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Responses of deciduous broadleaf trees to defoliation in a CO_2 enriched atmosphere

JOHN C. VOLIN,[1,2] ERIC L. KRUGER[3] and RICHARD L. LINDROTH[4]

[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 CO_2 enrichment for tree responses to biotic disturbances such as folivory. We examined the combined effects of elevated CO_2 concentration ($[CO_2]$) 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 CO_2-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 CO_2 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 CO_2 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ and defoliation on only a few of the measured traits. Thus, the data do not support the hypothesis that atmospheric CO_2 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 $[CO_2]$.

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 CO_2 concentration ($[CO_2]$) 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 $[CO_2]$ 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 $[CO_2]$ (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 CO_2 availability (Stitt 1991).

Kruger et al. (1998) recently observed an interaction between elevated $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$, and defoliated seedlings exhibited a significant increase in both photosynthesis and mass allocation to leaves. These data indicate that atmospheric CO_2 enrichment could potentially enhance the ability of certain tree species to recover from folivory, and that responses to elevated $[CO_2]$ 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 $[CO_2]$ 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 CO_2-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 CO_2-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 CO_2-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 m^3 s^{-1}, providing about three complete air exchanges per minute. Treatments were arranged in a randomized complete-block design, and atmospheric $[CO_2]$ 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 $[CO_2]$ 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 CO_2 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 (Hammamatsu 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

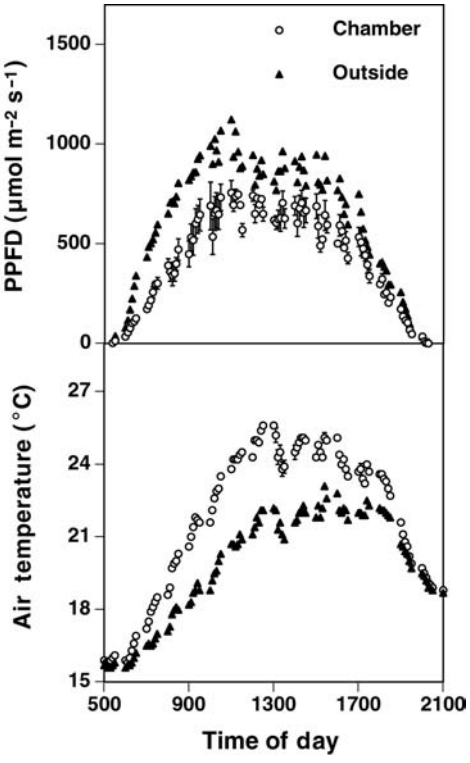

Figure 1. Diurnal patterns of mean photosynthetic photon flux density (PPFD) and air temperature in OTCs and chamberless plots during the June–August growth interval. We monitored PPFD near or at the top of the aspen canopies, whereas temperature was monitored at a height of 1 m in the center of the 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.

photon flux density (PPFD) was 25% lower inside than outside the OTCs (456 versus 613 μmol m^{-2} s^{-1}) (Figure 1).

In late June, 31 days after atmospheric CO_2 enrichment began, about 50% of the leaf area was removed from 36 plants per species in each OTC and chamberless plot. Defoliation entailed removing roughly half of each leaf lamina, along one side of the midrib, with hair-texturizing scissors (cf. Kruger et al. 1998, Lindroth and Kinney 1998). The treatment was completed within 3 days. All clipped foliage was placed on ice in the field and stored at 5 °C until it was measured for fresh area and dry mass.

Growth measurements

Trees were harvested on four occasions: at the start of CO_2 treatments in late May, immediately after defoliation in late June, 6 weeks after defoliation in early August and after leaf abscission in October. Three to four randomly chosen seedlings per species were harvested from each OTC and chamberless plot in late May. Seven, 18 and 29 maples per OTC or chamberless plot were harvested in June, August and October, respectively. For aspen, we harvested 9, 13 and 23 seedlings, respectively. At each harvest, seedlings were separated into leaves, stems and roots, and leaf area was measured with a Li-Cor LI-3100 leaf area meter. Soil was rinsed from roots and all components were dried to a constant mass at 70 °C before weighing. Relative growth rate (RGR; mg g^{-1} day^{-1}) was calculated for individual seedlings during intervals between the May, June and August harvests, where RGR = [ln(harvest dry mass) – ln(initial dry mass)]/days (Evans 1972). For defoliated seedlings, mid-season RGR (June to August) was calculated after adjusting initial mass for measured leaf loss.

