

Low soil temperature reduces the positive effects of high nutrient supply on the growth and biomass of white birch seedlings in ambient and elevated carbon dioxide concentrations

Titus Fondo Ambebe, Qing-Lai Dang, and Jacob Marfo

Abstract: To investigate the interactive effects of soil temperature (T_{soil}) and nutrient supply on the responses of growth and biomass of white birch (*Betula papyrifera* Marsh.) to atmospheric carbon dioxide concentration ($[\text{CO}_2]$), seedlings were grown under two $[\text{CO}_2]$ (360 and 720 $\mu\text{mol}\cdot\text{mol}^{-1}$), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C one month later), and three nutrient regimes (Low: N–P–K = 4:1.8:3.3 $\text{mg}\cdot\text{L}^{-1}$; Intermediate: N–P–K = 80:35:66 $\text{mg}\cdot\text{L}^{-1}$; and High: N–P–K = 160:70:132 $\text{mg}\cdot\text{L}^{-1}$) for 4 months. Low T_{soil} reduced leaf and total biomass at high nutrient supply and root biomass at intermediate and high nutrient supply. There were significant three-factor interactive effects on root collar diameter (RCD), stem biomass, and leaf mass ratio. Low T_{soil} reduced RCD at high nutrient supply and stem biomass at intermediate and high nutrient supply in elevated $[\text{CO}_2]$ while intermediate and high T_{soil} enhanced them. Values of leaf mass ratio were lowest at low T_{soil} and low nutrient supply in elevated $[\text{CO}_2]$. The effect of T_{soil} was generally insignificant at low nutrient supply, but the responses of growth and biomass remained significantly higher under high than low nutrient supply at all T_{soil} .

Key words: boreal forest, global change, $T_{\text{soil}} \times \text{nutrition}$, $\text{CO}_2 \times T_{\text{soil}} \times \text{nutrition}$.

Résumé : Afin d'examiner les effets interactifs entre la température du sol (T_{sol}) et l'apport en nutriments sur les réactions de croissance et en biomasse du bouleau blanc (*Betula papyrifera* Marsh) aux concentrations de bioxyde de carbone ($[\text{CO}_2]$), les auteurs ont cultivé des plantules sous deux teneurs $[\text{CO}_2]$ (360 et 720 $\mu\text{mol}\cdot\text{mol}^{-1}$) et trois T_{sol} (5, 15 et 25 °C au départ, avec des augmentations à 7, 17 et 27 °C, un mois plus tard), et trois régimes nutritifs (Faible: N–P–K = 4:1.8:3.3 $\text{mg}\cdot\text{L}^{-1}$, Intermédiaire; N–P–K = 80:35:66 $\text{mg}\cdot\text{L}^{-1}$, et Élevé; N–P–K = 160:70:132 $\text{mg}\cdot\text{L}^{-1}$), pendant quatre mois. La basse température du sol réduit la biomasse foliaire et totale avec nutriments élevés et la biomasse racinaire avec des apports intermédiaires et élevés. On observe des effets trifactoriels significatifs sur le diamètre racinaire au collet (RCD), et le rapport de la biomasse de la tige sur la biomasse foliaire. La faible T_{sol} réduit le RCD avec des nutriments élevés et la biomasse de la tige avec des nutriments intermédiaires et élevés, avec un $[\text{CO}_2]$ élevé, alors que les T_{sol} intermédiaires et élevés les augmentent. Les valeurs de ratio de la masse foliaire sont plus faibles à T_{sol} faible et faible apport en nutriments en présence de $[\text{CO}_2]$ élevé. L'effet de T_{sol} demeure généralement non significatif à faibles apports de nutriments, mais les réactions de croissance et de biomasse demeurent significativement plus élevées avec des teneurs en nutriments élevées que faibles, à toutes les températures du sol.

Mots-clés : forêt boréale, changement global, $T_{\text{sol}} \times \text{nutrition}$, $\text{CO}_2 \times T_{\text{sol}} \times \text{nutrition}$.

[Traduit par la Rédaction]

Introduction

Global atmospheric carbon dioxide concentration ($[\text{CO}_2]$) has gradually risen from 280 $\mu\text{mol}\cdot\text{mol}^{-1}$ in 1850 to 379 $\mu\text{mol}\cdot\text{mol}^{-1}$ in 2005, and is currently increasing at a rate of 1.9 $\mu\text{mol}\cdot\text{mol}^{-1}$ per year as a result of both natural and human-induced emissions (IPCC 2007). The majority of experimental evidence indicates that elevated $[\text{CO}_2]$ stimulates the growth (Bowes 1993; Cipollini et al. 1993; Johnsen and Major 1998; Zhang et al. 2006; Zhang and Dang

2007) and CO_2 assimilation rate (Bazzaz 1990; Drake et al. 1997; Zhang and Dang 2005; Zhang and Dang 2006) of C_3 plants. Common growth responses to high $[\text{CO}_2]$ include increases in plant biomass, root:shoot ratio (R:S ratio), leaf area, numbers of leaves and branches, plant height, and root length (Norby et al. 1986; Bazzaz et al. 1990; Cipollini et al. 1993; Stulen and den Hertog 1993; Centritto et al. 1999; Pritchard et al. 1999; Liu et al. 2006; Zhang et al. 2006; Zhang and Dang 2007). However, the magnitude of response varies considerably among species. In any case, the allocation of assimilated carbon in elevated $[\text{CO}_2]$ appears to depend greatly upon the prevailing environmental conditions (Saxe et al. 1998; Zhang et al. 2006; Zhang and Dang 2007).

Soil temperature (T_{soil}) is a key environmental factor limiting tree growth in the boreal forest (Bonan 1992). Low T_{soil} reduces shoot growth (Landhäusser et al. 2001; Zhang and Dang 2007), and tends to increase the relative allocation

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T.F. Ambebe, Q. Dang,¹ and J. Marfo. Faculty of Forestry and the Forest Environment, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada.

¹Corresponding author (e-mail: qdang@lakeheadu.ca).

Table 1. Analysis of variance for the effects of [CO₂], soil temperature (T_{soil}), nutrient supply (N), and their interactions on morphology, biomass, and biomass ratios of white birch.

