Responses of deciduous broadleaf trees to defoliation in a CO₂ enriched atmosphere

JOHN C. VOLIN,1,2 ERIC L. KRUGER3 and RICHARD L. LINDROTH4

1 Division of Biological Sciences, Florida Atlantic University, 2912 College Avenue, Davie, FL 33314, USA
2 Author to whom correspondence should be addressed (jvolin@fau.edu)
3 Department of Forest Ecology and Management, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA
4 Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA

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Summary  Relatively little is known about the implications of atmospheric CO₂ enrichment for tree responses to biotic disturbances such as folivory. We examined the combined effects of elevated CO₂ concentration ([CO₂]) and defoliation on growth and physiology of sugar maple (Acer saccharum Marsh.) and trembling aspen (Populus tremuloides Michx.). Seedlings were planted in the ground in eight open-top chambers. Four chambers were ventilated with CO₂-enriched air (ambient + 283 µmol mol⁻¹) and four chambers were supplied with ambient air. After 6 weeks of growth, half of the leaf area was removed on a subset of seedlings of each species in each CO₂ treatment. We monitored subsequent biomass gain and allocation, along with leaf gas exchange and chemistry. Defoliation did not significantly affect final seedling biomass in either species or CO₂ treatment. Growth recovery following defoliation was associated with increased allocation to leaf mass in maple and a slight enhancement of mean photosynthesis in aspen. Elevated [CO₂] did not significantly affect aspen growth, and the observed stimulation of maple growth was significant only in mid-season. Correspondingly, simulated responses of whole-tree photosynthesis to elevated [CO₂] were constrained by a decrease in photosynthetic capacity in maple, and were partially offset by reductions in specific leaf area and biomass allocation to foliage in aspen. There was a significant interaction between [CO₂] and defoliation on only a few of the measured traits. Thus, the data do not support the hypothesis that atmospheric CO₂ enrichment will substantially alter tree responses to folivory. However, our findings do provide further indication that regeneration-stage growth rates of certain temperate tree species may respond only moderately to a near doubling of atmospheric [CO₂].

Keywords: Acer saccharum, biomass allocation, elevated carbon dioxide, nitrogen, photosynthesis, Populus tremuloides, specific leaf area, stomatal conductance.

Introduction

Trees face numerous biotic stresses acting at a variety of spatial and temporal scales. These stresses may influence how forests acclimate to long-term changes in climate and atmospheric chemistry. Many studies have compared tree responses to elevated CO₂ concentration ([CO₂]) in the presence and absence of abiotic stresses such as drought, nutrient imbalance, temperature extremes and gaseous oxidants (Saxe et al. 1998). Much less is known about the effects of increased [CO₂] on the behavior and overall resiliency of trees following biotic insults such as severe folivory (Trumble et al. 1993).

In several herbaceous species, defoliation was found to reverse the decline in photosynthetic capacity often observed in plants subjected to long-term exposure to elevated [CO₂] (Peet 1984, Rogers et al. 1995, Stirling and Davey 1995, Bryant et al. 1998). This reversal is thought to result from a defoliation-induced shift in the supply of assimilate relative to its demand (source/sink ratio) that partially or fully relieves an opposing imbalance brought about by increased CO₂ availability (Stitt 1991).

Kruger et al. (1998) recently observed an interaction between elevated [CO₂] and defoliation in sugar maple (Acer saccharum Marsh.) and trembling aspen (Populus tremuloides Michx.) saplings grown in pots. However, the species differed in their response to defoliation in elevated [CO₂] presumably because of their contrasting ecologies (Bazzaz and Miao 1993, Norby et al. 1996, Hättenschwiler and Körner 2000). In sugar maple, which is late successional, relatively slow-growing and shade-tolerant—but not in trembling aspen, which is early successional, fast-growing and shade-intolerant—defoliated seedlings grew more rapidly than non-defoliated seedlings in elevated [CO₂], and defoliated seedlings exhibited a significant increase in both photosynthesis and mass allocation to foliage. These data indicate that atmospheric CO₂ enrichment could potentially enhance the ability of certain tree species to recover from folivory, and that responses to elevated [CO₂] might be largest in the presence of stresses that decrease assimilate source/sink ratios.

The present paper summarizes our exploration of the effects of elevated [CO₂] on the responses of maple and aspen to defoliation. Our principal objective was to resolve the interplay...
among photosynthetic, morphological and allocational changes contributing to growth variation among treatments. Our central hypothesis was that net biomass gain would be relatively less affected by defoliation in CO2-enriched air than in ambient air, because post-defoliation regrowth would be accelerated by enhanced light-saturated photosynthesis. As a corollary, we postulated that this differential response to defoliation would be most pronounced for species that otherwise exhibit a decline in photosynthetic capacity in CO2-enriched air. Because the behavior of potted saplings in an artificially lit growth room might differ considerably from that of a forest-grown tree, we planted mixed stands of maple and aspen seedlings in the ground in open-top chambers (OTCs) ventilated with either ambient or CO2-enriched (ambient + 283 µmol mol–1) air. We monitored biomass gain and allocation, and leaf gas exchange of defoliated and non-defoliated seedlings throughout one growing season. To determine possible associations among treatment effects on photosynthetic traits and assimilate source/sink ratios, we also monitored changes in foliar starch concentration and [hexose]/[sucrose] ratio, which are thought to signal feedback or end-product inhibition of photosynthesis, and hence source–sink imbalance (Van Oosten and Besford 1996, Moore et al. 1999).