Initial dry mass was estimated for each seedling harvested in June and August based on allometric relationships between seedling mass (or its natural logarithm) and either stem height (for aspen) or leaf area (for maple). Relationships between $\log_e$ of aspen mass and height did not vary significantly among CO_2 treatments or growth environments at either the May or June harvest; therefore one global relationship was generated at each harvest. The r^2 of these regressions ranged from 0.74 to 0.88 ($P < 0.001$). For maple, total area of the first flush, which was fully expanded at the time of the May harvest, was a better predictor of seedling mass than stem height. In May, no significant variation was detected among CO_2 treatments or growth environments in the relation between mass and leaf area. In June, both the slope and intercept differed significantly between OTCs and chamberless plots, and accordingly, separate relationships were used. The r^2 of these regressions ranged from 0.66 to 0.83 ($P < 0.001$).

Gas exchange measurements

At the June and August harvests, net photosynthesis and stomatal conductance were measured in situ (at the growth [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 [CO_2] and ambient + ~290 μmol mol^{-1} [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 [CO_2], and then repeated the measurement while exposing the leaf to air from the other 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 ($A_{area,av}$).

For aspen, the influence of intra-crown shading on mean photosynthesis was incorporated using previously measured

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 sympodial 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 ($A_{mass,av}$) and seedling mass ($A_{seedling,av}$) were also generated as means of treatment-level estimates from the June and August harvests, where $A_{mass,av} = A_{area,av}SLA$ and $A_{seedling,av} = A_{mass,av}LMR$ (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-

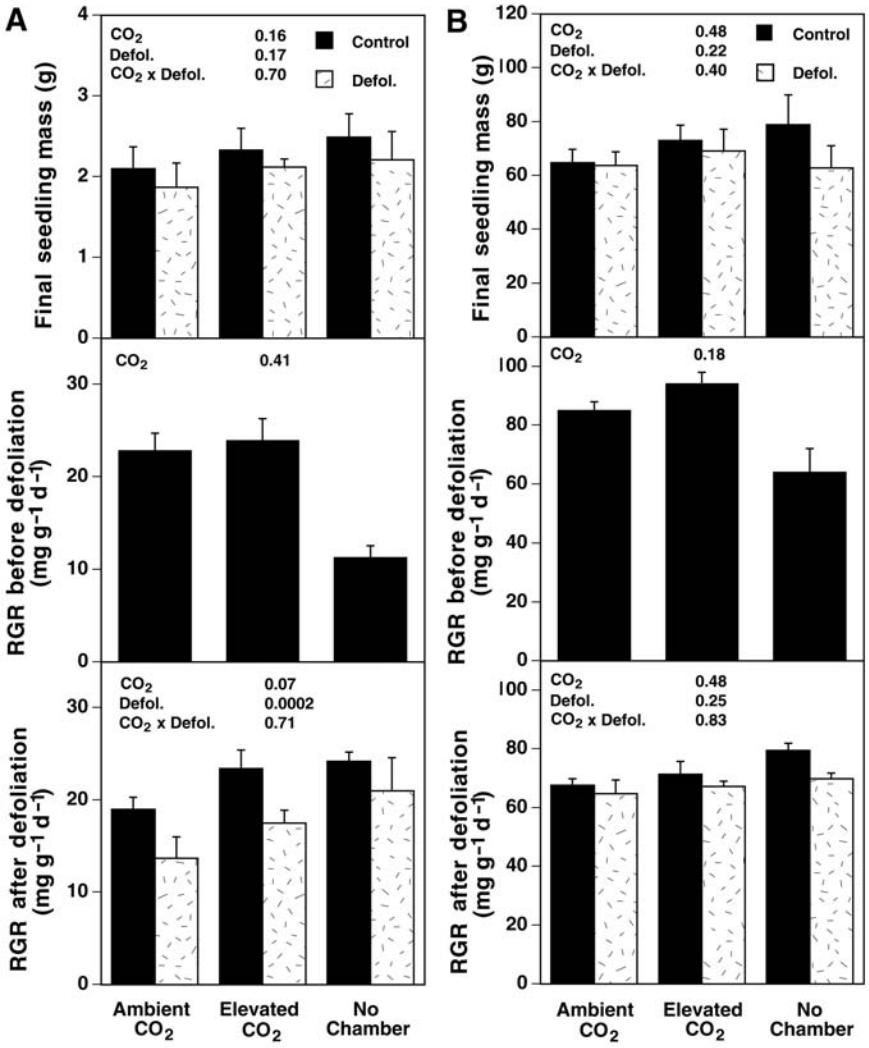

Figure 2. Final dry mass and relative growth rate (RGR) of defoliated (Defol.) and non-defoliated sugar maple (A) and trembling aspen (B) seedlings in OTCs ventilated with either ambient or CO_2-enriched (ambient + 283 μmol mol^{-1}) air. Data on seedlings grown in chamberless plots are also included. Within a species, columns represent treatment means with standard errors, based on $n = 4$ and $n = 2$ OTC and chamberless plot means, respectively. Final mass (stem + root) was measured on seedlings harvested in the fall, after leaf abscission. Seedling RGR was measured during the 30 days before defoliation (conducted in late June) and the 40-day period following defoliation (late June through early August). The *P*-values for treatment main effects and their interaction are provided in each panel, based on a mixed-effects linear model run separately for each species on the OTC data only.