Source	CO ₂	T_{soil}	N	CO ₂ × T_{soil}	CO ₂ × N	T_{soil} × N	CO ₂ × T_{soil} × N
Morphology							
Height	0.1513	0.0188	0.0013	0.0457	0.8737	0.0857	0.2106
RCD	0.2407	0.0288	0.0073	0.1108	0.8973	0.0430	0.0381
Biomass							
Stem	0.3766	0.0253	0.0025	0.0204	0.9104	0.0676	0.0131
Leaf	0.8663	0.0156	0.0123	0.1321	0.4289	0.0357	0.1866
Root	0.5871	0.0035	0.0162	0.666	0.1796	0.0066	0.8161
Total	0.9456	0.0142	0.0081	0.0962	0.3628	0.0318	0.3206
Mass ratios							
Stem	0.3776	0.1794	0.1235	0.0682	0.1837	0.2371	0.9551
Leaf	0.2372	0.0516	0.0434	0.7181	0.2817	0.0333	0.0426
Root	0.5277	0.0055	0.0203	0.3165	0.1406	0.1078	0.6871
R:S	0.7417	0.0065	0.0250	0.2607	0.1887	0.1476	0.1401

Note: Seedlings were grown under two [CO₂] (360 and 720 $\mu\text{mol}\cdot\text{mol}^{-1}$), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three N ($N\text{-P-K} = 4:1.8:3.3 \text{ mg}\cdot\text{L}^{-1}$, $80:35:66 \text{ mg}\cdot\text{L}^{-1}$, and $160:70:132 \text{ mg}\cdot\text{L}^{-1}$) for 4 months. RCD and R:S represent root collar diameter and root:shoot ratio, respectively.

of carbon to roots because nutrient and water uptake are reduced (Lambers et al. 1995; Leuschner et al. 2007). T_{soil} may rise as a part of global climatic response to elevated [CO₂] (Peterjohn et al. 1994; Pregitzer and King 2005). A small change in T_{soil} can have a profound impact on physiological processes and growth of trees (Moorby and Nye 1984; Clarkson et al. 1992; Pregitzer and King 2005; Zhang and Dang 2007).

As atmospheric [CO₂] and T_{soil} rise, nutrient requirements of plants may change, owing to the increased growth (Reddy et al. 1997; Lawlor 2005). The response of plants to global change may be limited in nutrient-poor soils, although the evidence is not as clear as might be expected (Steffen and Canadell 2005). Because of the large variation in T_{soil} across the boreal forest, mineral nutrition is likely to be a complex issue under climate change associated with rising atmospheric [CO₂].

T_{soil} and mineral nutrition are, thus, two major growth-limiting factors that are likely to change under the future scenario of global atmospheric [CO₂] elevation. Interestingly, their effects are known to interact with each other and with [CO₂] and are therefore of particular interest for understanding and predicting global change effects on the boreal forest. King et al. (1999) and Zhang and Dang (2006, 2007) have explored interactive effects between T_{soil} and nutrient supply, [CO₂] and nutrient supply, and [CO₂] and T_{soil} on the growth of coniferous and deciduous boreal species. In the present study, we investigated the interactive effects of T_{soil} and nutrient supply on growth and biomass responses of white birch to elevated [CO₂]. Nutrient and [CO₂] elevation have synergistic effects on biomass production of white birch (Zhang et al. 2006). Since low T_{soil} reduces root capacity for the uptake of nutrients (Paré et al. 1993), we hypothesized that low T_{soil} will reduce the positive effect of high nutrient supply on the responses of growth and biomass to atmospheric [CO₂] elevation. This is the first study to examine the interactive effects of three environmental factors, including T_{soil} , on a major boreal tree species.

Materials and methods

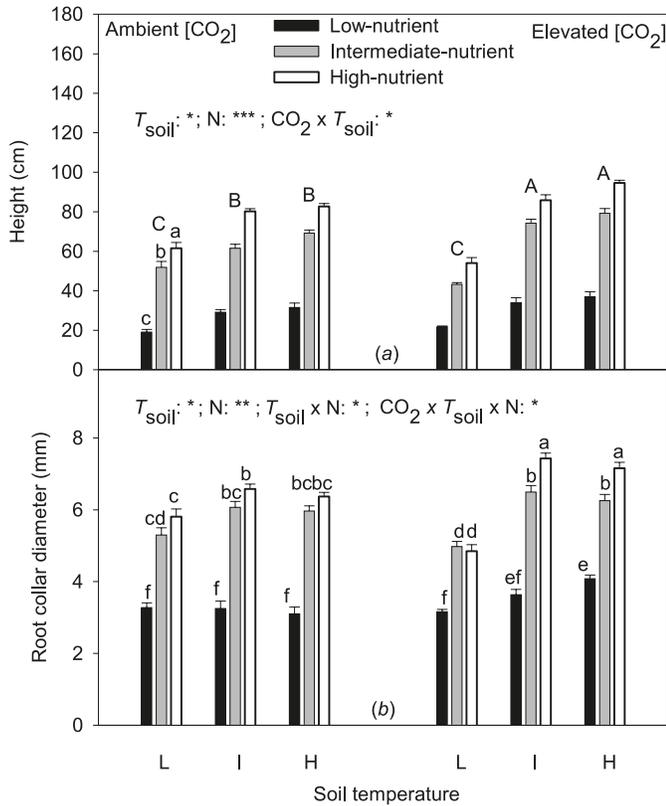
Plant materials

White birch seeds (*Betula papyrifera* Marsh.) were germinated in germination trays (52 cm × 26 cm × 6 cm) containing a 1:1 (v/v) mixture of peat moss and vermiculite (Sun Gro Horticulture Canada Ltd, Seba Beach, Alberta, Canada) in a greenhouse. The environmental conditions in the greenhouse were temperatures at 26 °C (day) – 16 °C (night) (± 2 °C) and a 15 h light period. The natural light was supplemented using high-pressure sodium lamps (model LR48877, P.L. Systems, Grimsby, Ontario, Canada) in early morning and late afternoon to maintain the photoperiod. The light intensity at plant level averaged 660 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, as measured with a LI-190SA quantum sensor (Li-COR, Lincoln, Nebraska, USA). The growing medium was kept moist by misting with a spray bottle. The seedlings were about 2.5 cm tall when relatively uniform-sized seedlings were selected and transplanted into pots of 13.5 cm tall and 11–9.5 cm top–bottom diameters. The growing medium used was as described previously (Zhang and Dang 2005). The pots were mounted in soil temperature control boxes as described in the following section. The germination phase lasted 8 weeks and there were no fertilizer applications during this phase.

