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Leaf respiration at different canopy positions in sweetgum (*Liquidambar styraciflua*) grown in ambient and elevated concentrations of carbon dioxide in the field

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Summary Trees exposed to elevated CO_2 partial pressure ($[CO_2]$) generally show increased rates of photosynthesis and growth, but effects on leaf respiration are more variable. The causes of this variable response are unresolved. We grew 12-year-old sweetgum trees (*Liquidambar styraciflua* L.) in a Free-Air CO_2 Enrichment (FACE) facility in ambient $[CO_2]$ (37/44 Pa daytime/nighttime) and elevated $[CO_2]$ (57/65 Pa daytime/nighttime) in native soil at Oak Ridge National Environmental Research Park. Nighttime respiration (R_N) was measured on leaves in the upper and lower canopy in the second (1999) and third (2000) growing seasons of CO_2 fumigation. Leaf respiration in the light (R_L) was estimated by the technique of Brooks and Farquhar (1985) in the upper canopy during the third growing season. There were no significant short-term effects of elevated $[CO_2]$ on R_N or long-term effects on R_N or R_L, when expressed on an area, mass or nitrogen (N) basis. Upper-canopy leaves had 54% higher R_N (area basis) than lower-canopy leaves, but this relationship was unaffected by CO_2 growth treatment. In August 2000, R_L was about 40% of R_N in the upper canopy. Elevated $[CO_2]$ significantly increased the number of leaf mitochondria (62%), leaf mass per unit area (LMA; 9%), and leaf starch (31%) compared with leaves in ambient $[CO_2]$. Upper-canopy leaves had a significantly higher number of mitochondria (73%), N (53%), LMA (38%), sugar (117%) and starch (23%) than lower-canopy leaves. Growth in elevated $[CO_2]$ did not affect the relationships (i.e., intercept and slope) between R_N and the measured leaf characteristics. Although no factor explained more than 45% of the variation in R_N, leaf N and LMA were the best predictors for R_N. Therefore, the response of R_N to CO_2 treatment and canopy position was largely dependent on the magnitude of the effect of elevated $[CO_2]$ or canopy position on these characteristics. Because elevated $[CO_2]$ had little or no effect on N or LMA, there was no effect on R_N. Canopy position had large effects on these leaf characteristics, however, such that upper-canopy leaves exhibited higher R_N than lower-canopy leaves. We conclude that elevated $[CO_2]$ does not directly impact leaf respiration in sweetgum and that barring changes in leaf nitrogen or leaf chemical composition, long-term effects of elevated $[CO_2]$ on respiration in this species will be minimal.

Keywords: carbohydrates, cytochrome c oxidase, daytime respiration, forest trees, Free-Air CO_2 Enrichment (FACE), nighttime respiration, number of mitochondria.

Introduction

Terrestrial plant photosynthesis and respiration are key components of the global carbon cycle (Amthor 1995). The magnitude of carbon fluxes between the atmosphere and terrestrial biosphere are large, because photosynthesis assimilates about 120 Pg C $year^{-1}$, whereas plant respiration and soil respiration each release about 60 Pg C $year^{-1}$ to the atmosphere (Amthor 1995). Therefore, it is important to examine not only the sensitivity of photosynthesis to elevated CO_2 partial pressure ($[CO_2]$), but also to examine further the less frequently studied and more poorly understood short-term and long-term effects of atmospheric CO_2 enrichment on leaf respiration (Amthor 1991, Wullschleger et al. 1994).

Stimulatory effects of elevated $[CO_2]$ on net photosynthesis have been frequently and clearly demonstrated (Saxe et al. 1998, Norby et al. 1999, Curtis et al. 2000), as well as mechanistically explained in large part by the direct effect of elevated $[CO_2]$ on Rubisco kinetics (Farquhar and Sharkey 1982). However, the effects of elevated $[CO_2]$ on leaf respiration are less well understood, particularly the mechanisms that regulate respiratory responses to changes in atmospheric $[CO_2]$. In some studies, a short-term effect of elevated $[CO_2]$ on dark respiration has been observed, in which respiration rates are immediately and significantly reduced (Amthor et al.

1992, Thomas and Griffin 1994, Drake et al. 1999, Baker et al. 2000, Hamilton et al. 2001). However, in other studies, an immediate suppression of dark respiration by elevated [CO_2] was not observed (Tjoelker et al. 1999, Amthor 2000, Amthor et al. 2001, Jahnke 2001, Burton and Pregitzer 2002). To date, there is no mechanism to explain a direct suppression of dark respiration by elevated [CO_2], although the role of cytochrome c oxidase has been explored (Gonzalez-Meler et al. 1996, Gonzalez-Meler and Siedow 1999).

Long-term effects on respiration may occur after extended growth in elevated [CO_2] and may be mediated through effects of elevated [CO_2] on growth rate, nonstructural carbohydrate concentration and tissue composition. Contrasting results, in which elevated [CO_2] was shown to increase (Thomas and Griffin 1994, Wang et al. 2001), decrease (Wullschleger et al. 1992, Bunce and Ziska 1996), or have no effect on (Lewis et al. 1999, Hamilton et al. 2001) leaf respiration have confounded efforts to predict long-term plant response to atmospheric CO_2 enrichment. Further complications arose because the direction of long-term leaf respiratory response to elevated [CO_2] depended on whether respiration was calculated on a leaf area or leaf mass basis (Poorter et al. 1992). A meta-analysis of leaf respiratory responses to elevated [CO_2] (Wang and Curtis 2002) indicated that leaf respiration on a mass basis was significantly reduced (18%), but was not significantly affected on an area basis, when all plants were included in the analysis. Woody plants showed no significant respiratory response to elevated [CO_2] on either an area or a mass basis (Wang and Curtis 2002). Caution should be exercised, however, when interpreting meta-analyses because studies that may have relied on a flawed methodology (e.g., lack of correction for diffusion errors in the gas exchange system) are equally weighted with correctly conducted studies.