Materials and methods

Plant material and treatments

Sugar maple germinants were collected during summer 1993 from large gaps in a mesic hardwood forest in southwestern Wisconsin, USA, and transplanted to 10 experimental plots established in a former alfalfa field at the University of Wisconsin Agricultural Research Station in Madison, WI. The plots were located on a silt loam soil (typic arguidoll, Hole 1976) with N and organic matter concentrations of 1.8 mg g–1 and 4.7%, respectively (based on a composite sample of the upper 20 cm of soil at the outset of the study). Eight plots were prepared to accommodate the OTCs (4.66 m diameter, no rain cap, as described by Heagle et al. 1989) that were constructed in March 1994. Two additional plots (4×4 m) remained chamberless.

In April 1994, half-sib seeds of trembling aspen, obtained from the North Central Forest Experiment Station (Grand Rapids, MN), were germinated in flats in a greenhouse. Aspen germinants were transplanted to the OTCs and chamberless plots in late May. Equal numbers of both species (125 trees per species per OTC) were planted directly in the soil, in a uniform interspersion, at an overall density of 33 trees m–2. Seedlings were watered daily throughout the growing season (May through October).

Operation of the OTCs and the experimental design are detailed in Lindroth et al. (1997). Ambient air was pumped through the OTCs at a rate of 3 m3 s–1, providing about three complete air exchanges per minute. Treatments were arranged in a randomized complete-block design, and atmospheric [CO2] was continuously elevated by an average of 283 µmol mol–1 (SE = 12 µmol mol–1, based on n = 336 instantaneous samples) throughout the growing season. Daytime [CO2] in the elevated and ambient OTCs averaged 640 µmol mol–1 and 357 µmol mol–1, respectively. Carbon dioxide was supplied from a pressurized 12-Mg receiver (Praxair, Danbury, CT).

Throughout the study, air temperature was continuously monitored in one OTC per CO2 treatment and in one chamberless plot with shielded thermocouples attached to a data logger (Model LI-1000, Li-Cor, Lincoln, NE). Thermocouples were located 1 m above ground in the center of the OTC or chamberless plot. On average, air temperature was 2.1 °C higher in the OTCs than in the chamberless plot during the growing season, with maximum differentials typically occurring at midday (Figure 1). During July and early August, seedling light environments were also monitored continuously with gallium arsenide photodiodes (Hamamatsu Photonics, Middlesex, NJ) connected to data loggers. The photodiodes, calibrated against a Li-Cor LI-160 quantum sensor, were placed atop aspen or maple crowns at various locations within each OTC as well as in one chamberless plot. Data from the most exposed sensors indicated that the mean photosynthetic

![Figure 1. Diurnal patterns of mean photosynthetic photon flux density (PPFD) and air temperature in OTCs and chamberless plots during the June–August growth interval. We monitored PPFD near or at the top of the aspen canopies, whereas temperature was monitored at a height of 1 m in the center of the OTC or chamberless plot. The PPFD data from the OTCs are means (and 1 SE) of n = 4 OTC means. The PPFD was monitored in only one chamberless plot. Temperature data are from a thermocouple in one OTC and one chamberless plot. During the growth interval, skies tended to be clear in the mornings and overcast or partly cloudy in the afternoons.](image-url)
Gas exchange measurements

At the June and August harvests, net photosynthesis and stomatal conductance were measured in situ (at the growth \( [\text{CO}_2] \)) with an LCA-2 portable infrared gas analyzer (Analytical Development Corporation, Hoddesdon, U.K.) on a fully expanded leaf from four or five randomly chosen seedlings per species per defoliation treatment and OTC. Gas exchange was also measured on the same number of seedlings in chamberless plots at the August harvest. To facilitate treatment comparisons, leaves of similar age were chosen for photosynthetic measurements: first-flush leaves for maple and recently mature foliage (leaf plastochron index of 10–15) for aspen. These cohorts constituted the majority of total leaf area in the species at the two harvests. A magnesium perchlorate column was added to the intake pump and cuvette return stream (Bunce and Ward 1985). Leaf temperature and leaf-to-air vapor pressure gradient (VPG) in the cuvette were not controlled, and averaged 24.7 °C (range 17–31 °C) and 1.2 kPa (range 0.5–2.4 kPa), respectively. Measurements were conducted between 0800 and 1100 h.

During measurements, all aspen leaves were exposed to sunlight (PPFD > 1500 µmol m\(^{-2}\) s\(^{-1}\)), and a subset of leaves was also illuminated with a descending PPFD sequence between 1000 and 20 µmol m\(^{-2}\) s\(^{-1}\) provided by a red LED array (Quantum Devices, Barneveld, WI) attached to the cuvette. The LED array was used for all maple measurements. Every leaf was measured at a PPFD of 1000 µmol m\(^{-2}\) s\(^{-1}\), and a subset was also illuminated with a descending PPFD sequence between 600 and 20 µmol m\(^{-2}\) s\(^{-1}\). Within this PPFD range, preliminary tests on leaves of both species failed to reveal any significant difference in gas exchange response to red light versus sunlight (modulated with neutral density filters, data not shown). Light-saturated photosynthesis was measured in all plots, but light response measurements were limited to three of the four OTC blocks plus both chamberless plots. The relationship between net photosynthesis and PPFD was characterized by a non-rectangular hyperbola (Hanson et al. 1988). Because gas exchange was not measured in complete darkness, the light-response model output presented does not include dark respiration.

At both the June and August harvests, we measured light-saturated gas exchange at ambient \( [\text{CO}_2] \) and ambient + ~290 µmol mol\(^{-1}\) \( [\text{CO}_2] \) on an additional pair of sunlit leaves from each species and defoliation treatment in all OTCs. We first measured leaves at their growth \( [\text{CO}_2] \), and then repeated the measurement while exposing the leaf to air from the other \( [\text{CO}_2] \) treatment.