Experimental design and growth conditions

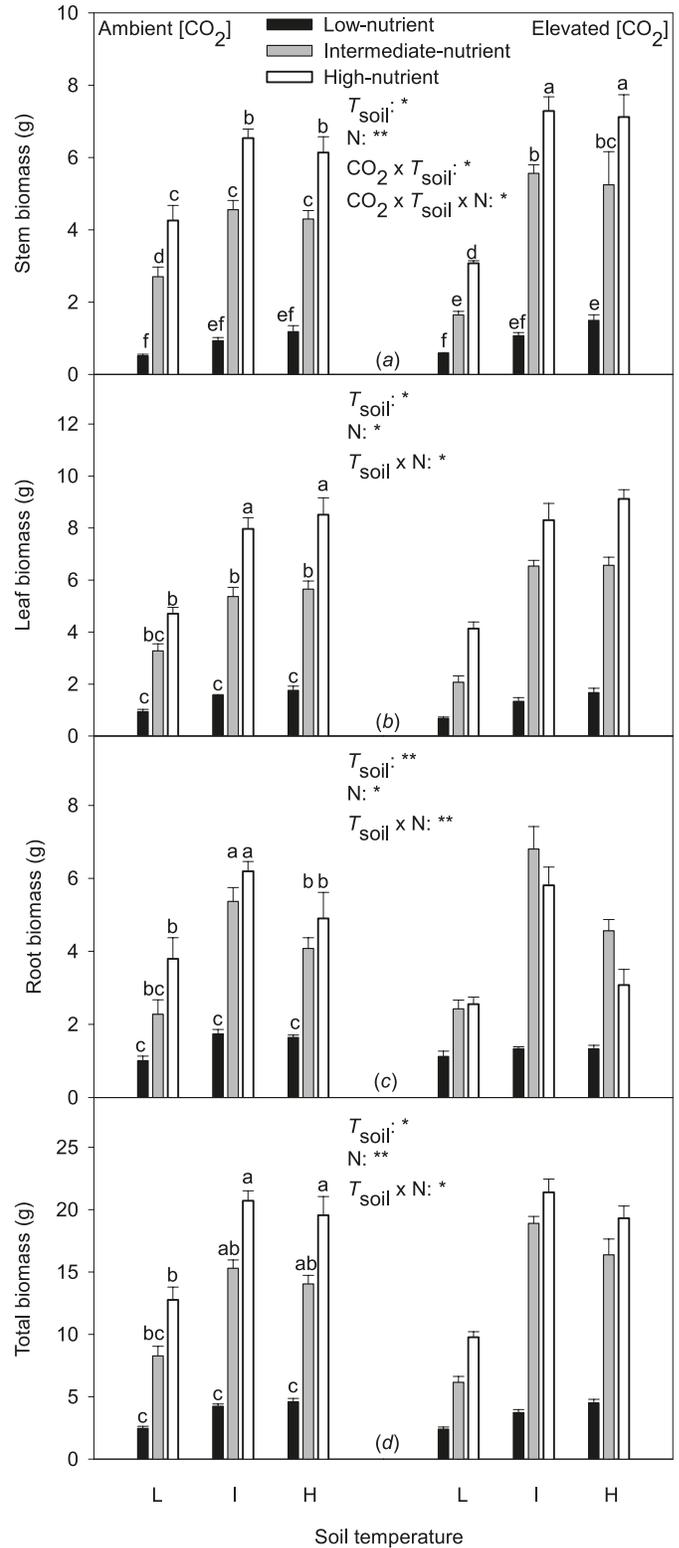
The experiment was conducted in greenhouses at Lakehead University (48°25'N, 89°16'W) from 1 January to 30 April 2008. The treatments were composed of two [CO₂] (360 $\mu\text{mol}\cdot\text{mol}^{-1}$ (ambient) and 720 $\mu\text{mol}\cdot\text{mol}^{-1}$ (elevated)), three T_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, at the beginning of February), and three nutrient regimes (Low: $N\text{-P-K} = 4:1.8:3.3 \text{ mg}\cdot\text{L}^{-1}$; Intermediate: $N\text{-P-K} = 80:35:66 \text{ mg}\cdot\text{L}^{-1}$; and High: $N\text{-P-K} = 160:70:132 \text{ mg}\cdot\text{L}^{-1}$). According to Zhang et al. (2006) and Perry and Hickman (2001), our intermediate nutrient treatment should result in foliage nutrient concentrations similar to values found in white birch trees naturally growing on rich sites while the low and high nutrient treatment should

Fig. 1. Effects of [CO₂], soil temperature (*T*_{soil}), and nutrient supply (*N*) on (a) seedling height and (b) root collar diameter (means ± SE, *n* = 7) of white birch. Seedlings were grown under two [CO₂] (360 and 720 μmol·mol⁻¹), three *T*_{soil} (5, 15, and 25 °C initially, increased to 7, 17, and 27 °C, respectively, one month later), and three *N* (N–P–K = 4:1.8:3.3 mg·L⁻¹, 80:35:66 mg·L⁻¹, and 160:70:132 mg·L⁻¹) for 4 months. The upper-case letters indicate CO₂ × *T*_{soil} interactions. In Figs. 1a and 1b, the lower-case letters indicate *N* effect and CO₂ × *T*_{soil} × *N* interactions, respectively. Different letters above the bars represent significantly different means under Scheffé’s post hoc test (*p* ≤ 0.05). L, I, and H represent the low, intermediate, and high *T*_{soil}, respectively.