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 [CO_2], 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 CO_2-enriched air versus OTCs with ambient air (Figures 3–5). However, maple foliage in elevated [CO_2] exhibited signs of decreased photosynthetic capacity at both harvests (Table 2); that is, light-saturated photosynthesis (A_{max}) was lower in leaves grown in elevated [CO_2] ($P = 0.006$ and $P = 0.10$ in June and August, respectively) than in leaves grown in ambient air when leaves were measured at the same external [CO_2]. 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 [CO_2] (C_i/C_a).

For aspen, the response of light-saturated photosynthesis to CO_2 enrichment varied with leaf temperature (Figure 3). When measured at the growth [CO_2], light-saturated photosynthesis was positively related to leaf temperature in seedlings grown in OTCs with CO_2-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 CO_2 enrichment increased from 40% at 19 °C to 75% at 29 °C. When aspen leaves from the two CO_2 treatments were measured at the same external [CO_2], 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 C_i/C_a to be lower in seedlings in the OTCs with CO_2-enriched air.

Light compensation point (LCP) of aspen leaves was also sensitive to leaf temperature (T_{leaf}; °C), where $\ln LCP = 0.204T_{leaf} - 1.98$, $r^2 = 0.51$, $P < 0.001$, 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.

Measure	Harvest	Ambient [CO_2]		Elevated [CO_2]		Chamberless		*P*-Values		
		Control	Defol.	Control	Defol.	Control	Defol.	CO_2	Defol.	[CO_2] × Defol.
Sugar maple										
Specific leaf area	June	16.9 (0.3)	–	16.2 (0.3)	–	19.1 (0.3)	–	0.05	–	–
(m^2 kg^{-1})	August	15.5 (0.4)	16.2 (0.3)	14.8 (0.4)	15.0 (0.5)	15.7 (0.4)	14.6 (1.3)	0.02	0.22	0.57
Leaf mass ratio	June	0.43 (0.002)	0.27	0.42 (0.004)	0.27	0.47 (0.018)	0.31	0.30	–	–
	August	0.30 (0.015)	0.27 (0.025)	0.33 (0.014)	0.29 (0.025)	0.33 (0.023)	0.32 (0.005)	0.37	0.01	0.84
Stem mass ratio	June	0.21 (0.009)	0.27	0.21 (0.014)	0.27	0.22 (0.016)	0.28	0.99	–	–
	August	0.23 (0.003)	0.24 (0.008)	0.23 (0.016)	0.26 (0.016)	0.20 (0.023)	0.26 (0.007)	0.41	0.08	0.59
Root mass ratio	June	0.36 (0.010)	0.46	0.37 (0.014)	0.46	0.31 (0.002)	0.41	0.83	–	–
	August	0.47 (0.014)	0.49 (0.027)	0.44 (0.026)	0.45 (0.022)	0.46 (0.045)	0.42 (0.039)	0.23	0.40	0.79
Fraction of mass allocated to leaves	–[1]	0.17 (0.034)	0.25 (0.055)	0.26 (0.029)	0.31 (0.037)	0.23 (0.001)	0.32 (0.045)	0.20	0.10	0.75
Fraction of mass allocated to stems	–[1]	0.24 (0.009)	0.21 (0.023)	0.25 (0.031)	0.25 (0.044)	0.20 (0.050)	0.24 (0.001)	0.33	0.40	0.53
Fraction of mass allocated to roots	–[1]	0.58 (0.041)	0.55 (0.079)	0.48 (0.049)	0.44 (0.058)	0.58 (0.050)	0.44 (0.066)	0.18	0.42	0.91
Trembling aspen										
Specific leaf area	June	25.8 (0.9)	–	23.5 (0.5)	–	21.5 (0.2)	–	0.07	–	–
(m^2 kg^{-1})	August	21.3 (0.7)	21.0 (0.6)	19.3 (0.6)	19.6 (0.8)	18.4 (0.2)	16.4 (0.9)	0.08	0.95	0.60
Leaf mass ratio	June	0.60 (0.002)	0.44	0.59 (0.008)	0.42	0.60 (0.013)	0.43	0.12	–	–
	August	0.41 (0.006)	0.40 (0.014)	0.39 (0.005)	0.38 (0.005)	0.39 (0.002)	0.40 (0.027)	0.03	0.23	0.91
Stem mass ratio	June	0.26 (0.006)	0.36	0.26 (0.009)	0.37	0.22 (0.008)	0.32	0.85	–	–
	August	0.43 (0.007)	0.43 (0.005)	0.43 (0.004)	0.44 (0.008)	0.40 (0.013)	0.39 (0.040)	0.45	0.40	0.56
Root mass ratio	June	0.14 (0.008)	0.20	0.15 (0.005)	0.22	0.18 (0.005)	0.25	0.15	–	–
	August	0.16 (0.003)	0.16 (0.010)	0.18 (0.004)	0.18 (0.004)	0.21 (0.016)	0.21 (0.013)	0.04	0.28	0.56
Fraction of mass allocated to leaves	–[1]	0.40 (0.006)	0.40 (0.016)	0.37 (0.005)	0.38 (0.008)	0.38 (0.004)	0.40 (0.028)	0.007	0.33	0.38
Fraction of mass allocated to stems	–[1]	0.44 (0.007)	0.43 (0.006)	0.45 (0.004)	0.44 (0.011)	0.41 (0.013)	0.40 (0.041)	0.16	0.12	0.46
Fraction of mass allocated to roots	–[1]	0.16 (0.003)	0.16 (0.011)	0.18 (0.004)	0.18 (0.005)	0.21 (0.016)	0.21 (0.014)	0.06	0.79	0.54