Simulations

We assessed the implications of treatment variation in light response for mean photosynthetic performance by simulating diurnal photosynthetic patterns during the interval between July and August harvests. Photosynthetic light-response models, incorporating species- and treatment-specific values of light-saturated photosynthesis, apparent quantum yield and light compensation point, were combined with data on the temporal distribution of PPFD measured atop either aspen or maple crowns. For each species/treatment/harvest, an estimate of net photosynthesis was generated for every appropriate PPFD measurement, and these values were averaged to generate a grand mean for photosynthesis per unit leaf area \( (A_{\text{mean}}) \).

For aspen, the influence of intra-crown shading on mean photosynthesis was incorporated using previously measured photon flux density (PPFD) was 25% lower inside than outside the OTCs (456 versus 613 µmol m\(^{-2}\) s\(^{-1}\)) (Figure 1).
spatial distributions of light transmittance within the crowns of defoliated and non-defoliated saplings (Kruger et al. 1998). Based on these distributions, every PPFD measure was converted to a crown PPFD distribution, and each value from the latter was then input into the light-response model to generate a weighted crown mean for photosynthesis. Self-shading was not considered to be an important constraint in the symподial crown that typified maples during the interval, and thus mean photosynthesis was calculated solely on the basis of PPFD measured atop the maple canopy.

When any of the light-response variables for a given species/treatment was found to be sensitive to leaf temperature (°C) (based on covariance or separate-slopes analysis of gas exchange data), the variable was adjusted accordingly for every PPFD-based photosynthesis estimate using the coincident measure of air temperature (Figure 1) as a proxy for leaf temperature. Simultaneous monitoring of the temperature of sunlit aspen leaves and surrounding air (E. McDonald, USDA Forest Service North Central Experiment Station, and E. Kruger, unpublished data) indicated that the two converged (to within 0.5 °C) under relatively turbulent conditions similar to those in our OTCs.

We acknowledge that although this simulated mean reflects the constraint of PPFD and, when applicable, leaf temperature on photosynthesis under otherwise favorable conditions, it does not incorporate the potentially important but presently unknown influences of other factors (e.g., spatial or age-related variation in leaf structure and photosynthetic traits, diurnal variation in stomatal sensitivity to VPG and enzyme activation).

Simulated values for mean photosynthesis per unit leaf mass ($A_{\text{mass,av}}$) and seedling mass ($A_{\text{seedling,av}}$) were also generated as means of treatment-level estimates from the June and August harvests, where $A_{\text{mass,av}} = A_{\text{mass,av,SLA}}$ and $A_{\text{seedling,av}} = A_{\text{mass,av,LMR}}$ (here SLA and LMR are harvest means for specific leaf area and leaf mass ratio, respectively).

**Leaf chemical analyses**

In each OTC, three to four leaves per species and defoliation treatment from among the recently mature (aspen) and first-flush (maple) leaves measured for photosynthesis, including the pair measured at both CO$_2$ concentrations, were harvested immediately after gas exchange measurement at the June and August harvests. Leaves were transported on ice to the laboratory, flash frozen in liquid nitrogen, freeze-dried, ground and then stored at −80 °C until analyzed. Foliar nitrogen was measured by Kjeldahl analysis. Digestions were conducted as described by Parkinson and Allen (1975), and nitrogen concentrations were quantified by the micro-Nesslerization technique of Lang (1958). Glycine p-toluene-sulfonic acid (5.665% N) served as the nitrogen standard. For total nonstructural carbohydrate analyses, we used the enzymatic method of M.M. Schoeneberger et al., USDA Forest Service Rocky Mountain Research Station (unpublished data), for starch and soluble sugars (hexoses and sucrose), as described by Lindroth et al. (1993). Briefly, leaf tissue (25 mg) was extracted in 80% ethanol. Soluble sugars and starch were then enzymatically converted to glucose and measured indirectly using an assay that reduces NADP to NADPH in amounts proportional to the glucose content in each sample.

**Statistical analyses**

Treatment main effects and their interactions were analyzed by species based on a 2 × 2 factorial split-plot in a randomized complete-block design. These analyses were confined to OTC data. A linear mixed-effects analysis of variance was used, in which treatment effects (whole plot = [CO$_2$] and subplot = defoliation) were fixed, and covariate (when appropriate), block and block × treatment (error) were random. This procedure employs the restricted maximum likelihood method to accurately estimate the variance components in a nested design. No significant block effect emerged ($P < 0.1$) in any of our analyses. As has commonly been observed (e.g., Walters et al. 1993, Kruger et al. 1998, Volin et al. 1998), the log$_e$ of seedling mass was a significant covariate in analyses of RGR and leaf mass ratio (LMR) in our study. Relationships among leaf and seedling attributes were examined by linear regression.

**Results**

**Seedling growth**

At the end of the growing season after leaf abscission, OTC-grown maple and aspen seedlings tended to be larger in CO$_2$-enriched air than in ambient air, but treatment differences were not significant (Figure 2). As a result of defoliation, which removed 22–30% of seedling mass, final mass tended to be lower in both species, but the effect was not significant. Trends in relative growth rate (RGR) during the June–August interval resembled those in final mass, although for maple, the mid-season responses of RGR to treatments, and defoliation in particular ($P = 0.0002$), were more pronounced. Chamberless seedlings grew less rapidly than their OTC counterparts early in the season, but this trend was reversed later, and chamberless seedlings were generally larger at the end of the year.

**Specific leaf area and biomass distribution**

In both species, elevated [CO$_2$] reduced specific leaf area (SLA) ($P < 0.08$), whereas defoliation had no effect on SLA (Table 1). At the June harvest, atmospheric CO$_2$ enrichment had no effect on biomass distribution to leaves, roots or stems in either species (Table 1). At the August harvest, biomass distribution in maple remained unaffected by elevated [CO$_2$] (Table 1); however, in aspen, atmospheric CO$_2$ enrichment led to a slight (~6%), yet significant, shift in allocation of biomass from leaves to roots (Table 1).