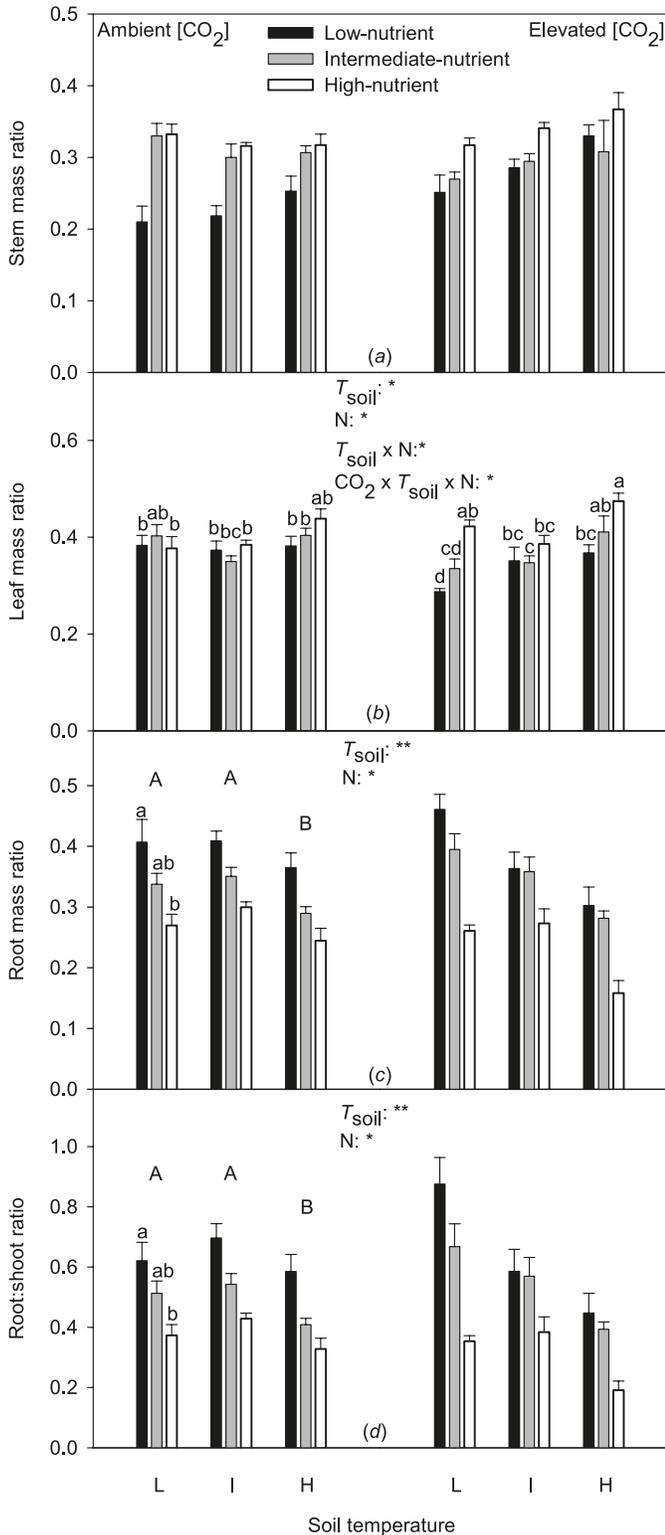
result in foliar nutrient concentrations lower and higher than values in natural white birch trees at nutrient poor and nutrient rich sites, respectively. The natural distribution range of a species is generally narrower than what its physiological capacity permits (physiological filter) because of biological limitations (biotic filter, Lambers et al. 2008). Thus, extending the treatment beyond both ends of the natural spectrum should permit the better examination of the physiological responses. The experiment was laid out in a split-split plot design where the [CO₂] treatments were the main plots, *T*_{soil} were the sub-plots, and nutrient treatments were the sub-sub-plots. The [CO₂] treatments were applied simultaneously in four separate environmentally controlled greenhouses, each pair representing two replications per treatment. Ten seedlings were assigned randomly to each treatment combination. The elevated [CO₂] was achieved using Argus CO₂ generators (Argus, Vancouver, B.C., Canada). *T*_{soil} was regulated by circulating heated or cooled water between the pots attached to the bottom of an insulated wooden box. The pots in each box were insulated

Fig. 2. Effects of [CO₂], soil temperature (*T*_{soil}), and nutrient supply (*N*) on (a) stem biomass, (b) leaf biomass, (c) root biomass, and (d) total biomass (means ± SE, *n* = 3). The letters in Figs. 2b, 2c, 2d indicate *T*_{soil} × *N* interactions. See Fig. 1 for other explanations.



with foam insulation sheets to minimize heat exchange between the growth medium and the air, and a drain hole was installed beneath each pot. A detailed description of the *T*_{soil}

Fig. 3. Effects of [CO₂], soil temperature (*T*_{soil}) and nutrient supply (*N*) on (a) stem mass ratio, (b) leaf mass ratio, (c) root mass ratio and (d) root:shoot ratio (means ± SE, *n* = 3). In Fig. 3a, the absence of labels indicates no significant effects. The upper-case letters indicate *T*_{soil} effect. See Fig. 1 for other explanations.



control system is provided by Cheng et al. (2000). Nutrient treatments were applied once a week in the form of solutions.

All treatments were subjected to day–night air temperatures of 26–16 °C and a 16 h photoperiod (the natural light was supplemented with high-pressure sodium lamps on cloudy days, early mornings and late evenings). All the environmental conditions were monitored and controlled with an Argus environmental control system (Vancouver, Canada). The seedlings were watered daily to keep the growing medium moist.

Measurements

Seedling height (*H*) and root collar diameter (RCD) were measured at the end of the experiment. Three randomly selected seedlings were then harvested. Seedlings were divided into leaves, root, and stem. The roots were rinsed free of growing medium. Each sample was oven-dried at 70 °C for 48 h and weighed. The ratios of stem (stem mass ratio), leaf (leaf mass ratio), and root (root mass ratio) biomass to total seedling biomass, and root biomass to shoot (leaf + stem) biomass (R:S ratio) were calculated.

Statistical analysis

Data analyses were performed using Data Desk 6.01 (Data Description 1996). Data were graphically examined for normality (probability plots) and equal variances among treatments (scatter plots). Those data that did not follow a normal distribution were square-root transformed and the transformed data satisfied the normal distribution assumption for analysis of variance (ANOVA). A three-way, split-split plot ANOVA was then used to test the effects of [CO₂], *T*_{soil}, nutrient supply, and their interactions. The statistical test was considered significant at *p* ≤ 0.05 and Scheffé’s post hoc test was used to determine significant differences between means.

Results

Morphology

There was significant interactive effect between [CO₂] and *T*_{soil} on *H* (Table 1). Although the intermediate and high *T*_{soil} treatments significantly increased *H* growth in both ambient and elevated [CO₂], the increases were greater under elevated than ambient [CO₂] (Fig. 1a). Elevated [CO₂] significantly enhanced *H* growth only at the intermediate and high, but not at the low *T*_{soil} (Fig. 1a). *H* increased with increases in nutrient supply (Fig. 1a). There were significant three-way interactions among [CO₂], *T*_{soil}, and nutrient supply on RCD (Table 1). RCD was significantly lower in the low nutrient treatment, and *T*_{soil} did not significantly affect RCD under the low nutrient regime at ambient [CO₂] (Fig. 1b). Furthermore, the [CO₂] elevation significantly enhanced RCD only under the intermediate and high *T*_{soil} (Fig. 1b).