[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., RGR_{organ}/RGR_{plant}) 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.

means (Figure 5). Across defoliation treatments and harvests, the apparent quantum yield of aspen leaves in CO_2-enriched air exceeded that in ambient air by an average of 33% ($P < 0.02$, Figure 5).

Across CO_2 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 C_i/C_a, in the remaining foliage (Table 2; June harvest). The largest proportional response of photosynthesis (~34% increase) occurred in leaves from OTCs with CO_2-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 C_i/C_a, tended to be largest in leaves from OTCs with CO_2-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 C_i/C_a slightly higher ($P = 0.02$ for measurements at elevated [CO_2]), in leaves of defoli-

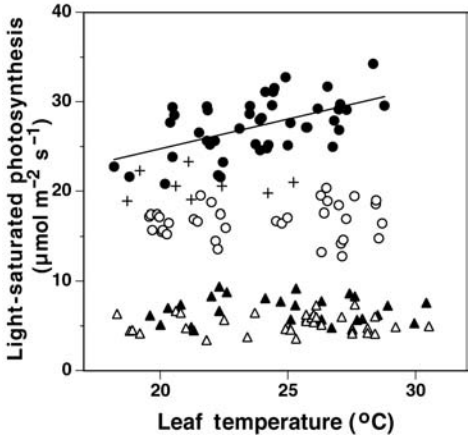

Figure 3. Relationships between light-saturated photosynthesis, measured at the growth [CO_2], and leaf temperature for maple (▲, △) and aspen (●, ○) in OTCs ventilated with ambient (open symbols) or CO_2-enriched (solid symbols) air. For aspen, data from chamberless plots are also presented (+). For maple, data for chamberless trees and trees in OTCs ventilated with ambient air, which were statistically indistinguishable, are all presented as open triangles. For clarity, defoliation treatments are not highlighted. In OTCs with CO_2-enriched air, aspen photosynthesis was significantly and positively correlated with leaf temperature (T_{leaf}; °C) across defoliation treatments and harvests ($A_{area} = 11.4 + 0.67T_{leaf}$, $r^2 = 0.32$, $P = 0.0007$).

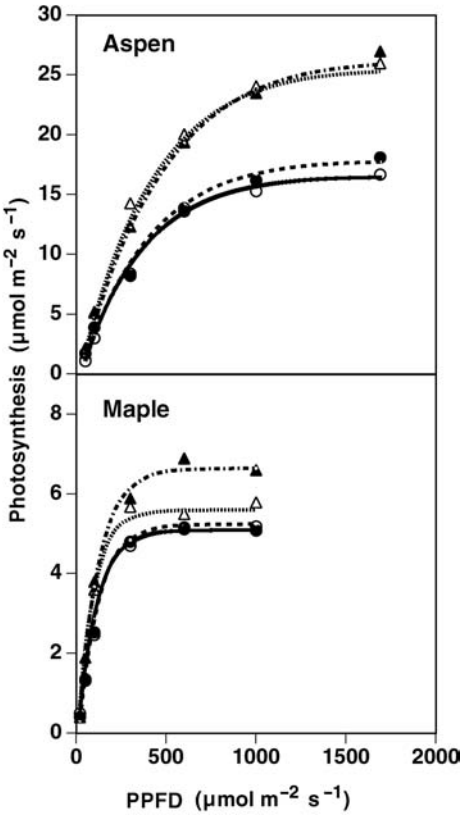

Figure 4. Representative leaf, photosynthetic light response curves for defoliated (solid symbols) and non-defoliated (open symbols) maple and aspen in OTCs with ambient (●, ○) and CO_2-enriched (▲, △) air. Curves, drawn only for illustrative purposes, are based on treatment means calculated from all measurements at a particular PPFD pooled across the late June and early August campaigns. Data were obtained from recently mature leaves of trembling aspen and first-flush leaves of sugar maple.