Several explanations for the differential long-term respiratory response in leaves have been suggested. For example, growth in elevated [CO_2] may reduce leaf nitrogen and protein concentrations, and increase leaf carbohydrate concentrations, all of which may affect leaf respiration rates (Amthor 1991). Confounding these effects, leaf biochemistry may also vary with leaf age and position in the canopy (Tissue et al. 2001). And, because most measurements are area-based, respiration rates are sensitive to increased leaf mass per unit area, which is often associated with growth in elevated [CO_2]. Recent studies also indicate that an increased number of mitochondria per unit cell area for plants grown in elevated [CO_2] may affect respiratory responses to elevated [CO_2] (Robertson et al. 1995, Griffin et al. 2001*a*). Additionally, it has been proposed that elevated [CO_2] may directly affect respiration through effects on the activity of mitochondrial enzymes, such as cytochrome c oxidase, which may be reduced in plants grown in elevated [CO_2] (Azcon-Bieto et al. 1994, Gonzalez-Meler and Siedow 1999).

In this study, we measured the effects of elevated [CO_2] and canopy position on leaf respiration at night (R_N) in sweetgum trees in a closed canopy forest. We measured cell ultrastructure (mitochondrial number per unit area of cell) and biochemistry (cytochrome c oxidase activity) in leaves at different canopy positions during different seasonal growth periods to provide a more mechanistic explanation for the dark respiratory response to growth in elevated [CO_2]. We also estimated leaf respiration in the light (R_L) to determine whether elevated [CO_2] affected daytime respiration. Our overall objective was to determine if, and why, enriched atmospheric [CO_2] might affect leaf respiration in sweetgum.

Materials and methods

Study site

Research was conducted in the Oak Ridge National Environmental Research Park at the Free-Air Carbon Dioxide Enrichment (FACE) facility in a plantation of sweetgum (*Liquidambar styraciflua* L.) trees in Roane County, Tennessee (35°54′ N, 84°20′ W). One-year-old sweetgum seedlings were planted in 1988 at a spacing of 2.3 × 1.2 m in a total area of 1.7 ha. During our study period in 1999 and 2000, the sweetgum trees were 14–16 m in height in a closed canopy (Norby et al. 2001) with the live canopy extending down to 8–9 m above ground. The CO_2 treatment of the experimental plots was initiated in April 1998, before leaf-out, and was fully functional in mid-May 1998 (Norby et al. 2001). In 1998, the CO_2 treatment set point was a constant 56.5 Pa CO_2, about 20 Pa above the global atmospheric CO_2 partial pressure. The CO_2 enrichment was maintained 24 h per day beginning in the spring before new leaves emerged and terminating after leaf senescence in the fall. In 1999 and 2000, this regime was modified to simulate a natural diurnal pattern of CO_2 partial pressure, with a higher nighttime set point of 64.5 Pa. In 1999 and 2000, the measured mean CO_2 partial pressures in the elevated [CO_2] treatment were 53.9 and 55.0 Pa during the day and 61.5 and 63.2 Pa at night, respectively (Norby et al. 2001). The elevated [CO_2] treatment was maintained in two 25-m-diameter experimental plots. Each plot enclosed about 120 trees and was equipped with a system of vertical pipes (with regularly spaced exit ports along the length) connected to a circular plenum supplied with CO_2 gas by a highly regulated, computer-based control system. Nearby, two control plots of equivalent plant composition had the same arrangement of vertical pipes and blowers, but received only ambient air, which averaged 39.4 Pa CO_2 during the day and 45.4 Pa at night. Additional information about the FACE site and CO_2 control system are described in Norby et al. (2001).

Nighttime leaf respiration rates

Leaf respiration at night (R_N) was measured about 2–4 h after dusk on mature leaves in the lower canopy (8–9 m height; deeply shaded leaves in the lower 10% of canopy) and upper canopy (14–16 m height; fully sunlit leaves in upper 10% of canopy) of the ambient and elevated [CO_2] treatments. Respiration rates typically decline throughout the night as a function of declining leaf temperature and carbohydrate availability. Here, R_N measurements at 2–4 h after dusk represent the average R_N for sweetgum at the FACE site, as has been demon-

strated at an adjacent field site (Amthor 2000). We selected four trees per plot (i.e., eight trees per CO_2 treatment), one branch per tree and two leaves per branch for measurements in both the lower and upper canopy. Lower- and upper-canopy leaves were measured in early season (May) and mid-season (July) in 2000, whereas only upper-canopy leaves were measured in mid-season (July) and late season (August) in 1999 and late season (August) in 2000. Fully expanded leaves attached to detached branches were placed in the cuvette of a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) for measurement of R_N. Inside the cuvette, leaves were maintained at 25 °C, ambient relative humidity and a gas flow rate of 300 μmol s^{-1}. All R_N measurements were conducted within 2 h of detaching branches from the tree. In a preliminary experiment, we found no difference in R_N of leaves on branches that were attached or detached from sweetgum trees over a 2-h period. We minimized the potential effects of CO_2 diffusion between the cuvette and outside environment on R_N by sealing the cuvette gaskets with vacuum grease. In addition, we ran 12 independent leak tests by measuring rates of gas exchange at 10 CO_2 concentrations ranging from 0 to 150 Pa in empty cuvettes. We used these data to develop an individual leak correction factor for each cuvette during each measurement period to correct leaf dark respiration rates to account for leaks.

To determine whether CO_2 had a short-term (direct) effect on respiration, we measured R_N on leaves grown in ambient [CO_2] (nighttime value of 44.5 Pa), but exposed to different CO_2 partial pressures. We measured R_N on leaves exposed to 44.5 Pa CO_2 for 20 min and then exposed to 64.5 Pa CO_2 for 20 min in the cuvette of the LI-6400. The ratio of R_N for leaves measured at elevated [CO_2] (64.5 Pa) compared with R_N measured at ambient [CO_2] (44.5 Pa) indicated the short-term effect of elevated [CO_2] on leaf dark respiration. The degree of reversibility of the short-term effect of CO_2 on R_N was determined by returning the CO_2 partial pressure in the LI-6400 cuvette to the growth CO_2 partial pressure (44.5 Pa) and comparing that R_N with R_N determined at 64.5 Pa CO_2. Long-term CO_2 effects (direct and indirect) were determined by measuring R_N at the growth CO_2 partial pressure (44.5 Pa or 64.5 Pa depending on CO_2 treatment) after 20 min of exposure to the appropriate CO_2 partial pressure. All R_N measurements were stable (i.e., calculated values of the coefficient of variation were less than 1%) over 20 min.