Biomass

There were significant three-way interactive effects among [CO₂], *T*_{soil}, and nutrient supply on stem biomass (Table 1). The [CO₂] elevation significantly increased stem biomass at high *T*_{soil} and high nutrient supply and under the

intermediate and high nutrient treatments at intermediate T_{soil} (Fig. 2a). In contrast, the $[\text{CO}_2]$ elevation decreased stem biomass under the low T_{soil} and intermediate and high nutrient supply, with no significant effects under low nutrient supply at any T_{soil} . There were significant two-way interactions between T_{soil} and nutrient supply on all other biomass parameters (Table 1). The general trend for nutrient effect was that leaf and total seedling biomass increased with increases in nutrient supply, but the effects of T_{soil} varied with nutrient regime; while the lowest T_{soil} resulted in the lowest leaf and total biomass at high nutrient supply, T_{soil} did not significantly affect leaf and total seedling biomass under low and intermediate nutrient supply (Figs. 2b and 2d). However, the interactive effects on root biomass were more complicated. Although the low nutrient treatment still resulted in the lowest root biomass at all T_{soil} , there was no significant difference between the intermediate and high nutrient regimes at any T_{soil} (Fig. 2c). The intermediate T_{soil} produced the highest root biomass at intermediate and high nutrient regimes, but T_{soil} had no significant effects on root biomass under low nutrient supply (Fig. 2c).

Biomass ratios

None of the three treatments had a significant effect on stem mass ratio (Table 1; Fig. 3a). However, there were significant three-way interactions among $[\text{CO}_2]$, T_{soil} , and nutrient supply on leaf mass ratio (Table 1): the leaf mass ratio was the lowest under the low nutrient and low T_{soil} and highest under high nutrient and high T_{soil} at elevated $[\text{CO}_2]$ (Fig. 3b). T_{soil} and nutrient supply significantly affected root mass ratio and R:S ratio (Table 1). The high T_{soil} significantly reduced both biomass allocation parameters whereas there was no significant difference between the low and intermediate T_{soil} treatments (Figs. 3c and 3d). Root mass ratio and R:S ratio decreased with increasing nutrient supply (Figs. 3c and 3d).

Discussion

Low T_{soil} significantly suppressed leaf, root, and total seedling biomass under high but not under low nutrient supply. However, biomass production remained significantly higher under the high than low nutrient supply at all T_{soil} . Our results are consistent with our prediction that low T_{soil} reduces the positive effect of high nutrient supply on biomass production. The lack of T_{soil} effect on biomass of the low nutrient grown seedlings may be attributed to the fact that this nutrient level was already highly limiting for growth (King et al. 1999). While leaf nitrogen concentrations for the intermediate and high nutrient treatments are in line with the levels found in natural non-nutrient limiting white birch forests (Perry and Hickman 2001), we found that the values in the low nutrient treatment were about 50% lower than the lowest level found in the field (T.F. Ambebe and Q.L. Dang, unpublished data).

The negative effects of low T_{soil} on biomass can be manifested through reductions in root growth and root function, which may lead to physiological drought and (or) nutrition stress (Bowen 1991; Grossnickle 2000). Low T_{soil} reduces the root capacity for absorbing water and nutrients (Paré et al. 1993; Lambers et al. 1995; Wan et al. 1999; Leuschner

et al. 2007). Increased water viscosity and decreased root permeability have been suggested to reduce root water uptake in cold soils (Kaufmann 1975, 1977; Kramer 1983). Low T_{soil} also affects soil processes associated with nutrient fluxes. For newly planted seedlings in the field the importance of T_{soil} for seedling establishment has been demonstrated (Örlander 1984). T_{soil} did not affect foliar water content in this experiment (data not shown) indicating that water stress was not a contributing factor for the slower growth and lower biomass production at low T_{soil} in this study.

Gavito et al. (2001) have found that $[\text{CO}_2]$ elevation enhances the biomass production of winter wheat at high T_{soil} but has no significant effect at low T_{soil} . The similar responses of net photosynthesis as biomass production suggest that low T_{soil} may have induced photosynthetic down-regulation (Gavito et al. 2001). Photosynthetic down-regulation at low T_{soil} may be triggered by an accumulation of non-structural carbohydrates in the leaf (Makino 1994; Eguchi et al. 2004) owing to an inability to use additional photosynthates for root growth (Tabbush 1986; King et al. 1999; Ainsworth et al. 2004). Low levels of leaf nitrogen owing to reduced root uptake capacity at low T_{soil} may also play a role (Stitt and Krapp 1999). In the present study, the low T_{soil} significantly reduced stem biomass of the intermediate and high nutrient grown seedlings with increasing $[\text{CO}_2]$, whereas the intermediate and high T_{soil} enhanced it. Similar findings were made for RCD. Our data partly support the findings of Gavito et al. (2001). Although elevated $[\text{CO}_2]$ is known to reduce plant growth at cold air temperatures (Idso et al. 1987; Idso and Kimball 1989; McKee and Woodward 1994), the decline in stem biomass and RCD under elevated $[\text{CO}_2]$ and low T_{soil} reported here is unprecedented, and provides a notable exception to the CO_2 fertilization hypothesis that stipulates that the rising atmospheric $[\text{CO}_2]$ has a positive effect on the growth of trees (Huang et al. 2007). We do not know the physiological mechanism underlining the negative CO_2 effect on stem growth at the low T_{soil} . The xylem is the primary component of the stem and functions to transport water and nutrients as well as to provide physical support to the canopy. The $[\text{CO}_2]$ elevation reduced transpiration rates to a greater extent at the low than at the other T_{soil} (T.F. Ambebe and Q.L. Dang, unpublished data) and presumably the transpirational demand and transport capacity of the xylem for water. This should at least provide a partial explanation to the negative effect of $[\text{CO}_2]$ elevation on stem growth (i.e., stem biomass and RCD), since there is no reason to believe that there should have been a corresponding increase for physical support to offset the reduced demand for water transport. Furthermore, the low T_{soil} in this study is lower than the lowest T_{soil} in all other published studies examining the interactive effects of T_{soil} and $[\text{CO}_2]$ (Gavito et al. 2001; Zhang and Dang 2005; Zhang and Dang 2007), which could have contributed to the discrepancy between our results and those of others. In addition, the growth enhancement by CO_2 elevation should be greater under non-limiting than under limiting nutrient availability (Maillard et al. 2001; Zhang et al. 2006; Huang et al. 2007). Our data are in agreement with this hypothesis and further reveal that trees growing in cold soils may not

experience the stem biomass and RCD growth increases that would be observed in those growing under warmer soil conditions.

