

Genetic variation of *Populus tremuloides* in ecophysiological responses to CO₂ elevation

Ning Liu, Qing-Lai Dang, and William H. Parker

Abstract: To investigate the genetic variation of trembling aspen (*Populus tremuloides* Michx.) in ecophysiological responses to [CO₂] elevation, 1-year-old seedlings of four provenances (three families per provenance) from northwestern Ontario were exposed to three [CO₂] levels in the greenhouse: ambient (360 ppm), 1.5 × ambient (540 ppm), and 2 × ambient (720 ppm). Biomass and foliage gas exchange were examined after 60 d of treatment. [CO₂] elevation significantly increased the rate of net CO₂ assimilation and photosynthetic water use efficiency. The stimulation was generally greater in the 540 ppm [CO₂] than in 720 ppm [CO₂]. The 720 ppm [CO₂] resulted in a 10% photosynthetic down-regulation, but no down-regulation was detected in the 540 ppm CO₂ treatment. The 540 ppm [CO₂] (but not the 720 ppm) treatment significantly decreased stomatal conductance and transpiration rate in the provenances from the Great Lakes – St. Lawrence Region but not in those from the Boreal Region. The intercellular to ambient CO₂ concentration ratio (Ci/Ca) was significantly higher under 720 ppm [CO₂] than under the other two [CO₂]. The CO₂ elevations generally increased the total and root biomass, and the stimulation was greater in the 540 ppm [CO₂] than in the 720 ppm [CO₂] treatment. The two provenances from the Great Lakes – St. Lawrence region generally had greater biomasses than those from the boreal region, while there were no significant differences between them in the physiological variables. However, we did not find any significant differences between provenances in the responses of biomass to [CO₂] treatments.

Key words: leaf gas exchange, biomass, trembling aspen, CO₂ elevation, photosynthetic down-regulation.

Résumé : Afin d'examiner la variation génétique du peuplier faux-tremble (*Populus tremuloides* Michx.) en relation avec les réactions écophysologiques au [CO₂] élevé, les auteurs ont exposé des plantules d'un an (trois familles par provenance) provenant du nord-ouest de l'Ontario, à trois concentrations de [CO₂], en serre, soient, ambient (360 ppm), 1,5 × ambient (540 ppm), et 2 × ambient (720 ppm). Ils ont mesuré la biomasse et l'échange gazeux du feuillage, après 60 jours de traitement. L'élévation du [CO₂] augmente significativement le taux net d'assimilation du CO₂ et l'efficacité photosynthétique de l'eau. La stimulation est généralement plus importante à 540 ppm [CO₂] qu'à 720 ppm [CO₂]. Avec 720 ppm [CO₂], on observe une régulation à la baisse, mais aucune diminution ne se manifeste avec 540 ppm [CO₂]. Le traitement avec 520 ppm [CO₂] (mais pas celui avec 720 ppm) diminue significativement la conductance stomatale et le taux de transpiration chez les provenances provenant de la région St-Laurent – Grands Lacs, mais non chez celles de la région boréale. Le ratio de la concentration en CO₂ intracellulaire sur celle du milieu ambient (Ci/Ca) est significativement plus élevé avec 720 ppm [CO₂] qu'en présence des deux autres [CO₂]. Des élévations en CO₂ augmentent généralement la biomasse racinaire et totale, et cette stimulation est plus importante avec 540 ppm [CO₂] qu'avec 720 ppm [CO₂]. Les deux provenances de la région St-Laurent – Grands Lacs montrent généralement des biomasses plus importantes que celles de la région boréale, alors qu'il n'y a pas de différence significative entre elles, dans les variables physiologiques. Cependant, les auteurs n'ont pas trouvé de différences significatives entre les provenances dans les réactions de la biomasse aux traitements [CO₂].

Mots clés : échange gazeux foliaire, biomasse, peuplier faux-tremble, élévation du CO₂, régulation à la baisse de la photosynthèse.

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Introduction

The global climate is changing mainly because of increases in greenhouse gases, particularly CO₂, in the atmosphere (Fol-

land et al. 2001). The atmospheric CO₂ concentration has risen from the preindustrial level of approximately 280 ppm in the late 18th century to 355 ppm in 1991 and continues to increase at a rate of 1.8 ppm per year (Watson et al. 1992). Based on the General Circulation models, the atmospheric CO₂ concentration will reach between 540 ppm and 900 ppm by year 2100 (Folland et al. 2001).

The increase in atmospheric CO₂ concentration can potentially increase forest productivity by directly affecting the physiological activities of trees (McGuire and Joyce 1995). The most consistent effect is an increase in the rate of photosynthetic CO₂ assimilation and a reduction in photorespiration, leading to increased rates of net photosynthesis and tree growth, at least in the short term (Saxe et al. 1998;

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Table 1. ANOVA results for the effects of [CO₂] on the rate of net CO₂ assimilation measured under growth [CO₂] (NA) and a common ambient 360 ppm [CO₂] (NA₃₆₀), instantaneous photosynthetic water use efficiency (WUE), stomatal conductance to water vapor (*g_s*), intercellular to surface CO₂ concentration ratio (Ci/Ca), and rate of transpiration (*E*) after 60 d of exposures to ambient, 540, and 720 ppm CO₂.

Source	df	NA		WUE		<i>g_s</i>		Ci/Ca		<i>E</i>		NA ₃₆₀	
		MS	<i>F</i>	MS	<i>F</i>	MS	<i>F</i>	MS	<i>F</i>	MS	<i>F</i>	MS	<i>F</i>
C	2	122.35	24.13***	24.06	44.12***	30 308	6.62**	0.163	80.14***	2.00	2.12	18.75	3.64**
P	3	4.21	1.96	0.85	0.38	544	0.03	0.002	0.19	0.02	0.01	5.66	2.12
C×P	6	4.84	0.67	2.31	1.76	16 259	2.26	0.007	1.92	1.93	2.67	2.17	0.57
F(P)	8	1.50	0.36	1.00	0.90	5 357	0.91	0.004	1.16	0.61	0.91	3.36	1.17
C×F(P)	16	4.19	1.09	1.11	1.10	5 859	0.78	0.003	0.78	0.67	0.84	2.86	0.77
Error	108	1.16		0.69		4 145		0.003		0.42		0.80	

Note: C, CO₂; F(P), family (nested within provenance); P, provenance; Error, experiment error; *, statistical significance at $p \leq 0.10$; **, statistical significance at $p \leq 0.05$; ***, statistical significance at $p < 0.01$.