Daytime leaf respiration rates

Leaf respiration rates in the light (R_L; rate of non-photorespiratory CO_2 efflux occurring in the light) were measured on mature leaves in the upper canopy in late season (August) in 2000. We selected four trees per plot (i.e., eight trees per CO_2 treatment), one branch per tree and one leaf per branch for R_L measurements. Based on the technique of Brooks and Farquhar (1985) to calculate R_L, we measured the initial slopes of net photosynthesis on leaves as a function of the intercellular CO_2 partial pressure at four CO_2 partial pressures (7.5, 10, 12.5 and 15 Pa) and two irradiances (1200 and 150 μmol m^{-2} s^{-1}), using the higher irradiance first. The point of intersection of the two slopes was used to determine R_L and Γ* (CO_2 compensation point when R_L = 0). Gas exchange was measured with a Li-Cor LI-6400. Temperature inside the leaf cuvette was maintained at 25 °C, relative humidity was ambient, and we used a gas flow rate of 500 μmol s^{-1}.

Leaf characteristics and biochemistry

Following respiration measurements, leaves were dried at 70 °C, ground to a fine powder and nitrogen was measured with a CN analyzer (NCS 2500, Carlo Erba, Milan, Italy). Soluble sugar and starch were determined colorimetrically by a phenol-sulfuric acid technique (Tissue and Wright 1995). Total nonstructural carbohydrate (TNC) was calculated as the sum of soluble sugar and starch. Leaf mass per unit area (LMA) was determined for leaves used for the respiration measurements. To link any changes in R_N or R_L with changes in key respiratory enzymes, additional leaves were collected for determination of cytochrome c oxidase activity, which is a key component of the mitochondrial electron transport chain. Cytochrome c oxidase extraction and measurement followed Azcon-Bieto et al. (1994). Samples were collected in July and August 2000 from leaves used to measure R_N, immediately frozen in liquid nitrogen and stored until analyzed. Cytochrome c oxidase activity was measured at 25 °C with a liquid-phase oxygen electrode system (Model 10, Rank Brothers, Cambridge, U.K.) and activity was calculated as the difference in oxygen loss rate from the sample solution before and after adding sodium azide.

Number of mitochondria

We counted the mitochondria per cell cross-sectional area to determine whether differences in R_N or R_L might be the result of changes in mitochondrial characteristics. Duplicate samples of leaves were collected from one tree per plot and placed in 2% (w/v) glutaraldehyde in 0.05 M potassium phosphate buffer (pH 7.2). The fixed material was washed in phosphate buffer, and post-fixed for 2 h at 5 °C in 2% (w/v) osmium tetroxide solution in the same phosphate buffer used for the glutaraldehyde fixative. Each sample was dehydrated in a graded series of acetone solutions, infiltrated with catalyzed epon, embedded in fresh catalyzed epon (Energy Beam Sciences, Agawam, MA), polymerized at 65 °C, and sectioned with a Porter-Blum MT-2 ultramicrotome (Ivan Sorvall, Norwalk, CT) fitted with a diamond knife (Griffin et al. 2001*a*). The sections were collected on copper grids, post stained with Reynolds lead citrate and observed with a Philips 201 transmission electron microscope (Einthoven, Netherlands). Random samples of sections of leaf tissues were examined and mitochondria were counted directly from the image on the microscope screen that contained a measuring grid for cell dimensions. In each sample, mitochondria were enumerated and expressed per 100 μm^2 of cell area, based on the quantification of 50 to 250 cells per sample. We counted only intact, structurally complete mitochondria, excluding mitochondria that were enclosed within digestive vacuoles (autophagosomes), which

would indicate that they were senescent and being digested, or nonfunctional. Mitochondrial samples were collected from leaves in the lower and upper canopy in mid-season (July) in 2000 and only from the upper canopy in late season (August) in 1999 and early season (May) and late season (August) in 2000.

Statistical analyses

A three-way analysis of variance (ANOVA) was used to test for the main effects and interactions of CO_2 treatment, canopy position and date on all parameters. In each analysis, plot was nested within each CO_2 treatment and added as a random effect to the model (Underwood 1981). The ANOVAs were performed with the general linear model function of Data Desk (Version 5.0, Data Description, Ithaca, NY). There was one degree of freedom for the main effects of CO_2 and canopy position, and for the date × canopy, CO_2 × canopy and date × CO_2 × canopy interactions. There were two degrees of freedom for plot, and for the plot × canopy and date × plot × canopy interactions. There were four degrees of freedom for the main effect of date and the date × CO_2 interaction and eight degrees of freedom for the date × plot interaction. Significant ($P < 0.05$) effects of plot or its interaction terms or CO_2 × canopy position × date interactions on parameters were not detected, so these results were not presented. An analysis of covariance (ANCOVA) was used to determine whether regression lines were significantly different from each other as a result of CO_2 treatment effects on the relationship between R_N and leaf characteristics. All data were normally distributed.

Results

Leaf respiration—R_N and R_L

The short-term, direct effect of elevated [CO_2] on R_N after the data were corrected for leaf chamber leaks was always less than 3% and not significant ($P = 0.6213$; data not shown). Before applying the empty chamber leak correction factor, however, there was an apparent significant 14% reduction in R_N attributable to a direct effect of elevated [CO_2], indicating that the diffusion of CO_2 from the leaf cuvette to the atmosphere affected the initial calculation of R_N.