Defoliation reduced leaf mass ratio (LMR) about 35% in maple and 28% in aspen. However, by the August harvest, the effects of defoliation on LMR in maple had diminished to less than 13% across growth environments as a result of a 19–47% increase ($P = 0.10$) in the fraction of biomass allocated to foliage. By August, there was no significant defoliation effect on biomass distribution in aspen. At the end of the growing sea-
son, stem mass ratio of leafless trees did not vary significantly among treatments (maple mean = 0.27, aspen mean = 0.54, data not shown).

**Leaf gas exchange**

When maple leaves were measured at their respective growth [CO₂], light-saturated photosynthesis and apparent quantum yield, averaged across defoliation treatments and harvests, were 19–25% higher (P < 0.05) in seedlings in OTCs with CO₂-enriched air versus OTCs with ambient air (Figures 3–5). However, maple foliage in elevated [CO₂] exhibited signs of decreased photosynthetic capacity at both harvests (Table 2); that is, light-saturated photosynthesis (A\text{max}) was lower in leaves grown in elevated [CO₂] (P = 0.006 and P = 0.10 in June and August, respectively) than in leaves grown in ambient air when leaves were measured at the same external [CO₂]. Although the decrease was accompanied by a decrease in stomatal conductance (P = 0.01 and P = 0.06 in June and August, respectively), there was no significant difference in the estimated ratio of intercellular to external [CO₂] (C𝑖/𝐶𝑎).

For aspen, the response of light-saturated photosynthesis to CO₂ enrichment varied with leaf temperature (Figure 3). When measured at the growth [CO₂], light-saturated photosynthesis was positively related to leaf temperature in seedlings grown in OTCs with CO₂-enriched air, whereas it was insensitive to temperature in seedlings grown in ambient air. Consequently, across defoliation treatments and harvests, stimulation of light-saturated photosynthesis by atmospheric CO₂ enrichment increased from 40% at 19 °C to 75% at 29 °C. When aspen leaves from the two CO₂ treatments were measured at the same external [CO₂], there was no consistent treatment difference in photosynthesis at a given leaf temperature (Table 2), despite a tendency for both light-saturated stomatal conductance and C𝑖/𝐶𝑎 to be lower in seedlings in the OTCs with CO₂-enriched air.

Light compensation point (LCP) of aspen leaves was also sensitive to leaf temperature (T\text{leaf}; °C), where lnLCP = 0.204T\text{leaf} − 1.98, r² = 0.51, P < 0.001, based on individual leaf data pooled across all treatments and harvests. There were no significant treatment effects on temperature-normalized LCP.
Table 1. Specific leaf area and leaf, stem and root biomass ratios of sugar maple and trembling aspen seedlings growing in OTCs and chamberless plots. Values are treatment means (with 1 SE in parentheses; n = 4 OTC/plot means) at the June and August harvests. Biomass ratio data for defoliated (Defol.) trees at the June harvest are estimates based on corresponding non-defoliated values. Also included are the fractions of mass gain allocated to leaves, stems and roots during the interval between harvests. The P-values are based on mixed-effects models conducted for each species separately on the OTC data only.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Harvest</th>
<th>Ambient [CO₂]</th>
<th>Elevated [CO₂]</th>
<th>Chamberless</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sugar maple</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Specific leaf area (m² kg⁻¹)</td>
<td>June</td>
<td>16.9 (0.3)</td>
<td>16.2 (0.3)</td>
<td>16.2 (0.3)</td>
<td>19.1 (0.3)</td>
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<td></td>
<td>August</td>
<td>15.5 (0.4)</td>
<td>14.8 (0.4)</td>
<td>15.0 (0.5)</td>
<td>15.7 (0.4)</td>
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<td>Leaf mass ratio</td>
<td>June</td>
<td>0.43 (0.002)</td>
<td>0.27</td>
<td>0.42 (0.004)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>0.30 (0.015)</td>
<td>0.27 (0.005)</td>
<td>0.33 (0.014)</td>
<td>0.29 (0.025)</td>
</tr>
<tr>
<td>Stem mass ratio</td>
<td>June</td>
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<td>0.27</td>
<td>0.21 (0.014)</td>
<td>0.27</td>
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<td></td>
<td>August</td>
<td>0.23 (0.003)</td>
<td>0.24 (0.008)</td>
<td>0.23 (0.016)</td>
<td>0.26 (0.016)</td>
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<td>Root mass ratio</td>
<td>June</td>
<td>0.36 (0.010)</td>
<td>0.46</td>
<td>0.37 (0.014)</td>
<td>0.46</td>
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<td></td>
<td>August</td>
<td>0.47 (0.014)</td>
<td>0.49 (0.027)</td>
<td>0.44 (0.026)</td>
<td>0.45 (0.022)</td>
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<tr>
<td>Fraction of mass allocated to leaves⁻¹</td>
<td>June</td>
<td>0.17 (0.034)</td>
<td>0.25 (0.055)</td>
<td>0.26 (0.029)</td>
<td>0.31 (0.037)</td>
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<td></td>
<td>August</td>
<td>0.24 (0.009)</td>
<td>0.21 (0.023)</td>
<td>0.25 (0.031)</td>
<td>0.25 (0.044)</td>
</tr>
<tr>
<td>Fraction of mass allocated to stems⁻¹</td>
<td>June</td>
<td>0.58 (0.041)</td>
<td>0.55 (0.079)</td>
<td>0.48 (0.049)</td>
<td>0.44 (0.058)</td>
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<td></td>
<td>August</td>
<td>0.43 (0.006)</td>
<td>0.36</td>
<td>0.26 (0.009)</td>
<td>0.37</td>
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<tr>
<td>Trembling aspen</td>
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<tr>
<td>Specific leaf area (m² kg⁻¹)</td>
<td>June</td>
<td>25.8 (0.9)</td>
<td>23.5 (0.5)</td>
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<td>August</td>
<td>21.3 (0.7)</td>
<td>21.0 (0.6)</td>
<td>19.3 (0.6)</td>
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<td>Leaf mass ratio</td>
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<td>0.44</td>
<td>0.59 (0.008)</td>
<td>0.42</td>
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<td>0.41 (0.006)</td>
<td>0.40 (0.014)</td>
<td>0.39 (0.005)</td>
<td>0.38 (0.005)</td>
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<tr>
<td>Stem mass ratio</td>
<td>June</td>
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<td>0.36</td>
<td>0.26 (0.009)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>0.43 (0.007)</td>
<td>0.43 (0.005)</td>
<td>0.43 (0.004)</td>
<td>0.44 (0.008)</td>
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<td>Root mass ratio</td>
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<td>0.20</td>
<td>0.15 (0.005)</td>
<td>0.22</td>
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<tr>
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<td>August</td>
<td>0.16 (0.003)</td>
<td>0.16 (0.010)</td>
<td>0.18 (0.004)</td>
<td>0.18 (0.004)</td>
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<tr>
<td>Fraction of mass allocated to leaves⁻¹</td>
<td>June</td>
<td>0.40 (0.006)</td>
<td>0.40 (0.016)</td>
<td>0.37 (0.005)</td>
<td>0.38 (0.008)</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>0.44 (0.007)</td>
<td>0.43 (0.006)</td>
<td>0.45 (0.004)</td>
<td>0.44 (0.011)</td>
</tr>
<tr>
<td>Fraction of mass allocated to stems⁻¹</td>
<td>June</td>
<td>0.16 (0.003)</td>
<td>0.16 (0.011)</td>
<td>0.18 (0.004)</td>
<td>0.18 (0.005)</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>0.16 (0.003)</td>
<td>0.16 (0.011)</td>
<td>0.18 (0.004)</td>
<td>0.18 (0.005)</td>
</tr>
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</table>

