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Stem respiration increases in CO_2-enriched sweetgum trees

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Summary

• Stem respiration in trees results in substantial CO_2 release to the atmosphere. Stem respiration is an important component of the carbon budget of forest stands that could be perturbed by CO_2 enrichment of the atmosphere.

• We measured stem respiration in a free-air CO_2 enrichment (FACE) experiment in a 15-yr-old sweetgum (*Liquidambar styraciflua*) plantation. The trees were exposed to ambient or 1.4× ambient [CO_2]. Stem respiration of 12 trees in ambient [CO_2] and 12 trees in CO_2-enriched plots was continuously monitored over a period of 1 year.

• CO_2 enrichment caused a 23% increase in annual growth respiration (R_g) and a 48% increase in maintenance respiration (R_m). During a 4-d period when CO_2 fumigation was turned off, both respiration and stem sucrose concentrations declined in the CO_2-enriched trees. Concentrations of other soluble sugars did not change.

• We conclude that elevated [CO_2] caused stem respiration to increase and that the increase was driven by increased substrate supply from leaves. We suggest that both R_g and R_m rate responses may result from changes in growth rates and live to dead tissue ratios rather than from mechanistic alterations of the respiratory processes.

Key words: stem respiration, free-air CO_2 enrichment (FACE), soluble carbohydrate concentrations, CO_2 enrichment, sucrose, *Liquidambar styraciflua* (sweetgum), growth respiration, maintenance respiration.

Introduction

Stem respiration in deciduous trees results in substantial amounts of photosynthetically fixed CO_2 being released back to the atmosphere. Stem respiration is important because the bulk of the biomass in a mature forest stand is in woody tissue, and respiration in the living cells of this tissue occurs continuously, even when trees are 'dormant'. Woody-tissue respiration is also a good indicator of growth phenology and can be used to calculate growth rates if the respiration required for maintenance is taken into consideration. Woody tissues account for about 25% of the total above-ground autotrophic respiration in some forests such as in northern coniferous sites (Lavigne *et al.*, 1997) and up to 50% of the above-ground autotrophic respiration in some temperate deciduous forests (Edwards *et al.*, 1981). Respiration in plants is generally partitioned into two functional components: growth respiration (R_g) and maintenance (R_m) respiration (McCree, 1970). However, this is an over simplification, because growth and maintenance may share some biochemical reactions, and in fact some respiration may occur without benefit to the plant (i.e. heat and CO_2 are produced with no contribution to growth or maintenance) (Amthor, 2000). The R_g component involves O_2 consumption and CO_2 production resulting from energy production that contributes both directly (biosynthesis) and indirectly (e.g. translocation of substrates used in growth and ion uptake) to the production of new tissues. The R_m component involves gas exchange during energy-requiring processes within living cells, including protein turnover, regulation of ion and metabolite gradients, and physiological adaptation to a changing environment (Penning de Vries, 1975). Partitioning these two components of respiration is important because R_g varies greatly through the year, and rates vary with phenology and environmental factors that control growth. Growth rate may increase as temperatures increase, but R_g per unit of tissue produced is

unaffected by temperature (Penning de Vries *et al.*, 1974). By contrast, R_m varies primarily with changes in temperature, but R_m also increases with increases in relative growth rate (Amthor, 1989; Lavigne & Ryan, 1997). While separation of the components of respiration is important, there is no highly accurate method for doing so. Amthor (2000) provides a comprehensive review of plant respiration with a detailed discussion of R_g and R_m.

Partitioning the relative contribution of R_g and R_m to total respiration, and the uncertainties surrounding the methods for doing so, must be taken into consideration when evaluating the response of stem respiration to environmental variables such as CO_2 enrichment. Our objectives were to quantify the relative amounts of CO_2 released from stems of trees growing in an atmosphere with elevated or ambient [CO_2], to propose mechanisms to explain treatment responses, and to assess the implications of observed treatment differences. Three hypotheses were tested: (1) CO_2 enrichment increases stem respiration by increasing substrate (carbohydrate) availability; (2) stem respiration increases under CO_2 enrichment because of increased stem growth rates; (3) maintenance respiration does not change in response to CO_2 enrichment.

