 elevations can be maintained or even enhanced by maintaining or increasing nutrient supply through fertilization, improved nutrient cycling or other means. These findings suggest that, at least for the fast-growing deciduous trees like white birch, nutrient supply is a primary determinant for the magnitude and direction (down- vs. up-regulation) of photosynthetic acclimation to CO_2 elevation although the report of down-regulation is much more common than up-regulation in the literature. However, there are other factors that influence how photosynthesis and growth respond to CO_2 elevations, e.g., growth habit, potential growth rates, plant and tissue age. Up regulations are generally found in fast-growing species [7–9]. Curtis et al. [30] also suggest that CO_2 acclimation responses are more apparent in fast-growing juvenile *Populus* than slow-growing matured *Pinus* spp. Furthermore, in many pine species, photosynthetic down regulation is only observed in older needles, but not young needles [31,32]. Thus results from short term studies on young plants should not be directly applied to old plants.

White birch responded to shading by reducing sink strength rather than increasing source strength. The shading reduced the flux density of photosynthetically active radiation to the lower canopy by 65% and thus must have lowered the photosynthetic capacity or source strength. Instead of increasing the photosynthetic capacity of unshaded leaves or reducing the degree of photosynthetic down regulation in the upper canopy (in the case of elevated $[CO_2]$) to compensate for the lost capacity in the lower canopy as proposed in the hypothesis, the seedlings reduced biomass allocation to roots to lower the sink strength. This finding is further corroborated by a corresponding decline in triose phosphate utilization. Studying the relationship between photosynthetic down-regulation and leaf-fruit ratio in *Citrus*, Nebauer et al. [33] also find that leaf-to-fruit ratio does not modulate photosynthesis but changes the allocation of photosynthates to the fruits. Our results are consistent with the idea that the capacities of photosynthetic production in leaves and sink growth appear to be closely coordinated such that a balance is maintained between source supply and sink demand [34–36]. However, these results could not rule out the transient effect of changes in source/sink ratio on photosynthetic rates [37].

It is worth noting that although the responses of photosynthetic traits to the CO_2 elevation was mainly nutrient-dependent, the CO_2 elevation substantially increased the rate of photosynthesis, photosynthetic water use efficiency and nitrogen use efficiency in both the high and low nutrient treatment in this study as in some other studies [38–46]. These findings suggest that the elevation of atmospheric CO_2 concentration may improve the carbon sequestration and productivity of forests, particularly on nutrient-rich site, and that fertilization will likely be more effective in improving site productivity in the future than it is today However, it should be cautioned that this is a short term study on young seedlings and the last conclusion should be taken with caution.

Acknowledgements

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