1 We calculated the percentage of acquired mass that was allocated to a given organ between June and August harvests as (Δ organ mass/Δ seedling mass)×100. We adopted this approach rather than allometry (i.e., mass of organ Y = a(mass of organ X)ᵇ, Ledig et al. 1970, McConnaughay and Coleman 1999) because our intent was to compare patterns of allocation at the whole-seedling level. Because an allometric coefficient (b) is the ratio of RGRs of two plant components (i.e., RGRorg/RGRplant) during a growth interval, it does not reflect the proportionality of total mass allocation if organ mass ratios differ initially. Correspondingly, it is not a reasonable basis for comparison of whole-seedling allocation when organ mass ratios vary among species or environments at the beginning of the interval, which was the case in our study.

Across defoliation treatments and harvests, the apparent quantum yield of aspen leaves in CO₂-enriched air exceeded that in ambient air by an average of 33% (P < 0.02, Figure 5). Across CO₂ treatments, defoliation of maple induced a rapid increase in light-saturated photosynthesis (P < 0.05) and stomatal conductance (P < 0.07), but no change in Cᵢ/Cᵢₐ in the remaining foliage (Table 2; June harvest). The largest proportional response of photosynthesis (~34% increase) occurred in leaves from OTCs with CO₂-enriched air. However, the effect disappeared during the 40-day interval between June and August harvests. Defoliation did not significantly alter apparent quantum yield or light compensation point in maple (Figure 5).

Residual aspen foliage responded to defoliation with a 10–20% stimulation (P < 0.006) of light-saturated photosynthesis and an accompanying 15–25% increase (P < 0.11) in stomatal conductance (Table 2; June harvest). The response, which did not include a significant change in Cᵢ/Cᵢₐ, tended to be largest in leaves from OTCs with CO₂-enriched air, and, like the response of maple, it did not persist through the period between harvests. By the August harvest, light-saturated photosynthesis was 7% lower (P = 0.01), and Cᵢ/Cᵢₐ slightly higher (P = 0.02 for measurements at elevated [CO₂]), in leaves of defoli-
ated versus non-defoliated seedlings (Table 2). As in maple, defoliation did not significantly alter the apparent quantum yield or light compensation point of aspen leaves (Figure 5).

Reconciling treatment effects on growth and photosynthesis
Simulated means for photosynthesis per unit leaf area ($A_{area,av}$), leaf mass ($A_{mass,av}$) and seedling mass ($A_{seedling,av}$) were generated for the 40-day growth interval following defoliation (Table 3). For maple, trends in $A_{area,av}$ generally resembled those measured at saturating PPFD (Table 2, Figure 3), except for the absence of any response to defoliation. For maple, $A_{seedling,av}$ was 21–24% higher in elevated [CO2] than in ambient [CO2]. Across CO2 treatments, the 17–18% reduction in mean LMR of defoliated maples led to a similar decrease in $A_{seedling,av}$.

For aspen, elevated [CO2] had a smaller effect on $A_{area,av}$ (~36% stimulation) than on light-saturated photosynthesis (~52% stimulation at 22.7 °C, Figure 3). The difference in average photosynthesis between CO2 treatments was diminished further (to 18–26%) when expressed on a leaf mass basis. The slight positive effect of defoliation on $A_{mass,av}$ was more than offset by a lower mean LMR, leading to a marked reduction in $A_{seedling,av}$. Depending on defoliation treatment, $A_{seedling,av}$ was 12–19% higher in CO2-enriched OTCs than in ambient OTCs.

During the period following defoliation, $A_{seedling,av}$ explained 89–93% of the variation in RGR among treatments and environments for each species ($P = 0.002$) (Figure 6).