Materials and Methods

Study site

Stem respiration measurements were conducted in a sweetgum (*Liquidambar styraciflua* L.) monoculture plantation on the Oak Ridge National Environmental Research Park in eastern Tennessee, USA. The study site has been described by Norby *et al.* (2001) and environmental monitoring at the site was described by Wullschleger & Norby (2001). The plantation was established in October 1988. Four 25-m diameter plots were laid out in 1997, each of which had about 90 trees within the 20-m diameter measurement area. An additional ambient plot was not used in this respiration study. Exposure to elevated [CO_2] began in April 1998 using a free-air CO_2 enrichment (FACE) system (Hendrey *et al.*, 1999; Norby *et al.*, 2001). When the respiration measurements began in June 2000, the basal area of the plantation was 35 m^2 ha^{-1}, the average tree height was 15 m, and the leaf area index was 6.7. In the two treatment plots the set-point [CO_2] at the top of the canopy was 565 μmol mol^{-1} during the day and 645 μmol mol^{-1} at night, although night exposures were discontinued in 2001 because of interference with soil respiration measurements. The system was turned off during the dormant season (November–March). Average growing season concentrations (June 21–October 31, 2000; April 1–June 20, 2001) are shown in Table 1.

Table 1 Average day and night CO_2 concentrations in the two treatment regimes from 21 June 2000–21 June 2001 (excluding the nongrowing season, November–March)

	CO_2-enriched plots (μmol mol^{-1})	Control plots (μmol mol^{-1})
2000		
Night	633	468
Day	548	389
2001		
Night	450	446
Day	541	393

Measurements

Stem respiration was measured continuously from 21 June 2000–21 June 2001 with an automated, open-flow system. Six trees in each of two treatment plots and six trees in each of two control plots were measured at 1.3 m height. The average diameter of the control trees selected for respiration measurements was 14.0 cm and the average diameter of selected CO_2-enriched trees was 14.3 cm. All trees had canopies that were dominant or codominant.

The automated respiration system comprised sample chambers on the trees, infrared gas analysers (IRGAs), and a manifold and switching system connected by tubing. Aluminum sample chambers were sealed to the north-facing sides of the trees at a height of 1.3 m with a permanently flexible sealant and rubber straps. The chambers were 16.5 cm long, 9.3 cm wide and 5.3 cm deep, covering a bark surface of 153 cm^2. The [CO_2] in air pumped from the sample chambers (sample gas) was compared with the concentration in air from adjacent empty chambers (reference gas) with IRGAs (LI-6252; LiCor, Lincoln, NE, USA) operated in differential mode. Each LI-6252 was connected in tandem to a Li-800 IRGA (Li-Cor), which monitored only reference gas concentrations. The reference gas concentration data were fed directly into the LI-6252. This was necessary to correct for the nonlinear response of the LI-6252 analyser to changing reference gas concentrations. One set of analysers was used to measure stem respiration in the control plots, and another set was used to measure stem respiration in the CO_2-enriched plots. The analysers were switched between control plots and CO_2-enriched plots on two occasions, and no interaction between treatment and analyzer was detectable. The analysers were calibrated at about 2-wk intervals.

The system was checked at regular intervals (every day when possible) for proper operation. Current and previously collected data could be easily accessed by fiber optic cables that were connected to a computer near the study site. The most common problem with the measurements was caused by leaks that occasionally developed between the chambers and the stems of the trees. For example, during storms with high winds and during very rapid diameter growth, when the seal between the chamber and the tree would sometimes be broken. This was generally easy to recognize by carefully examining recently collected data. A leak was indicated by erratic data or unexpectedly low delta [CO_2] values. Checks for leaks were

also performed periodically (usually monthly) by comparing flow rates before and after each chamber. When leaks were detected the data was discarded.

When infrared analysers are operated in the differential mode, it is necessary to maintain equal flow rates and volumes of reference and sample gases. This was accomplished with Model FC260V mass flow controllers (Millipore, Allen, TX, USA) and sample and reference lines (4 mm internal diameter plastic tubing) of equal lengths. The mass flow controllers were calibrated with a soap film bubble calibrator (Gilian Calibrator 2; Sensidyne, Clearwater, FL, USA) to maintain a volume flow of 900 cm^3 min^{-1} at 25°C. Each chamber was equipped with a manifold on both the intake and exit ports for mixing the chamber air. The intake port openings and the diameters of tubing leading into the intake ports were approximately three times larger than that of the exit ports in order to prevent pressure reduction within the chambers. Data from periodic point-in-time measurements using a push–pull, closed-loop system agreed closely with data from this open system. Air entering each respiration chamber and reference line was pulled through a single 0.02 m^3 plastic mixing jar to dampen the periodic but rapid changes in [CO_2] in the CO_2-enriched plots.