Aber et al. 2001). The average stimulation of photosynthesis by CO₂ enrichment is 62% for conifers and 53% for deciduous trees, with significant variations between species and between genotypes (Saxe et al. 1998).

Photosynthetic down-regulation is a form of acclimation to increasing [CO₂]. Long-term CO₂ exposure studies suggest that down-regulation of photosynthesis occurs over time (Lambers et al. 1998; Centritto and Jarvis 1999; Klus et al. 2001). However, photosynthetic down-regulation does not occur in all species (Curtis et al. 2000; Herrick and Thomas 2001). Significant changes in other physiological processes, phenology, and growth are also observed in plants grown under elevated [CO₂] (Aber et al. 2001).

Elevated [CO₂] can also be viewed as an additional selection pressure that may affect the genetic structure of the boreal forest (Colombo et al. 1998). The genetic structure of forests will change if the frequencies of current genotypes change in response to the environment change. In the context of changing environment, a better understanding of genotype and environment interactions is critical for understanding possible evolutionary responses to climate changes (Klus et al. 2001). Although the intraspecific variation of woody plants in photosynthetic responses to elevated [CO₂] has been documented (Curtis and Wang 1998; Saxe et al. 1998), such variation is still not well understood. Several studies have shown clonal differences in the response of stomatal conductance to CO₂ enrichment in poplar hybrids (Radoglou and Jarvis 1990), in biomass allocation (Lindroth et al. 2001), net assimilation (Wang et al. 2000), and wood chemistry in *Populus tremuloides* Michx. (Kaakinen et al. 2004). At the provenance level, *Picea mariana* (Miller) B.S.P. has demonstrated weak genetic variation (Johnsen and Seiler 1996), while *Pinus ponderosa* Dougl. ex P. & C. Laws. has shown evident genetic variation in net assimilation rate in response to elevated [CO₂] (Houpis et al. 1999). Furthermore, studies have determined that the basic organization of inheritable variation within a species is at population and family levels (Johnsen and Seiler 1996; Klus et al. 2001). The partitioning of variation among families and populations plays a critical role in determining the magnitude of responses to natural selection (Klus et al. 2001). For example, *Plantago lanceolata* L. has expressed strong photosynthetic variation in response to elevated CO₂ at both provenance and family levels (Klus et al. 2001).

Some species do not show significant provenance differ-

ences in physiological or morphological traits in response to elevated CO₂, for example, *Picea sitchensis* (Bong.) Carr. (Centritto and Jarvis 1999) and *Fagus sylvatica* L. (Leveranz et al. 1999). The different responses between different species are not well understood (Thompson 1998). The complexity of interspecific and intraspecific variations in the responses of woody plants to a changing environment has been a challenge to tree improvement programs. Predictions based on experiments under the current climate may not be appropriate for the future climate. Given the long rotation of trees, the risk of applying such predictions in tree improvement programs can be huge. Therefore, the genetic variation of trees in response to elevated CO₂ needs to be investigated to provide guidelines for current and future tree improvement programs.

Trembling aspen (*Populus tremuloides* Michx.) is an important tree species in the boreal forest both ecologically and economically. The purpose of this study was to investigate the genetic variation of trembling aspen in growth and ecophysiological responses to CO₂ enrichment.