Foliar nitrogen and carbohydrate concentrations
Elevated [CO2] caused changes in leaf chemistry of both defoliated and non-defoliated maples (Table 4), including decreases in area-based N concentration ($P = 0.04$ at August harvest) and mass-based N concentration ($P = 0.008$ at August harvest) and large increases in concentrations of starch ($P = 0.0001$ at August harvest) and total nonstructural carbohydrates ($P = 0.0002$ at August harvest). To determine if maple leaf nitrogen concentration ([N]) was diluted by CO2-mediated increases in total nonstructural carbohydrates (TNC), we recalculated [N] in the absence of TNC. At neither harvest was there a significant effect of CO2 treatment on “adjusted” [N] (Table 4).

For aspen, there was no significant CO2 effect on area-based leaf [N]. However, mass-based leaf [N] was 14–18% less in seedlings in elevated [CO2] than in OTCs ventilated with ambient air ($P < 0.04$) at the two harvests (Table 4). When defoliation treatments were pooled, leaf [starch] more than doubled ($P = 0.05$), and TNC concentrations increased by 22% ($P = 0.06$), in response to elevated [CO2] at the first harvest. However, in August, the only significant effect of CO2 enrichment on leaf carbohydrates was a relatively small increase in [sugar]. Comparisons of treatment variation in “adjusted” [N]
indicated that most of the CO$_2$-induced decrease in aspen leaf [N] did not result from TNC dilution.

Defoliation had minimal effects on maple leaf N and carbohydrate concentrations at both harvests (Table 4). For aspen, on the other hand, defoliation resulted in more than a 50% reduction in [starch] ($P = 0.002$), and a slight but significant reduction in [sugar] and [hexose]/[sucrose] ratio at the June harvest (Table 4), resulting in a 21% decrease in [TNC] ($P = 0.0006$) across CO$_2$ treatments. However, by August, only leaf [sugar] differed significantly between defoliation treatments in aspen, with slightly higher values in defoliated seedlings than in control seedlings, when averaged across CO$_2$ treatments.

**Relationships between leaf photosynthesis and nitrogen or carbohydrate concentration**

When data from all treatments and harvests were pooled, variation in mass-based light-saturated photosynthesis, measured at ambient [CO$_2$], was positively related to leaf [N] for both maple ($r^2 = 0.40, P = 0.0002$) and aspen ($r^2 = 0.32, P = 0.002$) (based on individual leaf measurements, regressions not shown). In addition, the efficiency of N use in photosynthesis (PNUE), calculated as the ratio of $A_{\text{mass}}$ (measured at 360 µmol mol$^{-1}$ CO$_2$) to leaf [N], was lower in maple seedlings in the CO$_2$-enriched OTCs than in the ambient OTCs at both harvests ($P < 0.08$, Table 2).

Defoliated maple seedlings had a significantly higher PNUE than non-defoliated seedlings at the June harvest ($P < 0.02$). Decreases in PNUE in seedlings in elevated [CO$_2$] generally coincided with increases in [starch] and [TNC] (Table 4), but there was no significant trend, either within or across harvests, between maple PNUE and [sugar], [starch] or [TNC], or [hexose]/[sucrose] ratio. Aspen PNUE did not vary significantly among treatments (Table 2) and was unrelated to any measure of leaf carbohydrate chemistry.

**Discussion**

Contrary to our findings with potted maple and aspen saplings (Kruger et al. 1998), CO$_2$ enrichment did not markedly alter responses to defoliation by either species in the present study. Specifically, in our OTCs there was no significant CO$_2$ influence on the magnitude of photosynthetic stimulation, proportion of mass allocated to leaves or growth recovery by defoliated maples. We currently have no rationale for this variation in maple behavior, and there is relatively little published data (on trees) with which to compare our results. Love-loclock et al. (1999) found that the negative impact of a 40% reduction in leaf area on growth of *Copaifera aromatica* Dwyerq. was accentuated in elevated [CO$_2$]. Of course, variable outcomes among studies may stem from differences in the manner of defoliation. Our protocol involved an extensive, one-time removal of foliage, and it remains to be seen whether the influences of CO$_2$ enrichment on tree responses vary with mode of folivory (Trumble et al. 1993). We note that, at least with respect to leaf chemistry, responses of aspen and maple to our defoliation methods resembled those induced by lepidopteran folivory, particularly during late-instar feeding (Roth et al. 1998).

In both CO$_2$ environments, the commonly observed stimulation of light-saturated photosynthesis following defoliation (e.g., Heichel and Turner 1983, Tschaplinski and Blake 1989, Reich et al. 1993, Syvertsen 1994, Hart et al. 2000) was ephemeral, especially for maple. Post-defoliation recovery of
growth potential in maple was largely dependent on the reestablishment of LMR, which was mediated by an increase in mass allocation to foliage. A similar shift in mass allocation following leaf loss has been observed in other species (e.g., Bassman and Dickmann 1982, Reich et al. 1993) and is consistent with prevailing models of carbon allocation (Wilson 1988, Cannell and Dewar 1994).

In contrast to maple, LMR of defoliated and non-defoliated aspen rapidly converged toward the prevailing ratio of mass allocated to foliage (~0.4) during the growth interval (cf. Kruger et al. 1998). The rate of convergence depended on RGR, indicating that it is mediated, in part, by the inherently high rate of mass-based photosynthesis in aspen leaves. Madgwick (1975) observed similar regrowth behavior in defoliated *Liriodendron tulipifera* L., another early successional, fast-growing tree species.