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 [CO_2] than in ambient [CO_2]. Across CO_2 treatments, the 17–18% reduction in mean LMR of defoliated maples led to a similar decrease in $A_{seedling,av}$.

For aspen, elevated [CO_2] 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 CO_2 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 CO_2-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 [CO_2] caused changes in leaf chemistry of both defoliated and non-defoliated maples (Table 4), including decreases in area-based N conconcentration ($P = 0.04$ 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 CO_2-mediated increases in total nonstructural carbohydrates (TNC), we recalculated [N] in the absence of TNC. At neither harvest was there a significant effect of CO_2 treatment on "adjusted" [N] (Table 4).

For aspen, there was no significant CO_2 effect on area-based leaf [N]. However, mass-based leaf [N] was 14–18% less in seedlings in elevated [CO_2] 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 [CO_2] at the first harvest. However, in August, the only significant effect of CO_2 enrichment on leaf carbohydrates was a relatively small increase in [sugar]. Comparisons of treatment variation in "adjusted" [N]

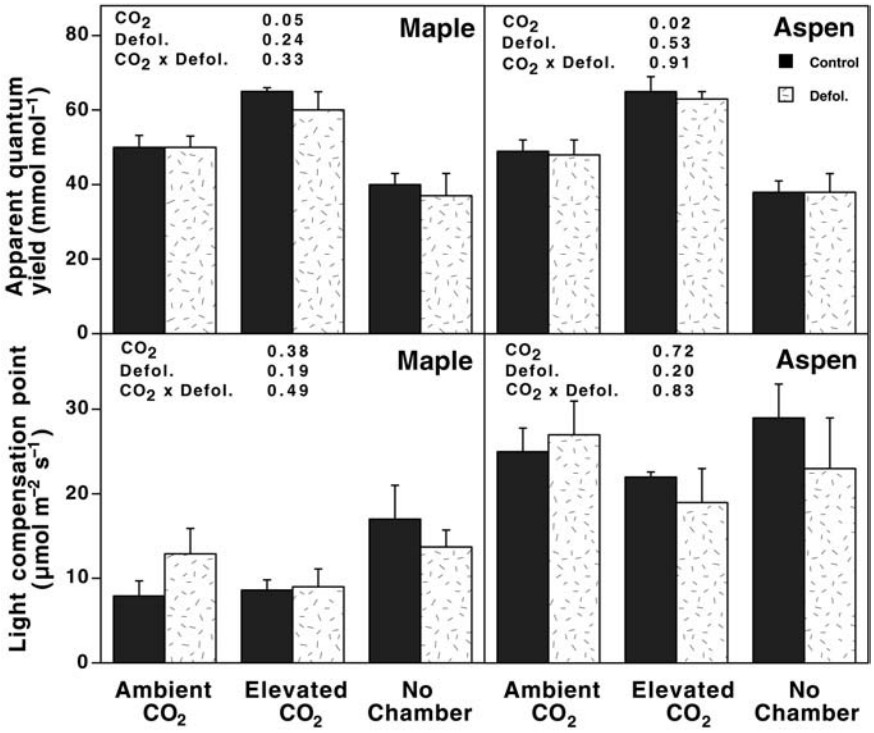

Figure 5. Components of the photosynthetic response of maple and aspen foliage to variation in incident PPFD, including apparent quantum yield and light compensation point. Columns represent treatment means with standard errors of $n = 3$ and $n = 2$ OTC and chamberless plot means, respectively. Each OTC mean is itself a mean of measurements conducted at both the June and August harvests. Leaf PPFD responses were measured on chamberless plots only at the August harvest. Only data from first-flush maple and recently mature aspen leaves are presented. The *P*-values for treatment main effects and their interaction are provided in each panel, based on a mixed-effects linear model performed only for OTC data. Abbreviation: Defol. = defoliation.

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_{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. Lovelock 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 invoved 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

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.