There was no significant effect of elevated [CO_2] on R_N when leaves were measured at their respective growth CO_2 partial pressures and calculated on a leaf area, mass or nitrogen basis (Tables 1 and 2). There were significant canopy position effects on R_N, however, as upper-canopy leaves had 54% higher R_N, expressed on a leaf area basis, than lower-canopy leaves, but this relationship was unaffected by the growth CO_2 partial pressure (Tables 1 and 2). There were no differences in R_N, expressed on a leaf mass or nitrogen basis, attributable to canopy position (Tables 1 and 2). There were no significant CO_2 treatment effects on R_L (mean ± SE, $n = 2$ plots; 0.489 ± 0.137 μmol m^{-2} s^{-1} in ambient [CO_2] and 0.299 ± 0.164 μmol m^{-2} s^{-1} in elevated [CO_2]) or on the CO_2 compensation point (mean ± SE, $n = 2$ plots; 5.94 ± 0.30 Pa in ambient [CO_2] and 6.37 ± 0.52 in elevated [CO_2]) of mature upper canopy leaves in August 2000 (Table 1).

Leaf characteristics and biochemistry

Leaves grown in elevated [CO_2] exhibited 9% higher leaf mass per unit area (LMA) than leaves in ambient [CO_2] (Table 3). There was no significant effect of elevated [CO_2] on leaf N on a leaf area or leaf mass basis (Tables 1 and 3), but upper-canopy leaves exhibited higher LMA (38%) and N on a leaf area basis (53%; Tables 1 and 3) than lower-canopy leaves. In May 2000, there were significant CO_2 × canopy position interactions (Table 1) on N on a leaf mass basis, which increased in the lower canopy and decreased in the upper canopy in response to elevated [CO_2] (Table 3). Leaves grown in elevated [CO_2] exhibited significantly higher concentrations of starch (31%) and TNC (18%) on a leaf area basis than leaves in ambient [CO_2] (Tables 1 and 3). Upper-canopy leaves had much higher concentrations of sugar (117%), starch (23%) and TNC (71%) on a leaf area basis than lower-canopy leaves (Tables 1 and 3). Although canopy position had similar effects on leaf carbohydrates on both a mass basis and leaf area basis, leaf carbohydrates, on a mass basis, were not significantly affected by growth in elevated [CO_2] (data not shown). In general, LMA and concentrations of N and carbohydrate increased significantly from early season to late season (Tables 1 and 3). Canopy position and CO_2 treatment did not significantly affect cytochrome c oxidase activity on a fresh mass basis (Tables 1 and 3).

Number of mitochondria

The number of mitochondria per unit leaf area increased 62% in elevated [CO_2] and upper-canopy leaves had 73% more mitochondria than lower-canopy leaves (Tables 1 and 3). The relative increase in the number of mitochondria in response to growth in elevated [CO_2] was greater in the lower canopy (159%) than in the upper canopy (27%). Although elevated [CO_2] and canopy position affected the number of mitochondria, there was no observable change in the size of the mitochondria attributable to these parameters. Therefore, because the mitochondria did not vary in size, the number of mitochondria represent an accurate estimate of changes in mitochondrial densities, as found in previous studies (Griffin et al. 2001*a*). In general, the number of mitochondria increased from early season (May) to mid- and late season (July and August) as the leaves matured (Table 3).

Relationships between R_N and leaf characteristics, biochemistry and number of mitochondria

Leaf nighttime respiration (R_N) was positively correlated with gross respiratory carbon substrate (sugar and starch) concentrations. Leaf soluble sugar concentration explained 25% (ambient [CO_2]) and 18% (elevated [CO_2]) of the variation in R_N (Figure 1A, Table 4), whereas leaf starch concentration accounted for 21% (ambient [CO_2]) and 13% (elevated [CO_2]) of the variation in R_N (Figure 1B, Table 4). Growth at elevated [CO_2] did not affect the slope or intercept of the R_N versus sol-

Table 1. The *P*-values for three-way ANOVA used to test for main effects (date, CO_2 and canopy position) and their interactions for listed leaf parameters (number of mitochondria per 100 μm^2 cell area, cytochrome c oxidase activity, nighttime respiration (R_N), daytime respiration (R_L), CO_2 compensation point, leaf N, leaf mass per unit area (LMA), and concentrations of sugar, starch and total nonstructural carbohydrate (TNC)). There was one degree of freedom for CO_2, canopy, date × canopy and CO_2 × canopy effects, and four degrees of freedom for date and date × CO_2 effects. There were no significant ($P < 0.05$) effects of CO_2 × canopy × date, so these results were not presented. Significant *P*-values are shown in boldface. Abbreviations: NA indicates that *P*-values could not be calculated because of the limited data set.

Parameter	Date	CO_2	Canopy	Date × canopy	Date × CO_2	CO_2 × canopy
No. of mitochondria	**0.0002**	**0.0102**	**0.0037**	NA	0.8405	0.2261
Cytochrome c oxidase activity	0.7698	0.6952	0.7623	NA	0.2081	0.7276
R_N (μmol m^{-2} s^{-1})	**< 0.0001**	0.8099	**< 0.0001**	0.3368	0.5574	0.6384
R_N (μmol kg^{-1} s^{-1})	**< 0.0001**	0.7314	0.8708	0.3084	0.1110	0.6837
R_N (μmol kg N^{-1} s^{-1})	**< 0.0001**	0.6133	0.9038	0.0237	0.3462	0.8696
R_L (μmol m^{-2} s^{-1})	NA	0.3801	NA	NA	NA	NA
CO_2 compensation	NA	0.4810	NA	NA	NA	NA
N (g m^{-2})	**0.0007**	0.6946	**< 0.0001**	0.2611	0.5607	0.3678
N (mg g^{-1})	**< 0.0001**	0.9442	0.3346	**0.0003**	0.0797	**0.0032**
LMA (g m^{-2})	**< 0.0001**	**0.0126**	**< 0.0001**	**0.0002**	**0.0358**	0.2175
Sugar (g m^{-2})	**< 0.0001**	0.0797	**< 0.0001**	**0.0016**	0.3109	0.0538
Starch (g m^{-2})	< **0.0001**	**0.0075**	**0.0435**	**0.0003**	**0.0075**	0.3375
TNC (g m^{-2})	**< 0.0001**	**0.0044**	**< 0.0001**	**< 0.0001**	**0.0122**	0.0714