Despite their contrasting ecology, both slow-growing maple and fast-growing aspen exhibited only modest growth responses to a near doubling of atmospheric [CO$_2$] over one growing season. This finding does not support the concept that inherently fast-growing species (with greater sink strength) are more responsive to CO$_2$ enrichment than slower-growing species (Stitt 1991, Poorter 1993). However, our results were consistent with previous findings (Kruger et al. 1998) and, in the case of aspen RGR, with those of several other studies (Brown and Higginbotham 1986, Brown 1991, Volin and Reich 1996, Kinney and Lindroth 1997, Karnosky et al. 1998, Kubiske et al. 1998, Tjoelker et al. 1998, Volin et al. 1998, Zak

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Table 2. Light-saturated gas exchange characteristics of sugar maple and trembling aspen leaves measured in OTCs at the June and August harvests. Photosynthesis (µmol m$^{-2}$ s$^{-1}$), stomatal conductance (mmol m$^{-2}$ s$^{-1}$) and the ratio of intercellular [CO$_2$] to ambient [CO$_2$] (C$_i$/C$_a$) were assessed at each of the two growth CO$_2$ concentrations (CO$_2$ of air entering cuvette ~365 µmol mol$^{-1}$ and 650 µmol mol$^{-1}$). Leaves were first measured at their growth CO$_2$. When aspen leaves were measured at elevated [CO$_2$], photosynthesis and stomatal conductance were significantly and positively correlated with leaf temperature across all treatments and harvests ($P < 0.0005$). Therefore, for aspen leaves only, means for these two measures were normalized for temperature by covariance analysis. For both species, estimates of the efficiency of nitrogen use in photosynthesis (PNUE; µmol g$^{-1}$ s$^{-1}$) were calculated only for measurements at ambient [CO$_2$]. Values are treatment means (with 1 SE in parentheses; $n = 4$ OTC means). We calculated C$_i$/C$_a$ with the equations of von Caemmerer and Farquhar (1981). Abbreviation: Defol. = defoliation.

<table>
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<th>Measure</th>
<th>Measurement [CO$_2$]</th>
<th>Ambient [CO$_2$]</th>
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et al. 2000). Sugar maple, on the other hand, has behaved less predictably, being very sensitive to atmospheric CO2 enrichment in certain cases (e.g., Bazzaz et al. 1990, Tschaplinski et al. 1995) and minimally so in others (e.g., Lindroth et al. 1993, Reid and Strain 1994). Although the source of this variability is unknown, growth responsiveness of maple has been modified by various manipulations of its environment, including air temperature (Norby et al. 1999), soil water content (Tschaplinski et al. 1995) and soil NO3 availability (Kinney and Lindroth 1997).

We found that a decrease in photosynthetic capacity of maple negated much of the potential benefit of increased CO2 availability for mean photosynthesis. Decreases in SLA further diminished the CO2 stimulus when photosynthesis was expressed per unit leaf mass. Although no discernible adjustment in photosynthetic metabolism was observed for aspen, decreases in both SLA and mass allocation to leaves constrained its growth response to elevated [CO2]. In addition, the potential benefit of enhanced light-saturated photosynthesis in response to atmosphere CO2 enrichment was not fully realized because our aspen leaves were mostly exposed to subsaturating PPFDs (< 400 µmol m–2 s–1). Nevertheless, photosynthesis was stimulated to a lesser extent in elevated [CO2] during periods of low PPFD (above light compensation point) than during periods of high PPFD because of the increase in apparent quantum yield.

Based on our simulations, mean maple photosynthesis in elevated [CO2] benefited almost exclusively from a parallel increase in apparent quantum yield. Estimated differences in mean photosynthesis between CO2 treatments were greater at subsaturating PPFDs (< 200 µmol m –2 s–1) than at saturating PPFDs. Others (e.g., Long and Drake 1991, McMurtrie and Wang 1993, Kubiske and Pregitzer 1996) have emphasized the potential importance of increased quantum yield for canopy carbon balance in response to elevated [CO2], particularly when photosynthetic capacity is reduced.

The mechanism underlying the decreases in photosynthetic capacity of maple and other field-grown trees exposed to elevated [CO2] (e.g., Lewis et al. 1996, Rey and Jarvis 1998, Li et al. 1999, Norby et al. 1999) is unknown. Although this adjustment may sometimes be attributable to experimental design (Arp 1991, Gunderson and Wullschleger 1994, Curtis 1996), its occurrence in well-established trees rooted in fertile ground is not easily dismissed as artifact. In our study, leaf N status may have been involved; however, it did not seem to be the sole cause, because decreases in PNUE were larger than declines in leaf N concentration.

The decrease in maple PNUE in elevated [CO2] coincided with leaf starch accumulation, an indicator of increased photosynthetic carbon allocation to storage, suggesting that a change in the balance between photosynthesis and respiration has occurred in elevated [CO2]. The decrease in PNUE in elevated [CO2] coincided with leaf starch accumulation, an indicator of increased photosynthetic carbon allocation to storage, suggesting that a change in the balance between photosynthesis and respiration has occurred in elevated [CO2].

### Table 3

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source/sink ratio. Overall, however, there was no significant relationship in either species between photosynthetic capacity (or PNUE) and leaf starch or other potential signals of source–sink imbalance (Van Oosten and Besford 1996, Rey and Jarvis 1998, Moore et al. 1999). Centritto and Jarvis (1999) noted a lack of coupling between photosynthetic acclimation and leaf carbohydrate or nitrogen status. Perhaps some of the decrease in maple PNUE in elevated [CO₂] resulted from morphological rather than chemical changes. Based on a broad and positive relationship between PNUE and SLA (Reich et al. 1997), Peterson et al. (1999) concluded that decreases in SLA in response to elevated [CO₂] could compromise light-saturated photosynthesis, expressed on a mass basis, and PNUE.

We postulated that photosynthetic responses to defoliation would shed light on the nature of metabolic adjustments to elevated [CO₂]. Our premise was that a 50% reduction in leaf area would preclude development of an assimilate surplus in response to atmospheric CO₂ enrichment. However, we found that defoliation-induced stimulation of light-saturated photosynthesis did not always correspond with phytochemical manifestations of a shift in source–sink balance. For example, in maple, defoliation-induced increases in PNUE were not accompanied by a discernible change in carbohydrate status. In aspen, both defoliation and atmosphere CO₂ enrichment affected leaf carbohydrate concentrations, but PNUE appeared to be insensitive to these perturbations. Photosynthetic acclimation to elevated [CO₂] is often associated with decreases in amount and activation of ribulose bisphosphate carboxylase (e.g., Sims et al. 1998, Urban and Marek 1999). We did not collect data on leaf enzymes, but the relative stability of \( \frac{C_i}{C_a} \) across treatments indicated that photosynthetic capacity may have varied as a result of treatment differences in leaf Rubisco content or activity.