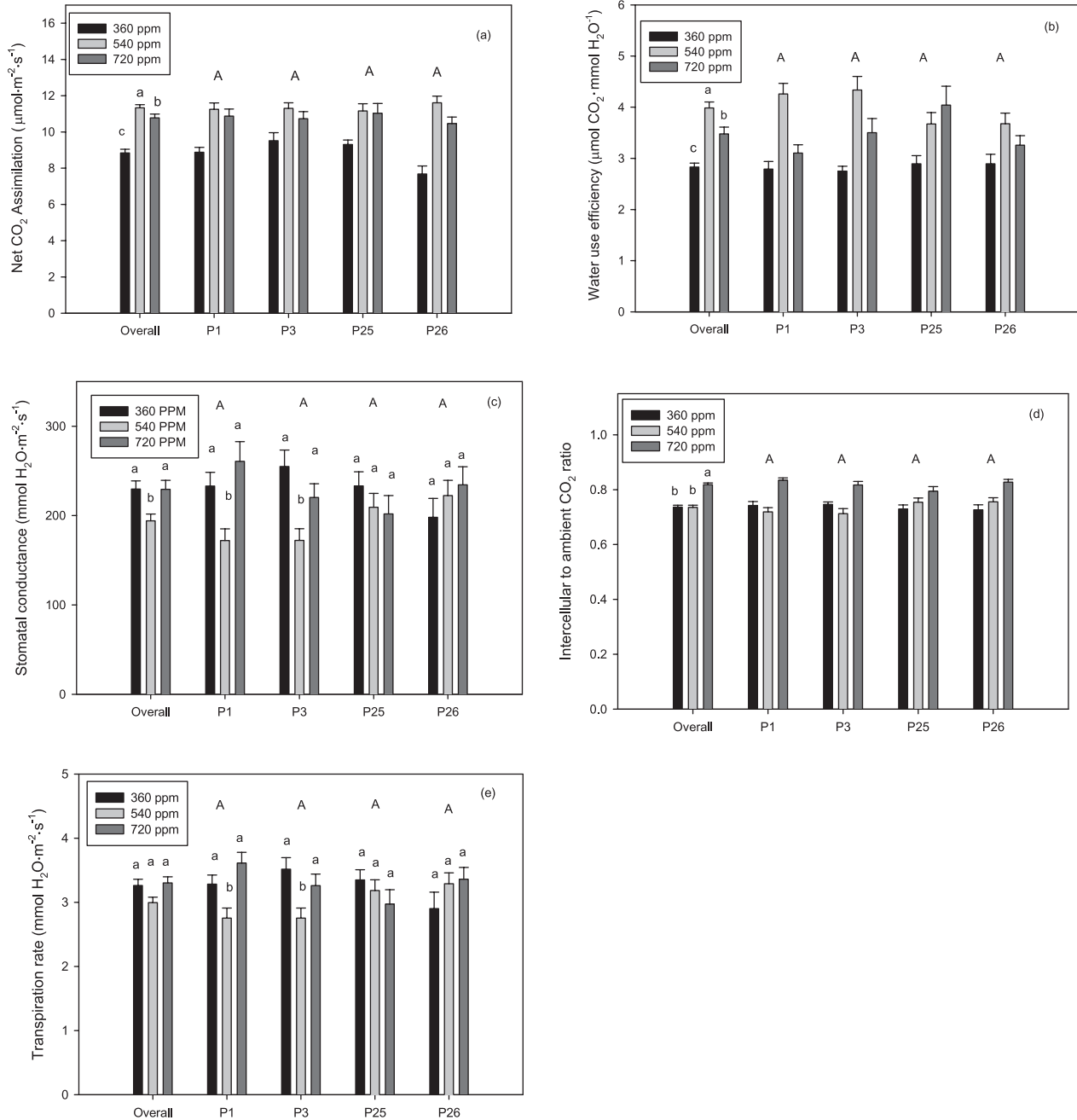
Material and methods

Plant materials

Seeds of four trembling aspen provenances were collected from northwestern Ontario: two from southwest of Thunder Bay representing the Great Lakes – St. Lawrence forest region (P1, 48°29'35"N, 90°48'57"W; P3, 48°27'45"N, 90°35'02"W) and two from the north shore of Lake Superior representing the boreal forest region (P25, 48°57'13"N, 87°58'13"W; P26, 49°03'38"N, 87°58'28"W) (Rowe 1972). Three open-pollinated families were selected for each provenance.

Climate data for the provenances were derived from a climate model for Ontario (Mackey et al. 1996). The mean daily maximum temperatures in the growing season (June–August) were 22.87, 22.57, 21.27, and 21.17 °C, respectively, for P1, P3, P25, and P26. The mean daily minimum temperatures in the growing season were 9.67, 9.07, 9.30, and 8.83 °C, respectively, for P1, P3, P25, and P26. The degree-days above 5 °C are 1390, 1310, 1248, and 1193, respectively, for P1, P3, P25, and P26, and the corresponding growing season lengths are 167, 162, 162, and 159 d. The mean monthly precipitation in the growing season was 95.47, 96.87, 85.60, and 89.03 mm, respectively, for P1, P3,

Fig. 1. Rate of net CO₂ assimilation (a), photosynthetic water use efficiency (b), stomatal conductance to water vapor (c), intercellular to surface CO₂ ratio (d), and rate of transpiration (e) (means \pm 1 SE) in four provenances of trembling aspen. One-year-old seedlings were exposed to three levels of CO₂ concentration (ambient, 540, and 720 ppm) for 60 d. Capital letters over the bars represent provenance differences, while lower-case letters represent [CO₂] effect. Different letters within the same group ([CO₂] or provenance) represent significant differences ($p < 0.05$).



P25, and P26. The soils in both areas were dry, shallow to moderately deep coarse sand.

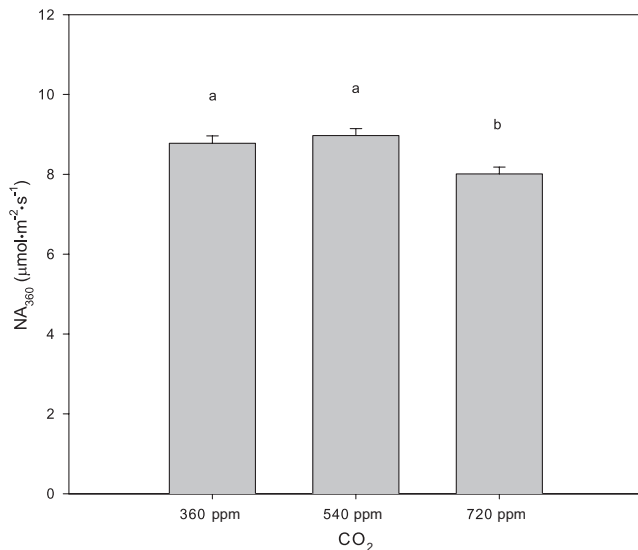
Seeds were sown in germination trays in the Lakehead University greenhouses. Irrigation and fertilization were applied as described by Hackleman et al. (2000). Twenty-five days after germination, 90 healthy seedlings per family were transplanted into styroblocks (45 cavities/block, 340 mL/cavity). After another 50 d, the seedlings were transplanted into 6-in. pots ((1 in. = 25.4 mm); total volume = 1.9 L). The growing medium was a mixture of peat and vermiculite (50:50 v/v).

The seedlings were grown for one growing season before dormancy was induced using 8 h periods and 15 °C day: 5 °C night temperatures.

Experimental design

The experiment was a nested split-plot design (Hicks 1993). The seedlings were exposed to three CO₂ concentrations in separate greenhouses: the ambient (360 ppm), 1.5 \times ambient (540 ppm), and 2 \times ambient (720 ppm). The CO₂ elevation was achieved using Argus[®] CO₂ generator (Van-

Fig. 2. Photosynthetic down-regulation in trembling aspen seedlings. The 1-year-old trees were exposed to three different CO₂ levels for 60 d. The rate of photosynthesis in this figure was measured at a common CO₂ concentration (360 ppm) for all the seedlings.



couver, British Columbia, Canada). There were three blocks nested in each CO₂ treatment. There were four provenances in each block, three families per provenance, and three seedlings per family. The locations of seedlings were randomized within each block. Other environmental conditions were set as follows for all the treatments: 24 °C day : 12 °C night temperatures, relative humidity 50%, and 16 h photoperiod (the natural light was supplemented by high-pressure sodium lamps on cloudy days, early mornings, and late evenings). The seedlings were fertilized to saturation every 3 d using the formulation of Hackleman et al. (2000). They were irrigated up to twice a day on hot days to keep the growing medium moist. All the environmental conditions, including CO₂ concentration, were monitored and controlled using an Argus[®] environment control system (Vancouver, Canada).