This study's general hypothesis was that low T_{soil} reduces the positive effect of higher nutrient supply on the responses of growth and biomass to elevated $[\text{CO}_2]$. The significant three-way interactions observed for RCD and stem biomass are in accordance with the hypothesis. Contrary to our expectation, however, the effects of $[\text{CO}_2]$ on biomass were, in general, non-significant. Unresponsiveness of biomass to elevated $[\text{CO}_2]$ may be attributed to photosynthetic down-regulation, as has been observed in oak (*Quercus geminata* Small) (Ainsworth et al. 2002) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Olszyk et al. 2003). A visual inspection of the root system at the end of the experiment showed that root growth was restricted by pot size under the intermediate and high T_{soil} . This finding suggests that photosynthetic down-regulation might have been initiated by a build-up of photosynthates in the leaf, owing to a pot-induced reduction in sink demand (Arp 1991; Teskey et al. 1995). Also, the lack of increases in biomass with elevated $[\text{CO}_2]$ could be related to the greater use of photosynthates in root respiration under warmer T_{soil} and the fact that biomass production under low T_{soil} was primarily limited by cold soil, but not CO_2 availability.

$[\text{CO}_2]$, T_{soil} , and nutrient supply had much smaller effects on biomass allocation than on growth and biomass production. King et al. (1999) found that T_{soil} has no significant effect on biomass allocation of trembling aspen and Peng and Dang (2003) have reported similar results for both coniferous and deciduous boreal species. It has also been shown that $[\text{CO}_2]$ does not change the biomass allocation between roots and shoots (Bosac et al. 1995; Tingey et al. 1996; Curtis and Wang 1998; Gavito et al. 2001; Zhang et al. 2006; Zhang and Dang 2007). Stulen and den Hertog (1993) have concluded that the significant response of biomass allocation parameters to $[\text{CO}_2]$ is due to low nutrient availability. In this study, elevated $[\text{CO}_2]$ reduced leaf mass ratio and tended to increase root mass ratio and R:S ratio in the low T_{soil} and low nutrient supply treatments. These results suggest that the low nutrient effect was most prominent under low T_{soil} in elevated $[\text{CO}_2]$. This is, however, not supported by the growth and biomass data, where the low nutrient treatment produced similar response at all T_{soil} . The present study is in agreement with the theory of functional equilibrium which predicts a reduction in leaf mass ratio and an increase in root mass ratio or R:S ratio in plants growing under elevated $[\text{CO}_2]$ (Brouwer 1983; Wilson 1988; Lambers et al. 2008).

In conclusion, low T_{soil} negatively affected leaf, root, and total biomass production of seedlings under high nutrient supply. More interestingly, low T_{soil} reduced the positive effect of high nutrient supply on the responses of stem biomass and RCD to elevated $[\text{CO}_2]$. Our data suggest that an increase in T_{soil} due to climate change could have a beneficial effect on the response to elevated $[\text{CO}_2]$ of trees growing under non-nutrient limiting cold soil conditions. There is considerable variation in T_{soil} within the boreal forest ranging from cold permafrost and wetland sites to warm southwest facing slopes. Moreover, soil fertility is likely to be enhanced by anthropogenic nitrogen deposition under future

changing climatic conditions (Galloway et al. 2004). The differences in response observed in this study could have important implications for the growth, biomass production and distribution of trees across the boreal forest ecosystem. However, as this short-term study with seedlings was conducted under strictly controlled environmental conditions, the results might differ from what would be found for trees growing in natural forests. The growing conditions in the greenhouse may be different from those in the field and trees rooted in the ground may not experience photosynthetic down-regulation as quickly as tree seedlings growing in pots (Curtis and Wang 1998; Norby et al. 1999; Wigley and Schimel 2000). Furthermore, seedlings may not accurately reflect the responses of mature trees owing to differences in ontogenetic age and (or) size (Hättenschwiler et al. 1997; Bond 2000; Cavender-Bares and Bazzaz 2000). Consequently, this study's findings should not be applied to natural forests without validation. Our results suggest a need for considering T_{soil} when examining the effects of nutrient availability on the responses of boreal trees to elevated $[\text{CO}_2]$. We hope that this work will form the basis for further research on the combined effects of T_{soil} and nutrient availability on the responses to elevated $[\text{CO}_2]$ of trees growing under natural forest conditions.

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References

- Ainsworth, E.A., Davey, P.A., Hymus, G.J., Drake, B.G., and Long, S.P. 2002. Long-term response of photosynthesis to elevated carbon dioxide in a Florida scrub – oak ecosystem. *Ecol. Appl.* **12**(5): 1267–1275. doi:10.1890/1051-0761(2002)012[1267:LTROPT]2.0.CO;2.
- Ainsworth, E.A., Rogers, A., Nelson, R., and Long, S.P. 2004. Testing the “source-sink” hypothesis of down-regulation of photosynthesis in elevated $[\text{CO}_2]$ in the field with single gene substitutions in *Glycine max*. *Agric. For. Meteorol.* **122**(1–2): 85–94. doi:10.1016/j.agrformet.2003.09.002.
- Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2 . *Plant Cell Environ.* **14**(8): 869–875. doi:10.1111/j.1365-3040.1991.tb01450.x.
- Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. *Annu. Rev. Ecol. Syst.* **21**(1): 167–196. doi:10.1146/annurev.es.21.110190.001123.
- Bazzaz, F.A., Coleman, J.S., and Morse, M.R. 1990. Growth responses of seven major co-occurring tree species of the north-eastern United States to elevated CO_2 . *Can. J. For. Res.* **20**(9): 1479–1484. doi:10.1139/x90-195.
- Bonan, G.B. 1992. Soil temperature as an ecological factor in boreal forests. In *A systems analysis of the Global Boreal Forest*. Edited by H.H. Shugart, R. Leemans, and G.B. Bonan. Cambridge University Press, Cambridge, UK. pp. 126–143.
- Bond, B.J. 2000. Age-related changes in photosynthesis of woody plants. *Trends Plant Sci.* **5**(8): 349–353. doi:10.1016/S1360-1385(00)01691-5. PMID:10908880.