uble sugar or starch regression. Leaf N concentration and LMA were also positively correlated with R_N and were better predictors of R_N than carbon substrate concentration. Leaf N concentration explained 38% of the variation in R_N in plants grown in ambient [CO_2] and 25% in plants grown in elevated [CO_2] (Figure 1C, Table 4). We found that LMA was the best predictor of R_N, accounting for 45% (ambient [CO_2]) and 33% (elevated [CO_2]) of the variation in R_N (Figure 1D, Table 4). The CO_2 treatments did not affect the slope or intercept of the regressions. Leaf ultrastructure (number of mitochondria per unit cell area; Figure 1E) and biochemistry (cytochrome c oxidase activity; Figure 1F) were not correlated with R_N, explaining 4% or less of the variation in R_N regardless of CO_2 treatment (Table 4). Similarly, R_L was not correlated with the number of mitochondria or cytochrome c oxidase activity (data not shown).

Overall, elevated [CO_2] significantly increased the number of mitochondria, LMA, starch and TNC of sweetgum leaves (Figure 2). Leaves in the upper canopy had higher numbers of mitochondria, R_N (area basis), N (area basis), LMA and carbohydrates than leaves in the lower canopy (Figure 3).

Discussion

During the second and third growing seasons of exposure of sweetgum trees to elevated [CO_2] in a FACE facility, leaf

Table 2. Effects of CO_2 treatment (ambient (Amb) and elevated (Elev)) and canopy position (upper and lower) on leaf nighttime respiration (R_N) of sweetgum measured over a 2-year period and expressed on an area, mass and nitrogen basis. Values are presented as means (± 1 SE) ($n = 2$ plots per CO_2 treatment) for the five measurement periods. Abbreviation: NA = data not available.

Parameter	CO_2	Canopy	July 1999	Aug 1999	May 2000	July 2000	Aug 2000
R_N (μmol m^{-2} s^{-1})	Amb	Upper	1.29 (0.06)	1.11 (0.06)	1.04 (0.10)	1.03 (0.06)	1.00 (0.05)
	Elev	Upper	1.44 (0.09)	1.11 (0.06)	1.13 (0.07)	1.01 (0.03)	0.99 (0.06)
	Amb	Lower	NA	NA	0.68 (0.04)	0.53 (0.07)	NA
	Elev	Lower	NA	NA	0.71 (0.05)	0.57 (0.04)	NA
R_N (μmol kg^{-1} s^{-1})	Amb	Upper	12.70 (0.67)	9.80 (0.49)	13.14 (0.98)	10.68 (0.44)	9.73 (0.67)
	Elev	Upper	12.96 (0.70)	9.05 (0.41)	14.38 (1.12)	8.76 (0.34)	8.18 (0.42)
	Amb	Lower	NA	NA	12.96 (0.86)	8.67 (0.75)	NA
	Elev	Lower	NA	NA	14.79 (1.09)	8.91 (0.66)	NA
R_N (μmol kg N^{-1} s^{-1})	Amb	Upper	710 (41)	512 (23)	664 (90)	647 (25)	549 (46)
	Elev	Upper	846 (53)	500 (27)	715 (54)	630 (33)	542 (33)
	Amb	Lower	NA	NA	665 (45)	457 (36)	NA
	Elev	Lower	NA	NA	702 (60)	514 (40)	NA

Table 3. Effects of CO_2 treatment (ambient (Amb) and elevated (Elev)) and canopy position (upper and lower) on leaf characteristics (nitrogen, leaf mass per unit area, sugar, starch, total nonstructural carbohydrates, cytochrome c oxidase activity and number of mitochondria) of sweetgum measured over a 2-year period. Values are presented as means (± 1 SE) (n = 2 plots per CO_2 treatment) for the five measurement periods. Abbreviation: NA = data not available.