Measurements

The foliar gas exchanges were measured after 60 d of treatment. The measurements were taken on the 6th leaf from the top (determined according to Thomas et al. 1997) using a PP-System CIRAS-1 open gas exchange system and a Parkinson broad-leaf chamber with automatic environmental control (PP-Systems, Haverhill, Massachusetts, USA). The measurements were taken at 22 ± 0.1 °C air temperature, 800 μmol·m⁻²·s⁻¹ photosynthetically active radiation, and at both the corresponding growth CO₂ and ambient (360 ppm) concentrations. The measurement sequence was randomized within and among blocks to avoid systematic errors (Lee and Rawlings 1982; Dang et al. 1994). In addition, the measurements were taken from 09:00 h to 16:30 h to eliminate diurnal errors based on our preliminary tests and Wang et al. (2000). Net CO₂ assimilation (NA), stomatal conductance (g_s), leaf transpiration rate (E), and the internal to leaf surface CO₂ concentration ratio (Ci/Ca) were calculated according to von Caemmerer and Farquhar (1981).

The instantaneous photosynthetic water use efficiency (WUE) was calculated as NA/ E .

Because of the large size of the trees and growing space restrictions in the greenhouse, the experiment was terminated following the measurements. All the trees were harvested and dried at 80 °C for 48 h to determine the dry mass of foliage, stem, and roots.

Data analysis

The data were analyzed using analysis of variance (ANOVA) using the SAS/STAT[®] 8.2 statistical software (SAS Institute Inc. 1989). The Student–Newman–Keuls range tests were used for post-hoc multiple comparisons when the ANOVA showed a significant provenance effect. The stomatal conductance and transpiration of provenance 1 (P1) and P3 showed obvious different response patterns from those of P25 and P26 (Figs. 1c, 1e). Therefore, the responses of those two variables were analyzed separately for each provenance, despite that ANOVA failed to detect significant interactions between provenance and CO₂ treatments at $p \leq 0.05$ (Table 1).

Results

Net CO₂ assimilation (NA)

Overall, the NA of trembling aspen seedlings showed a positive response to the CO₂ elevation, and the stimulation was generally greater in the 540 ppm treatment than in the 720 ppm treatment (Table 1; Fig. 1a). However, this trend was not statistically significant when each provenance was analyzed separately. There were also no significant differences between provenances or between families in the response (Table 1).

The measures taken under a common 360 ppm [CO₂] showed significant photosynthetic down-regulation in response to the 720 ppm CO₂ treatment but not to the 540 ppm [CO₂] treatment (Table 1; Fig. 2). The down-regulation was about 10%.

Photosynthetic water use efficiency (WUE)

Both CO₂ elevations increased the instantaneous WUE in trembling aspen, but the increase was greater in the 540 ppm [CO₂] than in the 720 ppm [CO₂] treatment (Table 1; Fig. 1b). This trend was in contrast with that of photosynthetic down-regulation as described above.

Stomatal conductance to water vapor (g_s)

In a general agreement with the trend of NA response but opposite to that of WUE response, the stomatal conductance was reduced by the 540 ppm [CO₂] but not by the 720 ppm [CO₂] elevation (Table 1; Fig. 1c). When each provenance was analyzed individually, however, the reduction in g_s was significant only in P1 and P3, but not in P25 and P26 (Fig. 1c).

Intercellular to ambient CO₂ concentration ratio (Ci/Ca)

The 720 ppm [CO₂] treatment significantly increased Ci/Ca ratio, while there was not a significant difference between the other two treatments (Table 1; Fig. 1d).

Table 2. ANOVA results for the effects of [CO₂] on total, shoot, stem, leaf, and root biomass of four provenances of trembling aspen seedlings that were exposed to three levels of CO₂ concentrations (ambient, 540, and 720 ppm) for 60 d.

Source	df	Total		Shoot		Stem		Leaf		Root	
		MS	F	MS	F	MS	F	MS	F	MS	F
C	2	2957	5.59**	1090	3.57*	333	3.59*	233	3.06	466	8.93***
P	3	4769	9.50***	2488	9.52***	739	12.82***	526	5.98**	381	6.91***
C×P	6	307	3.89	194	3.39	59	2.56	43	2.16	29	2.79
F(P)	8	323	2.53**	152	1.79	28	0.95	72	2.67**	39	3.08**
C×F(P)	16	127	0.61	85	0.67	29	0.84	27	0.70	13	0.72
Error	108	183		118		32		34		14	

Note: C, CO₂; F(P), family (nested within provenance); P, provenance; Error, experiment error; *, statistical significance at $p \leq 0.10$; **, statistical significance at $p \leq 0.05$; ***, statistical significance at $p < 0.01$.

Transpiration (*E*)

Overall, the CO₂ treatments did not significantly affect transpiration rate (Table 1). However, P1 and P3 showed distinctly different response patterns than P25 and P26 (Fig. 1e). Further analysis on individual provenances showed that the 540 ppm [CO₂] treatment significantly reduced *E* in P1 and P3, but not in P25 and P26 (Fig. 1e).

Biomass

Overall, the total biomass per tree and root biomass increased in response to the CO₂ elevations, and the response was stronger to the 540 ppm [CO₂] than to the 720 ppm [CO₂] treatment (Table 2; Fig. 3).

The provenances from different forest regions showed significantly different growth rates: the total biomass per tree and the biomass of all individual organs were significantly greater in P1 and P3 from the Great Lakes – St. Lawrence forest region (southwest of Thunder Bay) than in P25 and P26 from the boreal region (north shore of Lake Superior) (Table 2; Fig. 3). There were also significant family effects on total, leaf, and root biomass, but not on the total or stem biomass (Table 2).

Discussion

Overall, trembling aspen increased photosynthesis (NA) and photosynthetic water use efficiency (WUE) in response to CO₂ elevations. NA increased 28% and 22% in the 540 and 720 ppm [CO₂] treatments, respectively. These results are consistent with the range of responses reported for short-term CO₂ elevations (Curtis 1996; Curtis and Wang 1998; Lambers et al. 1998; Saxe et al. 1998; Tjoelker et al. 1998; Wang et al. 2000; Centritto et al. 2002; Lewis et al. 2002). Such increases were moderate compared with long-term field CO₂ enrichment results in the same species (Curtis and Wang 1998; Kubiske et al. 1997, 1998). The results support the finding that NA response in greenhouses is generally below growth-chamber but above open-top-chamber grown plants (Curtis 1996).