In maple, photosynthesis was insensitive to changes in temperature in both CO₂ treatments (Figure 3). Current biochemical models predict that C₃ photosynthesis becomes more responsive to variation in leaf temperature as atmospheric [CO₂] increases as a result of the inhibitory effect of elevated CO₂. Overall, however, there was no significant relationship in either species between photosynthetic capacity (or PNUE) and leaf starch or other potential signals of source–sink imbalance (Van Oosten and Besford 1996, Rey and Jarvis 1998, Moore et al. 1999). Centritto and Jarvis (1999) noted a lack of coupling between photosynthetic acclimation and leaf carbohydrate or nitrogen status. Perhaps some of the decrease in maple PNUE in elevated [CO₂] resulted from morphological rather than chemical changes. Based on a broad and positive relationship between PNUE and SLA (Reich et al. 1997), Peterson et al. (1999) concluded that decreases in SLA in response to elevated [CO₂] could compromise light-saturated photosynthesis, expressed on a mass basis, and PNUE.

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[CO₂] on photorespiration (Long 1991). We are uncertain as to the cause of this temperature insensitivity, but we note that there was a nonsignificant tendency for stomatal conductance and Cₒ to decrease with increasing VPD, which in turn was significantly and positively correlated with leaf temperature (data not shown). This tendency, which was not apparent in aspen, might offset an otherwise positive photosynthetic response to temperature in elevated [CO₂].

Compared with many OTC studies, two features of our experimental design enhance the potential relevance of the results for predicting forest response to global change (Norby et al. 1999). The seedlings were planted directly in the ground, and they were grown at densities typical of a regenerating hardwood stand in mesic temperate forests (cf. Kruger and Reich 1997). Several experiments have demonstrated that CO₂ responses of plants grown in isolation can differ markedly from those of plants with neighbors (Bazzaz and McConnaughay 1992, Wayne and Bazzaz 1995, 1997, Catoovsky and Bazzaz 1999), and in several of these studies competition muted the growth stimulation. We speculate that the presence of neighbors, by way of competitive resource depletion (Wayne and Bazzaz 1995), probably dampened the CO₂ response of aspen, especially as crowns began to coalesce toward the end of the growing season. Based on our measures of photosynthetic light response, a reduction in light availability by neighbors (or by the OTC) would decrease the benefit of CO₂ enrichment for carbon balance in aspen.

We were also concerned about a possible growth-mediated divergence of canopy structures and light environments between ambient and CO₂-enriched OTCs. This would pose particular problems for the assessment of treatment responses in maple, which was overtopped by aspen by mid-July. Our data indicate that such divergence was modest. For example, during the July–August growth interval, leaf area index (based on harvest data) averaged 1.7 and 1.5 in the elevated and ambient [CO₂] treatments, respectively, and the amount of light available to maple crowns did not differ between treatments (data not shown). Furthermore, LAI did not differ between treatments in late September (mean LAI = 2.5, data not shown). Based on these data, and the clumped nature of foliage in monopodial aspen crowns, we conclude that the light environment did not seriously confound treatment comparisons.

We did not monitor several aspects of tree carbon balance, including tissue dark respiration, exudate production and turnover of fine roots, emissions of volatile organic carbon, tissue construction costs, and carbon allocation to mycorrhizae. Some of these processes, such as fine root turnover, may be particularly responsive to CO₂ enrichment (Ceulemans and Mousseau 1994, Saxe et al. 1998, Norby et al. 1999). Moreover, they could be influenced by modest increases in air and soil temperatures in an OTC (Van Oijen et al. 1999). However, the relatively close correspondence between RGR and simulated means of photosynthesis per unit seedling mass (A.seedling,av; Figure 6) indicated that neither treatment- nor OTC-mediated variation in these components had a large impact on net biomass gain. The trends in Figure 6 also indicated that differences in growth between seedlings inside the OTCs and seedlings outside the OTCs stemmed primarily from differences in A.seedling,av, which was less in the OTCs because light attenuation (Figure 1) constrained A.area,av (Table 3). Collectively, our results and those of Roth et al. (1998) refute the hypothesis that atmospheric CO₂ enrichment will markedly alter the responses of sugar maple and trembling aspen regeneration to defoliation. Furthermore, these data augment a growing body of evidence (e.g., Curtis and Wang 1998, Kubiske et al. 1998, Olszyk et al. 1998, Tjoelker et al. 1998) indicating that, even in the short term, growth rates of certain key constituents of temperate forests may respond only moderately to rising [CO₂]. This finding contrasts with the large increases in growth observed in many OTC studies (reviewed by Norby et al. 1999) and at least one free-air CO₂ enrichment experiment (DeLucia et al. 1999). Although additional factors may be involved (Pregitzer et al. 1995, 2000, Wang et al. 1998), we attribute the lack of a pronounced growth response to atmospheric CO₂ enrichment primarily to the absence of an appreciable CO₂ effect on mean photosynthesis per unit leaf mass. Three limitations contributed to this absence: (1) a downward adjustment of photosynthetic capacity (in maple); (2) decreases in SLA (in both species); and (3) consequences of PPFD dynamics in a natural light environment, which decreased the opportunity to capitalize fully on increased CO₂ availability (in aspen). Although these limitations pertain to the behavior of seedlings during exposure to elevated [CO₂] for one growing season, they may reflect important constraints on forest growth responses to CO₂-enriched environments.

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