Parameter	CO_2	Canopy	July 1999	Aug 1999	May 2000	July 2000	Aug 2000
Leaf N (g m^{-2})	Amb	Upper	1.84 (0.05)	2.17 (0.07)	1.74 (0.16)	1.60 (0.07)	1.94 (0.10)
	Elev	Upper	1.72 (0.05)	2.23 (0.09)	1.62 (0.06)	1.65 (0.07)	1.86 (0.09)
	Amb	Lower	NA	NA	1.03 (0.04)	1.12 (0.08)	NA
	Elev	Lower	NA	NA	1.05 (0.06)	1.12 (0.04)	NA
Leaf N (mg g^{-1})	Amb	Upper	17.99 (0.22)	19.20 (0.61)	22.43 (2.04)	16.55 (0.41)	18.15 (0.44)
	Elev	Upper	15.55 (0.47)	18.19 (0.45)	20.11 (0.50)	14.20 (0.53)	15.43 (0.58)
	Amb	Lower	NA	NA	19.62 (0.53)	19.11 (0.72)	NA
	Elev	Lower	NA	NA	21.54 (0.76)	17.51 (0.59)	NA
LMA (g m^{-2})	Amb	Upper	102.6 (2.9)	113.5 (2.5)	77.9 (1.7)	95.9 (2.9)	106.5 (4.0)
	Elev	Upper	110.7 (1.0)	123.3 (3.3)	81.7 (3.3)	117.3 (3.9)	121.1 (4.2)
	Amb	Lower	NA	NA	52.9 (2.1)	58.9 (2.9)	NA
	Elev	Lower	NA	NA	49.0 (2.2)	64.0 (1.4)	NA
Sugar (g m^{-2})	Amb	Upper	8.06 (0.52)	12.46 (0.34)	6.51 (0.47)	11.81 (0.47)	12.13 (0.74)
	Elev	Upper	9.31 (0.43)	12.95 (0.52)	8.20 (0.43)	15.73 (0.50)	13.83 (0.60)
	Amb	Lower	NA	NA	1.49 (0.08)	5.58 (0.47)	NA
	Elev	Lower	NA	NA	1.26 (0.10)	5.59 (0.34)	NA
Starch (g m^{-2})	Amb	Upper	4.08 (0.29)	5.75 (0.76)	3.80 (0.41)	5.52 (0.33)	5.94 (0.42)
	Elev	Upper	6.44 (0.41)	6.44 (0.72)	4.30 (0.55)	9.44 (0.81)	8.55 (0.77)
	Amb	Lower	NA	NA	3.20 (0.21)	2.68 (0.16)	NA
	Elev	Lower	NA	NA	2.61 (0.23)	4.28 (0.52)	NA
TNC (g m^{-2})	Amb	Upper	12.15 (0.59)	18.22 (0.88)	10.31 (0.79)	17.33 (0.56)	18.07 (0.90)
	Elev	Upper	15.75 (0.61)	19.39 (0.99)	12.50 (0.92)	25.17 (1.01)	22.38 (1.15)
	Amb	Lower	NA	NA	4.69 (0.26)	8.26 (0.59)	NA
	Elev	Lower	NA	NA	3.87 (0.30)	9.87 (0.61)	NA
Cytochrome c oxidase activity (μmol O_2 g_{FW}^{-1} s^{-1})	Amb	Upper	NA	NA	NA	0.38 (0.05)	0.32 (0.07)
	Elev	Upper	NA	NA	NA	0.30 (0.06)	0.41 (0.10)
	Amb	Lower	NA	NA	NA	0.38 (0.05)	NA
	Elev	Lower	NA	NA	NA	0.34 (0.06)	NA
No. of mitochondria (per 100 μm^2 cell area)	Amb	Upper	NA	1.24 (0.20)	6.01 (0.22)	8.29 (0.56)	6.59 (0.15)
	Elev	Upper	NA	2.92 (0.42)	7.25 (0.54)	9.19 (0.32)	8.64 (0.75)
	Amb	Lower	NA	NA	NA	4.76 (0.43)	NA
	Elev	Lower	NA	NA	NA	7.40 (1.39)	NA

nighttime respiration (R_N) was unaffected by the CO_2 treatment. There were no short-term effects of elevated [CO_2] on R_N in sweetgum leaves, which was consistent with our finding that cytochrome c oxidase activity was unaffected by growth in elevated [CO_2], and is consistent with several recent studies (Amthor 2000, Amthor et al. 2001, Jahnke 2001, Tjoelker et al. 2001, Burton and Pregitzer 2002). It has been hypothesized that the reported direct suppression of respiration by elevated [CO_2] is attributable to the inhibition of respiratory enzymes such as succinate dehydrogenase and cytochrome c oxidase (Azcon-Bieto et al. 1994, Gonzalez-Meler et al. 1996, Gonzalez-Meler and Siedow 1999), although this has not been demonstrated in whole plants. A direct suppression of R_N of 7–14% in response to elevated [CO_2] has been observed in sweetgum trees growing in a FACE facility (Hamilton et al. 2001). However, a detailed field study of nine temperate deciduous trees, including sweetgum, found that the direct effect of a doubling of [CO_2] on respiration was not significant (mean decline in R_N of 1.5%), suggesting that the short-term effect of elevated [CO_2] on respiration was unlikely to significantly affect the carbon balance of temperate deciduous forests (Amthor 2000).

Elevated [CO_2] had no long-term effect on R_N in sweetgum on a leaf area or mass basis. In literature reviews, considerable variation has been observed in the response of leaf respiration to growth in elevated [CO_2] (Poorter et al. 1992, Amthor 1994, Curtis and Wang 1998), but a recent meta-analysis (Wang and Curtis 2001) concluded that woody plants were not significantly affected by elevated [CO_2] treatment. Long-term effects of elevated [CO_2] on R_N are generally mediated through changes in tissue composition, carbohydrate concentration, leaf growth rate, and ultrastructure. In our study with