It is interesting to note that photosynthetic down-regulation occurred in the 720 ppm but not in the 540 ppm [CO₂] treatment, suggesting that photosynthetic down-regulation may be a threshold response. Carbohydrate production generally increases with increasing [CO₂], and growth will increase accordingly (Lambers et al. 1998). However, if [CO₂] is so high that the increase in carbohydrate production exceeds the phloem capacity or sink demand (including increased

growth), triose-phosphate will accumulate in chloroplasts, causing feed-back inhibition of photosynthesis and subsequent photosynthetic down-regulation (Lambers et al. 1998).

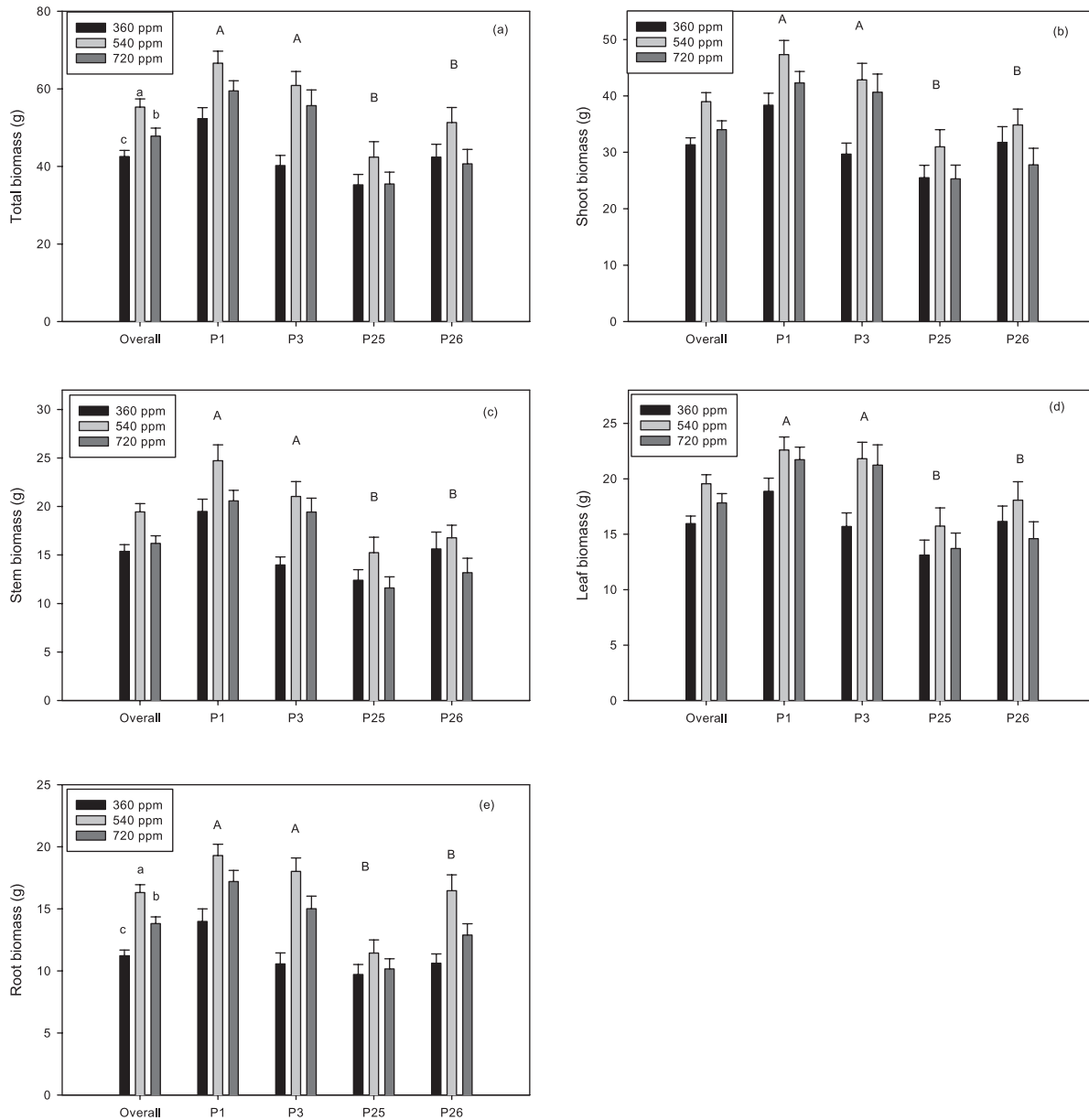
Some of the inconsistencies in the literature on photosynthetic down-regulation can be explained by the threshold response theory. Photosynthetic down-regulation is more likely to occur in greenhouse and growth chamber than in field FACE experiments (Saxe et al. 1998). However, [CO₂] in greenhouses and growth chambers is generally much higher than FACE experiments (ambient + 350 ppm vs. 550 ppm). The [CO₂] in FACE may be below the threshold to trigger photosynthetic down-regulation. The fact that photosynthetic down-regulation occurs in some species and (or) genotypes (Atkinson et al. 1997; Rey and Jarvis 1998; Roberntz and Stockfors 1998; Tissue et al. 1999; Urban and Marek 1999; Laitinen et al. 2000; Kubiske et al. 2002) but not in others (Liu and Teskey 1995; Ceulemans et al. 1997; Maier et al. 2002; Nowak et al. 2004; Wittig et al. 2005) suggests that the threshold may vary with species and genotype.

Pot limitation is often considered as the cause of photosynthetic down-regulation in closed-environment studies (Idso and Kimball 1989; Arp 1991; Long and Drake 1991; Teskey et al. 1995), but it was not a contributing factor in this study. If it was, the photosynthetic down-regulation would have been greater at 540 ppm [CO₂] than at 720 ppm [CO₂], because the trees at 540 ppm [CO₂] were larger and presumably would have been more limited by the pot than at 720 ppm [CO₂]. However, photosynthetic down-regulation occurred at 720 ppm but not at 540 ppm CO₂. Zhang and Dang (2005) also concluded that pot limitation was not responsible for the photosynthetic down-regulation in white birch and jack pine.