- Bosac, C., Gardner, S.D.L., Taylor, G., and Wilkins, D. 1995. Elevated CO₂ and hybrid poplar: a detailed investigation on root and shoot growth and physiology of *Populus euramericana*, 'Primo'. *For. Ecol. Manage.* **74**(1–3): 103–116. doi:10.1016/0378-1127(94)03506-R.
- Bowen, G.D. 1991. Soil temperature, root growth, and plant function. *In* Plant roots: the hidden half. *Edited by* Y. Eshel and U. Kafkafi. Marcel Dekker Inc., New York, N.Y. pp. 309–330.
- Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO₂. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**(1): 309–332. doi:10.1146/annurev.pp.44.060193.001521.
- Brouwer, R. 1983. Functional equilibrium: sense or nonsense? *Neth. J. Agric. Sci.* **31**(4): 335–348.
- Cavender-Bares, J., and Bazzaz, F.A. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia (Berl.)*, **124**(1): 8–18. doi:10.1007/PL00008865.
- Centritto, M., Lee, H.S.J., and Jarvis, P.G. 1999. Increased growth in elevated [CO₂]: an early, short-term response? *Glob. Change Biol.* **5**(6): 623–633. doi:10.1046/j.1365-2486.1999.00263.x.
- Cheng, S., Dang, Q.L., and Cai, T.B. 2000. A soil temperature control system for ecological research in greenhouses. *J. For. Res.* **5**(3): 205–208. doi:10.1007/BF02762403.
- Cipollini, M.L., Drake, B.G., and Whigham, D. 1993. Effects of elevated CO₂ on growth and carbon/nutrient balance in the deciduous woody shrub *Lindera benzoin* (L.) Blume (Lauraceae). *Oecologia (Berl.)*, **96**(3): 339–346. doi:10.1007/BF00317503.
- Clarkson, D.T., Jones, L.H., and Purves, J.V. 1992. Absorption of nitrate and ammonium ions by *Lolium perenne* from flowing solution cultures at low room temperatures. *Plant Cell Environ.* **15**(1): 99–106. doi:10.1111/j.1365-3040.1992.tb01462.x.
- Curtis, P.S., and Wang, X. 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia (Berl.)*, **113**(3): 299–313. doi:10.1007/s004420050381.
- Data Description. 1996. Data desk. Version 6.01. Data Description Inc., Ithaca, New York, N.Y.
- Drake, B.G., González-Meler, M.A., and Long, S.P. 1997. More efficient plants: a consequence of rising atmospheric CO₂? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**(1): 609–639. doi:10.1146/annurev.arplant.48.1.609. PMID:15012276.
- Eguchi, N., Fukatsu, E., Funada, R., Tobita, H., Kitao, M., Maruyama, Y., and Koike, T. 2004. Changes in morphology, anatomy, and photosynthetic capacity of needles of Japanese larch (*Larix kaempferi*) seedlings grown in high CO₂ concentrations. *Photosynthetica*, **42**(2): 173–178. doi:10.1023/B:PHOT.0000040587.99518.a8.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., and Vöosmarty, C.J. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**(2): 153–226. doi:10.1007/s10533-004-0370-0.
- Gavito, M.E., Curtis, P.S., Mikkelsen, T.N., and Jakobsen, I. 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *J. Exp. Bot.* **52**(362): 1913–1923. doi:10.1093/jexbot/52.362.1913. PMID:11520880.
- Grossnickle, S.C. 2000. Ecophysiology of northern spruce species: the performance of planted seedlings. NRC Research Press, Ottawa, Ont.
- Hättenschwiler, S., Miglietta, F., Raschi, A., and Körner, C. 1997. Morphological adjustments of mature *Quercus ilex* trees to elevated CO₂. *Acta Oecol.* **18**(3): 361–365. doi:10.1016/S1146-609X(97)80026-4.
- Huang, J.G., Bergeron, Y., Denneler, B., Berninger, F., and Tardif, J. 2007. Response of forest trees to increased atmospheric CO₂. *Crit. Rev. Plant Sci.* **26**(5): 265–283. doi:10.1080/07352680701626978.
- Idso, S.B., and Kimball, B.A. 1989. Growth response of carrot and radish to atmospheric CO₂ enrichment. *Environ. Exp. Bot.* **29**(2): 135–139. doi:10.1016/0098-8472(89)90045-2.
- Idso, S.B., Kimball, B.A., Anderson, M.G., and Mauney, J.R. 1987. Effects of atmospheric CO₂ enrichment on plant growth: the interactive role of air temperature. *Agric. Ecosyst. Environ.* **20**(1): 1–10. doi:10.1016/0167-8809(87)90023-5.
- IPCC. 2007. Summary for Policymakers. *In* Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Cambridge University Press, Cambridge, UK.
- Johnsen, K.H., and Major, J.E. 1998. Black spruce family growth performance under ambient and elevated CO₂. *New For.* **15**(3): 271–281.
- Kaufmann, M.R. 1975. Leaf water stress in Engelmann Spruce: influence of the root and shoot environments. *Plant Physiol.* **56**(6): 841–844. doi:10.1104/pp.56.6.841. PMID:16659406.
- Kaufmann, M.R. 1977. Soil temperature and drying cycle effects on water relations of *Pinus radiata*. *Can. J. Bot.* **55**(18): 2413–2418. doi:10.1139/b77-275.
- King, J.S., Pregitzer, K.S., and Zak, D.R. 1999. Clonal variation in above- and below-ground responses of *Populus tremuloides* Michx.: influence of soil warming and nutrient availability. *Plant Soil*, **217**(1–2): 119–130. doi:10.1023/A:1004560311563.
- Kramer, P.J. 1983. Water relations of plants. Academic Press, Orlando, Fla.
- Lambers, H., van den Boogarrd, R., Veneklaas, E.J., and Villar, R. 1995. Effects of global environmental change on carbon partitioning in vegetative plants of *Triticum aestivum* and closely related *Aegilops* species. *Glob. Change Biol.* **1**(6): 397–406. doi:10.1111/j.1365-2486.1995.tb00038.x.
- Lambers, H., Chapin, F.S., III, and Pons, T.L. 2008. Plant physiological ecology. Springer Science+Business Media LLC, New York, N.Y.
- Landhäusser, S.M., DesRochers, A., and Lieffers, V.J. 2001. A comparison of growth and physiology in *Picea glauca* and *Populus tremuloides* at different soil temperatures. *Can. J. For. Res.* **31**(11): 1922–1929. doi:10.1139/cjfr-31-11-1922.
- Lawlor, D.W. 2005. Plant responses to climate change: impacts and adaptation. *In* Plant responses to air pollution and global change. *Edited by* K. Omasa, I. Nouchi, and L.J. De Kok. Springer-Verlag, Tokyo, Japan. pp. 82–88.
- Leuschner, C., Moser, G., Bertsch, C., Röderstein, M., and Hertel, D. 2007. Large altitudinal increase in tree root/shoot ratio in tropical mountain forests of Ecuador. *Basic Appl. Ecol.* **8**(3): 219–230. doi:10.1016/j.baec.2006.02.004.
- Liu, N., Dang, Q.L., and Parker, W.H. 2006. Genetic variation of *Populus tremuloides* in ecophysiological responses to CO₂ elevation. *Can. J. Bot.* **84**(2): 294–302. doi:10.1139/B05-171.
- Maillard, P., Guehl, J.M., Muller, J.F., and Gross, P. 2001. Interactive effects of elevated CO₂ concentration and nitrogen supply on partitioning of newly fixed ¹³C and ¹⁵N between shoot and roots of pedunculate oak seedlings (*Quercus robur*). *Tree Physiol.* **21**(2–3): 163–172. PMID:11303647.
- Makino, A. 1994. Biochemistry of C₃ photosynthesis in high CO₂. *J. Plant Res.* **107**(1): 79–84. doi:10.1007/BF02344533.
- McKee, I.F., and Woodward, F.I. 1994. CO₂ enrichment responses