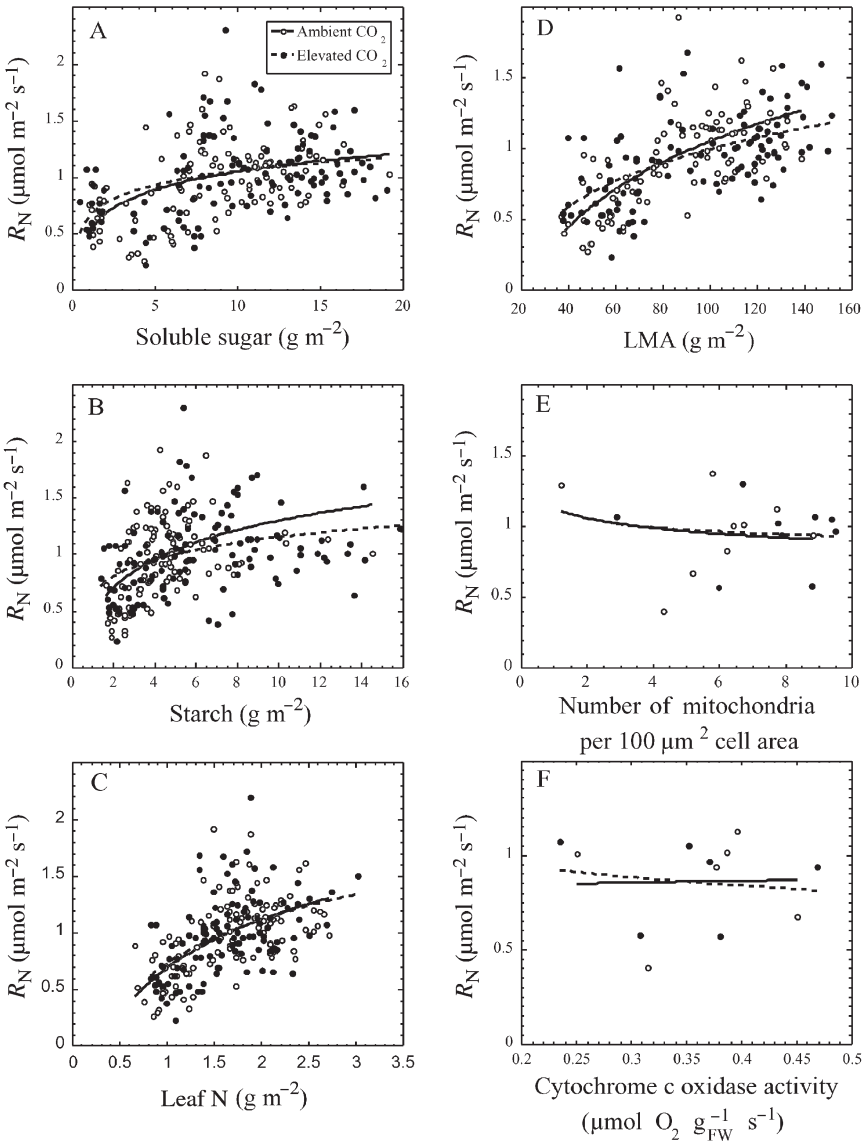

Figure 1. Log function regressions between R_N and (A) leaf soluble sugar, (B) leaf starch, (C) leaf nitrogen, (D) leaf mass per unit area (LMA), (E) number of mitochondria per unit cell area and (F) cytochrome c oxidase activity. Regressions were developed for plants grown in ambient [CO_2] (44 Pa nighttime) and elevated [CO_2] (65 Pa nighttime) treatments. Regression equations and r^2 values for each CO_2 treatment are presented in Table 4.

sweetgum, leaf N and soluble sugar concentrations were unaffected by growth in elevated [CO_2], but leaf starch concentration was increased by 31%. However, leaf starch was not a major determinant of R_N in sweetgum, and its increase was insufficient to affect R_N. Similarly, although LMA increased 9% in response to elevated [CO_2], and was the major determinant of R_N for sweetgum, the change in LMA was too small to significantly affect R_N.

Although the elevated [CO_2] treatment resulted in increased numbers of mitochondria per unit cell area, there was no change in R_N or R_L. It has been hypothesized that the increased number of mitochondria frequently observed in plants grown in elevated [CO_2] is a response to increased energy demand during daylight periods (Griffin et al. 2001*a*). A photosynthetic enhancement of 46–47% at elevated [CO_2] in sweetgum (Gunderson et al. 2002) implies greater cellular energy demand for sugar transport, ribulose bisphosphate (RuBP) regeneration, starch production and other cellular processes, which might be met by increasing the number of mitochondria. Therefore, increased R_L in response to increased numbers of mitochondria could satisfy the energy demands of these more metabolically active cells. Although we did not observe changes in R_L in response to elevated [CO_2], Wang et al. (2001) found that elevated [CO_2] increased R_L in *Xanthium strumarium* L., and that the indirect response of R_N to atmospheric CO_2 enrichment was variable depending on the developmental state. Increased numbers of mitochondria may be linked to processes other than ATP production, such as photorespiration, the glyoxalate cycle, stromal redox state and nitrogen assimilation, that are affected by atmospheric [CO_2] (Foyer and Noctor 2000).

Upper-canopy leaves had much higher R_N than lower-canopy leaves, reflecting greater metabolic activity, probably related to greater light availability and consequently greater photosynthesis (Gunderson et al. 2002) and transport of carbohydrates. In sweetgum, upper-canopy leaves contained 117%

Table 4. Summary of regression equations for the relationships between R_N and leaf characteristics, biochemistry and number of mitochondria (as shown in Figure 1) for leaves of *Liquidambar styraciflua*.

Independent variable	Dependent variable	CO_2	Regression equation	r^2
Soluble sugar ($g\ m^{-2}$)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	0.51 + 0.53 log sugar	0.25
		Elevated	0.64 + 0.41 log sugar	0.18
Starch ($g\ m^{-2}$)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	0.43 + 0.86 log starch	0.21
		Elevated	0.64 + 0.51 log starch	0.13
Leaf N ($g\ m^{-2}$)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	0.68 + 1.43 log leaf N	0.38
		Elevated	0.72 + 1.29 log leaf N	0.25
LMA ($g\ m^{-2}$)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	–2.02 + 1.53 log LMA	0.45
		Elevated	–1.07 + 1.03 log LMA	0.33
No. of mitochondria (per 100 μm^2)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	1.12 – 0.23 log mitos	0.04
		Elevated	1.08 – 0.16 log mitos	0.01
Cytochrome c oxidase activity ($\mu mol\ O_2\ g_{FW}^{-1}\ s^{-1}$)	R_N ($\mu mol\ m^{-2}\ s^{-1}$)	Ambient	0.89 + 0.07 log cyt c	0.01
		Elevated	0.69 – 0.35 log cyt c	0.02

more sugar and 23% more starch than lower-canopy leaves. Greater numbers of mitochondria and higher leaf N also suggest that upper-canopy leaves had greater energy demands and were metabolically more active than lower-canopy leaves. The relationship between leaf N and R_N is indirect and largely dependent on the positive relationship between leaf N and photosynthetic activity, giving rise to greater downstream metabolism. It has been clearly demonstrated that leaf N regulates photosynthesis (Field and Mooney 1986, Peterson et al. 1999), and therefore tree species allocate nitrogen within the canopy to maximize photosynthesis in regions of highest light (Pons and Bergkotte 1996, Kull and Kruijt 1999, Carswell et al. 2000). Increased leaf N is required to support increased rates of Rubisco carboxylation and RuBP regeneration via electron transport associated with increased rates of photosynthesis (Wullschleger 1993). Therefore, lower leaf N observed in the lower canopy may contribute to reductions in respiration with canopy depth in sweetgum, as has been found in