Photosynthetic down-regulation can also be influenced by other environmental factors. Murray et al. (2000) and Singaas et al. (2004) reported that photosynthetic down-regulation at elevated CO₂ occurs only when N supply is low. Huxman et al. (1998) found that photosynthetic down-regulation occurs only when the plants are well watered. However, moisture and nutrient should not have been causes for photosynthetic down-regulation in this study, because all the seedlings were well watered and well fertilized, and significant differences in water and nutrient regimes between CO₂ treatments were unlikely.

The CO₂ enrichments generally increased the root and total biomass of trembling aspen seedlings. The increase in total biomass was 30% and 12%, respectively, in the 540 ppm

Fig. 3. Total (a), shoot (b), stem (c), leaf (d), and root biomass (e) (means \pm 1 SE) in four provenances of trembling aspen. The one-year-old seedlings were exposed to three levels of CO₂ concentrations (360, 540, and 720 ppm) for 60 d. See Fig. 1 for other explanations.



and 720 ppm CO₂ treatment. The relative increase at 720 ppm CO₂ was comparable to the average value for shade-intolerant species (18%, Kerstiens 2001). The smaller enhancement at 720 ppm than at 540 ppm [CO₂] may be related to the photosynthetic down-regulation that resulted in a lower carbohydrate production at 720 ppm than at 540 ppm [CO₂].

Our growth and physiological data show that there was generally no significant genetic variation in the response of trembling aspen to [CO₂] at either provenance or family level with the exception of *E* and *g_s* which showed significant reductions in response to the 540 ppm CO₂ treatment in P1 and P3 but not in P25 or P26. These results suggest that the increases in atmospheric [CO₂] alone may not modify the genetic structure and distribution of trembling aspen in northwestern Ontario. However, the differences in *E* and

g_s response to [CO₂] suggest that increases in atmospheric [CO₂] in combination with changes in precipitation may give some provenances advantages over others. There is a great degree of inconsistency in the literature on the genetic variation of trembling aspen in response to [CO₂]. Some studies have found significant differences between genotypes (Ceulemans et al. 1995; Curtis 1996; Wang et al. 2000; Lindroth et al. 2001) in response to elevated [CO₂], while others have found no significant differences (Kubiske et al. 1998; Curtis et al. 2000). Kalina and Ceulemans (1997) reported different responses between clones of poplar. The reason(s) for the difference is unknown. However, the plant materials in different studies are from different regions and cover areas of different sizes. Therefore, the detectable degree of genetic difference or the lack of it may be related to the range of natural distribution that the mate-

rials cover or the region where the plant materials are obtained. Thus, results should not be extrapolated beyond the geographic region of the experimental materials without testing. Additionally, while almost all other studies use root cuttings, we used seedlings in this study. The greater genetic diversity in seeds than in cuttings may have an impact on the detectable differences in the response. Furthermore, we would like to point out that, because of the limitation of infrastructure, our experiment was pseudoreplicated. Although the continuous monitoring (data not shown) shows that there were no systematic differences in environmental conditions among the three greenhouses, readers may want to exercise prudence in using our results.

Our physiological results appear to lead to more contradictory conclusions than the biomass data. The lack of significant difference in the physiological variables tends to suggest that there would be no significant difference in growth between different provenances. However, the biomass of P1 and P3 was significantly greater than that of P25 and P26. The contradictory results may be related to differences in the amount of leaves per tree. P1 and P3 had significantly greater amounts of foliage than P25 and P26. At a given rate of photosynthesis, more leaves will result in greater carbohydrate production and thus faster biomass accumulation and growth. The results suggest that the rate of physiological processes should be measured in conjunction with total leaf area/biomass. Additionally, all our gas exchange measurements were taken on the 6th leaf on the terminal shoot as in Thomas et al. (1997) (6th–10th). Although those leaves were all fully expanded and experienced similar light conditions, it is not clear whether they are representative of all the leaves on the tree for all the provenances. Therefore, we recommend that the variation in photosynthesis among leaves at different canopy positions of the same tree be examined for each provenance before sample leaves are selected.