Harvest	Measure	Measurement $[CO_2]$	Ambient $[CO_2]$		Elevated $[CO_2]$		*P*-Values		
			Control	Defol.	Control	Defol.	CO_2	Defol.	$[CO_2]$ × Defol.
Sugar maple									
June	Photosynthesis	365	4.5 (0.5)	5.2 (0.3)	2.7 (0.6)	3.6 (0.1)	0.001	0.05	0.85
		653	7.9 (0.9)	8.4 (0.2)	4.7 (0.4)	6.3 (0.3)	0.006	0.04	0.24
	Conductance	365	83 (9)	96 (3)	51 (9)	64 (2)	0.001	0.07	0.99
		653	74 (8)	82 (1)	47 (5)	61 (5)	0.01	0.03	0.45
	C_i/C_a	365	0.67 (0.01)	0.67 (0.01)	0.71 (0.03)	0.69 (0.03)	0.25	0.18	0.48
		653	0.65 (0.03)	0.66 (0.03)	0.67 (0.02)	0.66 (0.03)	0.52	0.38	0.17
	PNUE	365	4.5 (0.1)	5.1 (0.2)	3.3 (0.8)	4.0 (0.5)	0.08	0.02	0.83
August	Photosynthesis	367	5.7 (0.7)	5.3 (0.4)	3.5 (0.4)	4.0 (0.4)	0.02	0.97	0.35
		655	8.9 (1.4)	9.5 (1.2)	6.5 (0.9)	7.0 (0.8)	0.10	0.57	0.98
	Conductance	367	126 (15)	126 (7)	85 (4)	87 (6)	0.03	0.80	0.88
		655	109 (14)	121 (11)	87 (3)	87 (8)	0.06	0.38	0.38
	C_i/C_a	367	0.72 (0.02)	0.74 (0.01)	0.75 (0.02)	0.73 (0.01)	0.48	0.99	0.11
		655	0.72 (0.03)	0.73 (0.01)	0.75 (0.03)	0.73 (0.03)	0.58	0.76	0.55
	PNUE	367	4.9 (0.6)	5.1 (0.5)	3.7 (0.3)	4.3 (0.2)	0.06	0.38	0.65
Trembling aspen									
June	Photosynthesis	365	17.0 (0.7)	18.7 (0.3)	14.9 (0.9)	17.8 (1.4)	0.11	0.006	0.28
		653	22.7 (1.8)	26.2 (1.4)	25.2 (1.4)	29.1 (1.1)	0.004	0.0005	0.76
	Conductance	365	715 (55)	895 (92)	552 (21)	639 (97)	0.02	0.11	0.55
		653	666 (93)	781 (38)	586 (14)	728 (57)	0.37	0.03	0.77
	C_i/C_a	365	0.74 (0.02)	0.75 (0.02)	0.77 (0.02)	0.74 (0.02)	0.99	0.24	0.14
		653	0.81 (0.02)	0.81 (0.02)	0.76 (0.02)	0.75 (0.01)	< 0.0001	0.34	0.67
	PNUE	365	15.1 (0.3)	16.9 (1.4)	16.1 (0.9)	16.4 (0.4)	0.81	0.27	0.43
August	Photosynthesis	367	16.3 (1.1)	17.5 (0.4)	16.3 (0.5)	15.5 (0.9)	0.09	0.77	0.10
		655	27.0 (1.7)	25.0 (1.4)	26.8 (1.6)	24.9 (1.3)	0.79	0.01	0.95
	Conductance	367	479 (57)	538 (33)	440 (15)	442 (19)	0.21	0.28	0.31
		655	518 (28)	532 (33)	438 (15)	450 (19)	0.07	0.43	0.94
	C_i/C_a	367	0.71 (0.02)	0.71 (0.02)	0.71 (0.02)	0.72 (0.02)	0.67	0.15	0.42
		655	0.74 (0.02)	0.77 (0.01)	0.72 (0.01)	0.75 (0.01)	0.10	0.02	0.91
	PNUE	367	12.4 (1.0)	13.1 (1.1)	13.0 (0.7)	13.1 (1.1)	0.92	0.29	0.34

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

Table 3. Simulated means for photosynthesis per unit leaf area ($A_{area,av}$), leaf mass ($A_{mass,av}$) and seedling mass ($A_{seedling,av}$) in OTCs ventilated with ambient and CO_2-enriched air, as well as in chamberless plots. Also included is the percent difference between values in OTCs ventilated with ambient and CO_2-enriched air. Values are means of treatment-level estimates generated at the June and August harvests. Abbreviations: $A_{mass,av} = A_{area,av}$SLA and $A_{seedling,av} = A_{mass,av}$LMR.