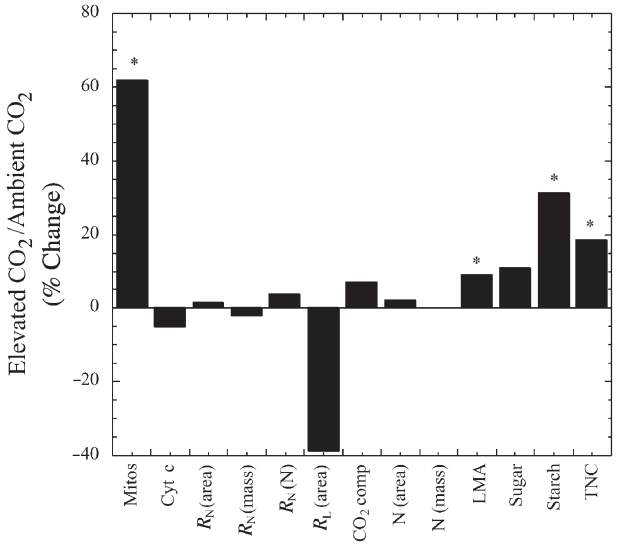

Figure 2. Relative effects of elevated [CO_2] on number of mitochondria per unit cell area (Mitos), cytochrome c oxidase activity (Cyt c), R_N (area basis), R_N (mass basis), R_N (N basis), R_L (area basis), CO_2 compensation point, leaf N (area basis), leaf N (mass basis), leaf mass per unit area (LMA), leaf soluble sugar, leaf starch and leaf total nonstructural carbohydrates (TNC). An asterisk indicates a significant CO_2 effect on a particular parameter.

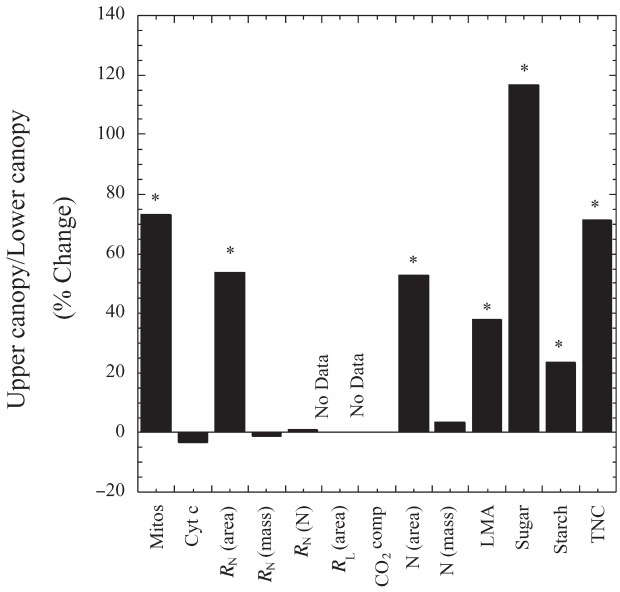

Figure 3. Relative effects of canopy position on number of mitochondria per unit cell area (Mitos), cytochrome c oxidase activity (Cyt c), R_N (area basis), R_N (mass basis), R_N (N basis), R_L (area basis), CO_2 compensation point, leaf N (area basis), leaf N (mass basis), leaf mass per unit area (LMA), leaf soluble sugar, leaf starch and leaf total nonstructural carbohydrates (TNC). An asterisk indicates a significant canopy position effect on a particular parameter.

Nothofagus fusca (Hook. f.) Ørst in New Zealand (Griffin et al. 2001*b*), deciduous temperate forest trees (Ellsworth and Reich 1993, Bolstad et al. 1999), conifers (Bond et al. 1999), and nine tropical rain forest trees in Brazil (Carswell et al. 2000).

Leaf mass per unit area (LMA) declined with depth in the canopy, indicating thinner leaves in the lower canopy, as has been observed in many trees (Hollinger 1996, Apple et al. 2000, Carswell et al. 2000, Griffin et al. 2001*b*, Tissue et al. 2001). The reduction in LMA in the lower canopy eliminated the canopy position effect on R_N on a mass basis, but because light absorption and photosynthetic processes largely regulate R_N, and are area-based, we used area-based estimates to develop relationships between R_N and other parameters. Growth at elevated [CO_2] did not affect the area-based relationship between R_N and leaf soluble sugar, starch, N, LMA, and number of mitochondria or cytochrome c oxidase activity on a fresh mass basis. Elevated [CO_2] had no effect on the intercept of the regressions (i.e., R_N was similar for trees grown in ambient and elevated [CO_2] when they had the same leaf N, LMA, etc.). This finding supports the results of our direct gas exchange measurements that there was no short-term effect of elevated [CO_2] on R_N. In addition, elevated [CO_2] had no effect on the slope of the regressions, indicating that [CO_2] did not affect the relative impact of individual leaf characteristics on R_N. Therefore, differences in R_N between trees in elevated and ambient [CO_2] or between different canopy positions were dependent on the magnitude of the effect of [CO_2] or canopy position on leaf characteristics. Because elevated [CO_2] had little effect on leaf soluble sugar, starch, N or LMA, it had no effect on R_N. Canopy position effects on these leaf characteristics were large, however, such that upper-canopy leaves exhibited higher R_N (area basis) than lower-canopy leaves. Nonetheless, no single parameter explained more than 45% of the variation in R_N, indicating that multiple factors determine R_N.

In summary, long-term exposure to elevated [CO_2] did not affect R_N or R_L in sweetgum trees growing in a plantation in the field. Canopy position affected R_N, partially through effects on leaf soluble sugar, starch, N and LMA, but the effects of canopy position on R_N were unaffected by CO_2 partial pressure. We conclude that elevated [CO_2] does not directly impact leaf respiration in sweetgum and that, barring changes in leaf nitrogen or leaf chemical composition, long-term effects of elevated [CO_2] on respiration in this species will be minimal.

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