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References

- Aber, J., Neilson, R.P., McNulty, S., Lenihan, J.M., Bachelet, D., and Drapek, R.J. 2001. Forest processes and global environmental change: predicting the effects of individual and multiple stressors. *Bioscience*, **51**: 735–751.
- Arp, W.J. 1991. Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant Cell Environ.* **14**: 869–875.
- Atkinson, C.J., Taylor, J.M., Wilkins, D., and Besford, R.T. 1997. Effects of elevated CO₂ on chloroplast components, gas exchange and growth of oak and cherry. *Tree Physiol.* **17**: 319–325.
- Centritto, M., and Jarvis, P.G. 1999. Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (*Picea sitchensis*). II. Photosynthetic capacity and nitrogen use efficiency. *Tree Physiol.* **19**: 807–814.
- Centritto, M., Lucas, M.E., and Jarvis, P.G. 2002. Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach (*Prunus persica*) seedlings in response to elevated carbon dioxide concentration and water availability. *Tree Physiol.* **22**: 699–706.
- Ceulemans, R., Jiang, X.N., and Shao, B.Y. 1995. Growth and physiology of one year old poplar (*Populus*) under elevated atmospheric CO₂ levels. *Ann. Bot. (Lond.)*, **75**: 609–617.
- Ceulemans, R., Taylor, G., Bosac, C., Wilkins, D., and Besford, R.T. 1997. Photosynthetic acclimation to elevated CO₂ in poplar grown in glasshouse cabinets or in open top chambers depends on duration of exposure. *J. Exp. Bot.* **48**: 1681–1689. doi:10.1093/jexbot/48.314.1681.
- Colombo, S.J., Buse, L.J., Cherry, M.L., Graham, C., Greifenhagen, S., McAlpine, R.S., Papadopol, C.S., Parker, W.C., Scarr, T., Mikaelian, M.T., and Flannigan, M.D. 1998. The impacts of climate change on Ontario's forests. Forest research information paper No. 143. Ontario Forest Research Institute, Sault St. Marie, Ont.
- Curtis, P.S. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environ.* **19**: 127–137.
- Curtis, P.S., and Wang, X.Z. 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*, **113**: 299–313. doi:10.1007/s004420050381.
- Curtis, P.S., Vogel, C.S., Wang, X.Z., Pregitzer, K.S., Zak, D.R., and Lussenhop, J. 2000. Gas exchange, leaf nitrogen, and growth efficiency of *Populus tremuloides* in a CO₂-enriched atmosphere. *Ecol. Appl.* **10**: 3–17.
- Dang, Q.L., Xie, C.Y., Ying, C., and Guy, R.D. 1994. Genetic variation of ecophysiological traits in red alder (*Alnus rubra* Bong.). *Can. J. For. Res.* **24**: 2150–2156.
- Folland, C.K., Karl, T.R., Christy, J.R., Clarke, R.A., Gruza, G.V., Jouzel, J., Mann, M.E., Oerlemans, J., Salinger, M.J., and Wang, S.-W. 2001. Observed climate variability and change. In *Climate change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Edited by J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell, and C.A. Johnson.* Cambridge University Press, Cambridge, U.K. pp. 22–83.
- Hackleman, A., Tull, A., and Moench, R. 2000. Trembling aspen propagation. Colorado State Service, Fort Collins, Colo. http://www.na.fs.fed.us/spfo/rngr/npr/poptre_co.htm (cited May 2001).
- Herrick, J.D., and Thomas, R.B. 2001. No photosynthetic down-regulation in sweetgum (*Liquidambar styraciflua* L.) after three years of CO₂ enrichment at the Duke Forest FACE experiment. *Plant Cell Environ.* **24**: 53–64. doi:10.1046/j.1365-3040.2001.00652.x.
- Hicks, C.R. 1993. *Fundamental concepts in the design of experiments.* 4th ed. Oxford University Press, Inc., New York.
- Houpis, J.L.J., Anderson, P.D., Pushnik, J.C., Ansel, D.J., Shepard, L.J., and Cape, J.N. 1999. Among-provenance variability of gas exchange and growth in response to long-term elevated CO₂ exposure. Special issue: Forest growth responses to the pollution climate of the 21st century. Contributions from the 18th IUFRO workshop on air pollution stress, Edinburgh, UK. *Water Air Soil*, **116**: 403–412.
- Huxman, T.E., Hamerlynck, E.P., Moore, B.D., Smith, S.D., Jordan, D.N., Zitzer, S.F., Nowak, R.S., Coleman, J.S., and Seemann, J.R. 1998. Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO₂: interaction with drought under glasshouse and field (FACE) exposure. *Plant Cell Environ.* **21**: 1153–1161. doi:10.1046/j.1365-3040.1998.00379.x.
- Idso, S.B., and Kimball, B.A. 1989. Growth response of carrot and radish to atmospheric CO₂ enrichment. *Environ. Exp. Bot.* **29**: 135–139. doi:10.1016/0098-8472(89)90045-2.
- Johnsen, K.H., and Seiler, J.R. 1996. Growth, shoot phenology and