Variable	Species	Defoliation treatment	Ambient [CO_2]	Elevated [CO_2]	% Difference	Chamberless
$A_{area,av}$ ($\mu mol\ m^{-2}\ s^{-1}$)	Maple	Control	3.2	4.0	25	4.0
		Defoliated	3.2	4.1	28	4.2
	Aspen	Control	6.5	8.8	35	9.5
		Defoliated	7.0	9.6	37	10.3
$A_{mass,av}$ ($nmol\ g^{-1}\ s^{-1}$)	Maple	Control	52.8	61.8	17	69.6
		Defoliated	54.0	64.5	19	71.2
	Aspen	Control	153	181	18	189
		Defoliated	164	206	26	200
$A_{seedling,av}$ ($nmol\ g^{-1}\ s^{-1}$)	Maple	Control	19.2	23.3	21	27.9
		Defoliated	14.7	18.3	24	22.3
	Aspen	Control	78.4	87.7	12	93.5
		Defoliated	69.2	82.5	19	82.7

et al. 2000). Sugar maple, on the other hand, has behaved less predictably, being very sensitive to atmospheric CO_2 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 NO_3^- availability (Kinney and Lindroth 1997).

We found that a decrease in photosynthetic capacity of maple negated much of the potential benefit of increased CO_2 availability for mean photosynthesis. Decreases in SLA further diminished the CO_2 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 [CO_2]. In addition, the potential benefit of enhanced light-saturated photosynthesis in response to atmosphere CO_2 enrichment was not fully realized because our aspen leaves were mostly exposed to subsaturating PPFDs (< 400 $\mu mol\ m^{-2}\ s^{-1}$). Nevertheless, photosynthesis was stimulated to a lesser extent in elevated [CO_2] during periods of low PPFD (above light compensation point) than during periods of high PPFD because of the increase in apparent quantum yield.

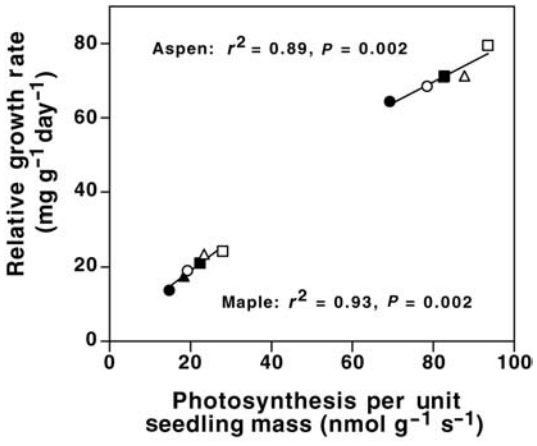

Figure 6. Relationships between RGR and simulated mean photosynthesis per unit seedling mass during the 40-day period following defoliation in late June. Symbols represent means (with + 1 SE) for defoliated (open symbols) and non-defoliated (solid symbols) treatments in OTCs ventilated with ambient (circles) and CO_2-enriched (triangles) air, and chamberless plots (squares). Separate regression analyses (based on $n = 6$ treatment means) were conducted for each species.

Based on our simulations, mean maple photosynthesis in elevated [CO_2] benefited almost exclusively from a parallel increase in apparent quantum yield. Estimated differences in mean photosynthesis between CO_2 treatments were greater at subsaturating PPFDs (< 200 $\mu mol\ m^{-2}\ s^{-1}$) 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 [CO_2], particularly when photosynthetic capacity is reduced.

The mechanism underlying the decreases in photosynthetic capacity of maple and other field-grown trees exposed to elevated [CO_2] (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 [CO_2] coincided with leaf starch accumulation, an indicator of increased

Table 4. Carbohydrate and nitrogen concentrations of first-flush sugar maple leaves and recently mature trembling aspen leaves in OTCs at the June and August harvests. Adjusted [N] ($[N]_{Adjusted}$) is a recalculation of leaf nitrogen concentration in the absence of total nonstructural carbohydrates (TNC). Sugar concentrations include sucrose and hexoses. Values are treatment means (with 1 SE in parentheses; $n = 4$ OTC means). The *P*-values are based on mixed-effects models conducted separately for each species.