- of wheat: interactions with temperature, nitrate and phosphate. *New Phytol.* **127**(3): 447–453. doi:10.1111/j.1469-8137.1994.tb03962.x.
- Moorby, H., and Nye, P.H. 1984. The effect of temperature variation over the root system on root extension and phosphate uptake by rape. *Plant Soil*, **78**(3): 283–293. doi:10.1007/BF02450362.
- Norby, R.J., O'neill, E.G., and Luxmoore, R.J. 1986. Effects of atmospheric CO₂ enrichment on the growth and mineral nutrition of *Quercus alba* seedlings in nutrient-poor soil. *Plant Physiol.* **82**(1): 83–89. doi:10.1104/pp.82.1.83. PMID:16665028.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W., and Ceulemans, R. 1999. Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell Environ.* **22**(6): 683–714. doi:10.1046/j.1365-3040.1999.00391.x.
- Olszyk, D.M., Johnson, M.G., Tingey, D.T., Rygielwicz, P.T., Wise, C., VanEss, E., Benson, A., Storm, M.J., and King, R. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO₂ and temperature for 4 years. *Can. J. For. Res.* **33**(2): 269–278. doi:10.1139/x02-186.
- Örlander, G. 1984. Some aspects of water relations in planted seedlings of *Pinus sylvestris*. Ph.D. thesis, Department of Silviculture, Swedish University of Agricultural Sciences, Umeå, Sweden.
- Paré, D., Bergeron, Y., and Camiré, C. 1993. Changes in the forest floor of Canadian southern boreal forest after disturbance. *J. Veg. Sci.* **4**(6): 811–818. doi:10.2307/3235619.
- Peng, Y.Y., and Dang, Q.L. 2003. Effects of soil temperature on biomass production and allocation in seedlings of four boreal tree species. *For. Ecol. Manage.* **180**(1–3): 1–9. doi:10.1016/S0378-1127(02)00486-3.
- Perry, E., and Hickman, G.W. 2001. A survey to determine the leaf nitrogen concentrations of 25 landscape tree species. *J. Arboric.* **27**(3): 152–159.
- Peterjohn, W.T., Melillo, J.M., Steudler, P.A., Newkirk, K.M., Bowles, F.P., and Aber, J.D. 1994. Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. *Ecol. Appl.* **4**(3): 617–625. doi:10.2307/1941962.
- Pregitzer, K.S., and King, J.S. 2005. Effects of soil temperature on nutrient uptake. *In* Nutrient acquisition by plants: an ecological perspective. Edited by H. BassiriRad. Springer-Verlag, Berlin, Germany. pp. 277–310.
- Pritchard, S.G., Rogers, H.H., Prior, S.A., and Peterson, C.M. 1999. Elevated CO₂ and plant structure: a review. *Glob. Change Biol.* **5**(7): 807–837. doi:10.1046/j.1365-2486.1999.00268.x.
- Reddy, V.R., Reddy, K.R., and Wang, Z. 1997. Cotton responses to nitrogen, carbon dioxide, and temperature interactions. *Soil Sci. Plant Nutr.* **43**: 1125–1130.
- Saxe, H., Ellsworth, D.S., and Heath, J. 1998. Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytol.* **139**(3): 395–436. doi:10.1046/j.1469-8137.1998.00221.x.
- Steffen, W., and Canadell, P. 2005. Carbon dioxide fertilization and climate change policy. Available from the Department of the Environment and Heritage, Australian Greenhouse Office, GPO Box 787, Canberra ACT 2601.
- Stitt, M., and Krapp, A. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ.* **22**(6): 583–621. doi:10.1046/j.1365-3040.1999.00386.x.
- Stulen, I., and den Hertog, J. 1993. Root growth and functioning under atmospheric CO₂ enrichment. *Vegetatio*, **104**–105(1): 99–115. doi:10.1007/BF00048147.
- Tabbush, P.M. 1986. Rough handling, soil temperature, and root development in outplanted Sitka spruce and Douglas fir. *Can. J. For. Res.* **16**(6): 1385–1388. doi:10.1139/x86-247.
- Teskey, R.O., Sheriff, D.W., Hollinger, D.Y., and Thomas, R.B. 1995. External and internal factors regulating photosynthesis. *In* Resource physiology of conifers. Edited by W.K. Smith and T.M. Hinckley. Academic Press, New York, N.Y. pp. 105–140.
- Tingey, D.T., Johnson, M.G., Phillips, D.L., Johnson, D.W., and Ball, J.T. 1996. Effects of elevated CO₂ and nitrogen on the synchrony of shoot and root growth in ponderosa pine. *Tree Physiol.* **16**(11_12): 905–914. PMID:14871783.
- Wan, X., Landhäusser, S.M., Zwiazek, J.J., and Lieffers, V.J. 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. *Tree Physiol.* **19**(13): 879–884. PMID:10562405.
- Wigley, T.M.L., and Schimel, D.S. 2000. The carbon cycle. Cambridge University Press, Cambridge, UK.
- Wilson, J.B. 1988. A review of evidence on the control of shoot:root ratio, in relation to models. *Ann. Bot. (Lond.)*, **61**(4): 433–449.
- Zhang, S., and Dang, Q.L. 2005. Effects of soil temperature and elevated atmospheric CO₂ concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. *Tree Physiol.* **25**(5): 523–531. PMID:15741145.
- Zhang, S., and Dang, Q.L. 2006. Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. *Tree Physiol.* **26**(11): 1457–1467. PMID:16877330.
- Zhang, S., and Dang, Q.L. 2007. Interactive effects of soil temperature and [CO₂] on morphological and biomass traits in seedlings of four boreal tree species. *For. Sci.* **53**(3): 453–460.
- Zhang, S., Dang, Q.L., and Yü, X. 2006. Nutrient and [CO₂] elevation had synergistic effects on biomass production but not biomass allocation of white birch seedlings. *For. Ecol. Manage.* **234**(1–3): 238–244. doi:10.1016/j.foreco.2006.07.017.