- physiology of diverse seed source of black spruce: I. Seedling responses to varied atmospheric CO₂ concentrations and the photoperiods. *Tree Physiol.* **16**: 367–373.
- Kaakinen, S., Kostianen, K., Ek, F., Saranpää, P., Kubiske, M.E., Sober, J., Karnosky, D.F., and Vapaavuori, E. 2004. Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone. *Glob. Change Biol.* **10**: 1513–1525. doi:10.1111/j.1365-2486.2004.00814.x.
- Kalina, J., and Ceulemans, E. 1997. Clonal differences in the response of dark and light reactions of photosynthesis to elevated atmospheric CO₂ in poplar. *Photosynthetica*, **33**: 51–61. doi:10.1023/A:1022123204974.
- Kerstiens, G. 2001. Meta-analysis of the interaction between shade-tolerance, light environment and growth response of woody species to elevated CO₂. *Acta Oecol.* **22**: 61–69. doi:10.1016/S1146-609X(00)01096-1.
- Klus, D.J., Kalisz, S., Curtis, P.S., Teeri, J.A., and Tonsor, S.J. 2001. Family and population level responses to atmospheric CO₂ concentration: gas exchange and the allocation of C, N, and biomass in *Plantago lanceolata* (Plantaginaceae). *Am. J. Bot.* **88**: 1080–1087.
- Kubiske, M.E., Pregitzer, K.S., Mikan, C.J., Zak, D.R., Maziasz, J.L., and Teeri, J.A. 1997. *Populus tremuloides* photosynthesis and crown architecture in response to elevated CO₂ and soil N availability. *Oecologia*, **110**: 328–336. doi:10.1007/s004420050166.
- Kubiske, M.E., Pregitzer, K.S., Zak, D.R., and Mikan, C.J. 1998. Growth and C allocation in response to atmospheric CO₂ and soil N availability. *New Phytol.* **140**: 251–260. doi:10.1046/j.1469-8137.1998.00264.x.
- Kubiske, M.E., Zak, D.R., Pregitzer, K.S., and Takeuchi, Y. 2002. Photosynthetic acclimation of overstory *Populus tremuloides* and understory *Acer saccharum* to elevated atmospheric CO₂ concentration: interactions with shade and soil nitrogen. *Tree Physiol.* **22**: 321–329.
- Laitinen, K., Luomala, E.M., Kellomäki, S., and Vapaavuori, E. 2000. Carbon assimilation and nitrogen in needles of fertilized and unfertilized field-grown Scots pine at natural and elevated concentrations of CO₂. *Tree Physiol.* **20**: 881–892.
- Lambers, H., Chapin, F.S., III, and Pons, T.L. 1998. *Plant physiological ecology*. Springer-Verlag, Inc., New York.
- Lee, C.S., and Rawlings, J.O. 1982. Design of experiments in growth chambers - uniformity trials in the North Carolina State University phytotron. *Crop Sci.* **22**: 551–558.
- Leveranz, J.W., Bruhn, D., and Saxe, S. 1999. Responses of two provenances of *Fagus sylvatica* seedlings to a combination of four temperature and two CO₂ treatments during their first growing season: gas exchange of leaves and roots. *New Phytol.* **144**: 437–454.
- Lewis, J.D., Lucash, M., Olszyk, D.M., and Tingey, D.T. 2002. Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure. *Plant Cell Environ.* **25**: 1411–1421. doi:10.1046/j.1365-3040.2002.00923.x.
- Lindroth, R.L., Roth, S., and Nordheim, E.V. 2001. Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO₂ enrichment. *Oecologia*, **126**: 371–379. doi:10.1007/s004420000521.
- Liu, S., and Teskey, R.O. 1995. Responses of foliar gas exchange to long-term elevated CO₂ concentrations in mature loblolly pine trees. *Tree Physiol.* **15**: 351–359.
- Long, S.P., and Drake, B.G. 1991. Effects of the long-term elevation of CO₂ concentration in the field on the quantum yield of photosynthesis of the C₃ sedge *Scirpus olneyi*. *Plant Physiol.* **96**: 221–226.
- Mackey, B.G., McKenny, D.W., Yang, Y.Q., McMahon, J.P., and Hutchinson, M.F. 1996. Site regions revisited: a climatic analysis of Hill's site regions for the province of Ontario using a parametric method. *Can. J. For. Res.* **26**: 333–354.
- Maier, C.A., Johnsen, K.H., Butnor, J., Kress, L.W., and Anderson, P.H. 2002. Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization. *Tree Physiol.* **22**: 1093–1106.
- McGuire, A.D., and Joyce, L.A. 1995. Responses of net primary production to changes in CO₂ and climate. In *Productivity of America's forests and climate change*. Edited by L.A. Joyce. USDA For. Serv. Gen. Tech. Rep. RM-271. pp. 9–45.
- Murray, M.B., Smith, R.J., Friend, A., and Jarvis, P.G. 2000. Effect of elevated [CO₂] and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). *Tree Physiol.* **20**: 421–434.
- Nowak, R.S., Ellsworth, D.S., and Smith, S.D. 2004. Functional responses of plants to elevated atmospheric CO₂ – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* **162**: 253–280. doi:10.1111/j.1469-8137.2004.01033.x.
- Radoglou, K.M., and Jarvis, P.G. 1990. Effects of CO₂ enrichment on four poplar clones. II. Leaf surface properties. *Ann. Bot. (Lond.)*, **65**: 627–632.
- Rey, A., and Jarvis, P.G. 1998. Long-term photosynthetic acclimation to increased atmospheric CO₂ concentration in young birch (*Betula pendula*) trees. *Tree Physiol.* **18**: 441–450.
- Roberntz, P., and Stockfors, J. 1998. Effects of elevated CO₂ concentration and nutrition on net photosynthesis, stomatal conductance and needle respiration of field-grown Norway spruce trees. *Tree Physiol.* **18**: 233–241.
- Rowe, J.S. 1972. *Forest regions of Canada*. Canadian Forest Service, Department of the Environment, Ottawa, Ont.
- SAS Institute Inc 1989. *SAS/STAT® user's guide*, version 6. Vol. 2. 4th ed. SAS Institute Inc., Cary, N.C.
- Saxe, H., Ellsworth, D.S., and Heath, J. 1998. Tree and forest functioning in an enriched CO₂ atmosphere, Tansley Review No. 98. *New Phytol.* **139**: 395–436. doi:10.1046/j.1469-8137.1998.00221.x.
- Singsaas, E.L., Ort, D.R., and Delucia, E.H. 2004. Elevated CO₂ effects on mesophyll conductance and its consequences for interpreting photosynthetic physiology. *Plant Cell Environ.* **24**: 41–50.
- 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. Ninckley. Academic Press, New York. pp. 105 – 140.
- Thomas, B.R., Macdonald, S.E., and Dancik, B.P. 1997. Variance components, heritabilities, and gain estimates for growth chamber and field performance of *Populus tremuloides*: gas exchange parameters. *Silvae Genet.* **46**: 309–317.
- Thompson, D.G. 1998. Getting the species and provenance right for climate change. *Ir. For.* **55**: 114–121.
- Tissue, D.T., Griffin, K.L., and Ball, J.T. 1999. Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO₂. *Tree Physiol.* **19**: 222–228.
- Tjoelker, M.G., Oleksyn, J., and Reich, P.B. 1998. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO₂ and temperature. *Tree Physiol.* **18**: 715–726.
- Urban, O., and Marek, M.V. 1999. Seasonal changes of selected

- parameters of CO₂ fixation biochemistry of Norway spruce under the long-term impact of elevated CO₂. *Photosynthetica*, **36**: 533–545. doi:10.1023/A:1007040020512.
- von Caemmerer, S., and Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**: 376–387.
- Wang, X.Z., Curtis, P.S., Pregitzer, K.S., and Zak, D.R. 2000. Genotypic variation in physiological and growth responses of *Populus tremuloides* to elevated atmospheric CO₂ concentration. *Tree Physiol.* **20**: 1019–1028.
- Watson, R.L., Filho, G.M., and Sanhueza, E. 1992. Greenhouse gases: sources and sinks. In *Climate change 1992: the supplementary report to the IPCC Scientific Assessment*. Edited by J.T. Houghton. Cambridge University Press, Cambridge, UK. pp. 25–46.
- Wittig, V.E., Bernacchi, C.J., Zhu, X.G., Calfapietra, C., Ceulemans, R., Deangelis, P., Gielen, B., Miglietta, F., Morgan, P.B., and Long, S.P. 2005. Gross primary production is stimulated for three *Populus* species grown under free-air CO₂ enrichment from planting through canopy closure. *Glob. Change Biol.* **11**: 644–656. doi:10.1111/j.1365-2486.2005.00934.x.
- Zhang, S.R., and Dang, Q.L. 2005. Effects of soil temperature and elevated CO₂ concentration on gas exchange, in vivo carboxylation and chlorophyll fluorescence in jack pine and white birch seedlings. *Tree Physiol.* **25**: 609–617.