Measure	Harvest	Ambient $[CO_2]$		Elevated $[CO_2]$		*P*-Values		
		Control	Defoliated	Control	Defoliated	CO_2	Defol.	$[CO_2] \times$ Defol.
Sugar maple								
$[N]_{area}$ (g m^{-2})	June	0.99 (0.12)	1.01 (0.07)	0.86 (0.05)	0.93 (0.06)	0.31	0.48	0.67
	August	1.16 (0.01)	1.04 (0.03)	0.96 (0.04)	0.94 (0.07)	0.04	0.43	0.10
$[N]_{mass}$ (%)	June	1.67 (0.17)	1.71 (0.10)	1.40 (0.07)	1.51 (0.11)	0.13	0.47	0.70
	August	1.81 (0.06)	1.68 (0.03)	1.41 (0.03)	1.40 (0.06)	0.008	0.08	0.11
$[N]_{Adjusted}$ (%)	June	1.96 (0.21)	1.97 (0.14)	1.69 (0.10)	1.88 (0.14)	0.33	0.50	0.54
	August	2.03 (0.06)	1.89 (0.05)	1.79 (0.04)	1.83 (0.12)	0.12	0.51	0.23
[Starch] (%)	June	5.7 (0.6)	4.6 (0.8)	8.6 (0.8)	10.0 (2.4)	0.01	0.93	0.37
	August	2.3 (0.4)	1.8 (0.3)	11.2 (1.5)	14.1 (2.0)	< 0.0001	0.36	0.20
[Sugars] (%)	June	9.0 (0.8)	8.5 (0.9)	8.8 (0.7)	8.5 (0.8)	0.95	0.64	0.92
	August	8.6 (0.3)	9.8 (1.0)	9.9 (0.8)	8.7 (1.7)	0.93	0.96	0.29
[TNC] (%)	June	14.7 (1.0)	13.1 (1.6)	17.4 (1.4)	19.7 (2.5)	0.02	0.85	0.28
	August	10.9 (0.5)	11.3 (0.9)	21.1 (1.6)	22.8 (3.6)	0.0002	0.62	0.76
[Hexose]/[Sucrose]	June	1.26 (0.26)	1.48 (0.50)	1.74 (0.65)	1.31 (0.27)	0.80	0.72	0.32
	August	1.93 (0.77)	1.60 (0.36)	2.01 (0.48)	1.68 (0.43)	0.90	0.55	0.99
Trembling aspen								
$[N]_{area}$ (g m^{-2})	June	1.13 (0.05)	1.13 (0.11)	0.95 (0.07)	1.09 (0.06)	0.29	0.10	0.12
	August	1.32 (0.05)	1.27 (0.09)	1.26 (0.08)	1.20 (0.10)	0.50	0.43	0.93
$[N]_{mass}$ (%)	June	2.90 (0.14)	2.90 (0.20)	2.22 (0.15)	2.56 (0.15)	0.02	0.15	0.14
	August	2.67 (0.21)	2.80 (0.05)	2.41 (0.10)	2.32 (0.10)	0.04	0.40	0.89
$[N]_{Adjusted}$ (%)	June	3.96 (0.25)	3.69 (0.31)	3.30 (0.19)	3.45 (0.22)	0.05	0.62	0.13
	August	3.45 (0.03)	3.48 (0.24)	3.25 (0.08)	3.07 (0.10)	0.03	0.52	0.42
[Starch] (%)	June	5.7 (1.6)	1.8 (0.3)	10.7 (1.9)	4.7 (1.3)	0.05	0.002	0.31
	August	1.1 (0.2)	2.1 (1.3)	3.8 (2.3)	1.6 (0.4)	0.49	0.63	0.22
[Sugars] (%)	June	21.9 (0.9)	19.5 (0.9)	22.2 (1.0)	21.1 (1.2)	0.23	0.01	0.24
	August	17.9 (1.3)	21.3 (0.8)	21.9 (1.5)	22.6 (1.5)	0.01	0.03	0.15
[TNC] (%)	June	26.6 (2.0)	21.3 (1.2)	32.9 (0.9)	25.8 (1.7)	0.06	0.0006	0.37
	August	19.0 (1.3)	23.4 (1.2)	25.6 (3.4)	24.2 (1.8)	0.18	0.40	0.12
[Hexose]/[Sucrose]	June	0.55 (0.01)	0.45 (0.01)	0.57 (0.10)	0.47 (0.07)	0.81	0.006	0.97
	August	0.55 (0.07)	0.56 (0.04)	0.58 (0.07)	0.58 (0.04)	0.73	0.82	0.90

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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$. Our premise was that a 50% reduction in leaf area would preclude development of an assimilate surplus in response to atmospheric CO_2 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 CO_2 enrichment affected leaf carbohydrate concentrations, but PNUE appeared to be insensitive to these perturbations. Photosynthetic acclimation to elevated $[CO_2]$ 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 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 CO_2 treatments (Figure 3). Current biochemical models predict that C_3 photosynthesis becomes more responsive to variation in leaf temperature as atmospheric $[CO_2]$ increases as a result of the inhibitory effect of elevated

[CO_2] 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 C_i 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 [CO_2].

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 CO_2 responses of plants grown in isolation can differ markedly from those of plants with neighbors (Bazzaz and McConnaughay 1992, Wayne and Bazzaz 1995, 1997, Catovsky 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 CO_2 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 CO_2 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 CO_2-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 [CO_2] 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 CO_2 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 CO_2 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 [CO_2]. 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 CO_2 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 CO_2 enrichment primarily to the absence of an appreciable CO_2 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 CO_2 availability (in aspen). Although these limitations pertain to the behavior of seedlings during exposure to elevated [CO_2] for one growing season, they may reflect important constraints on forest growth responses to CO_2-enriched environments.

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