A 16-port manifold and switching system allowed us to measure each tree of each treatment every 112 min over a period of 1 yr. Stem temperature at cambial depth on the north side of one tree in each treatment and adjacent air temperature were monitored continuously. The switching system was controlled and data (including delta [CO_2], flow rates, and temperatures) were logged with a CR10X measurement and control system (Campbell Scientific, Inc., Logan, UT, USA). Q_{10} values (described later) and stem temperatures were used to estimate respiration rates during power outage periods and when equipment was being serviced. Power outages were rare and lasted only a few hours. Equipment failure and service caused an 18-d curtailment of respiration data collection in early July 2000 and a 12-d curtailment in late August 2000. During the dormant season we did not collect respiration data between mid-December 2000 and mid-January 2001. Stem and air temperatures were recorded at all times except during power failures. During periods of power failure, regression analysis of air temperature vs stem temperature permitted the calculation of stem temperature from air temperatures recorded by the local the National Oceanic and Atmospheric Administration (NOAA) weather station.

The stem tissue under each chamber was assumed to be a pie-shaped wedge, and its volume was calculated for each tree based on stem circumference. Respiration data are expressed per unit of wood volume. Sap velocity studies indicated that about 73% of the wood was sapwood, with no treatment differences in relative sapwood area at a height of 1.3 m (Wullschleger & Norby, 2001). Growth respiration (R_g) was calculated by subtracting maintenance respiration (R_m) from measured total respiration (R_t); R_m was measured throughout the dormant season.

Baseline respiration rates at 10°C (R_{10}) and Q_{10} values were calculated for the dormant season and growing season separately for both the CO_2-enriched treatment and the control treatment. Q_{10} is defined as the change in the rate of respiration with a 10°C change in stem temperature as follows:

$$R = R_{10}Q_{10}(t_1 - t_2)/10 \qquad \text{Eqn 1}$$

where R is predicted respiration in μmol CO_2 m^{-3} of wood s^{-1}, R_{10} (the baseline respiration rate) is the respiration rate at t_2 (baseline temperature), Q_{10} is the respiratory temperature coefficient and t_1 is the varying stem temperature. We chose 10°C as our t_2 value. The Q_{10} and R_{10} values were calculated by regressing the log of average respiration rates for each plot during each measurement time interval against the average stem temperature for the same time interval.

The Q_{10} values and R_{10} rates established from the dormant season respiration data and dormant season stem temperatures were used to estimate R_m from stem temperatures measured continuously throughout the entire year.

Basal area increment (BAI) of each of the six respiration trees in each plot was calculated from stem circumference, which was measured at monthly intervals throughout the growing season with permanently mounted dendrometer bands (Norby *et al.*, 2001).

The CO_2 on–off experiment

In order to evaluate the response time of stem respiration and stem tissue carbohydrate concentrations to CO_2 enrichment, we stopped CO_2 enrichment for a 4-d period in June 2001. Equipment failure curtailed stem respiration measurements during the first and second days after CO_2 was turned back on. Measurements were continued on the third day after CO_2 was turned back on.

Stem tissue was collected for carbohydrate analysis 1 d before and 4 d after CO_2 was turned off. All samples were collected between 13 : 30 h and 14 : 00 h on each sample date. Samples were collected about 10 cm below and slightly to the side of the stem respiration chambers. Samples were collected from five of the six sample trees in each plot. Given no major change in weather conditions, we have found that carbohydrate concentrations are very stable over time if tissues are sampled at the same time of day. This was confirmed by analysing a subsample of control trees for sucrose concentration on the fourth day after CO_2 was turned off. Samples were collected with a 12-mm diameter increment corer and immediately frozen in dry ice and lyophilized for at least 48 h before being processed for analysis. The outer bark tissue and tissue just inside the cambium were discarded, leaving a 2-mm thick core on both sides of the cambium for analysis, which was then ground in a ball mill. Aqueous sorbitol was added as an internal standard to each approx. 50 mg of dried sample immediately before the extraction solvent was added. The

samples were then extracted twice with 5 ml of 80% aqueous ethanol, and the extracts were combined prior to analysis of soluble carbohydrates, as trimethylsilyl (TMS) derivatives by capillary gas chromatography. After air-drying 1 ml of the total extract, soluble carbohydrates were dissolved with 1 ml silylation-grade acetonitrile, and silylated with 1 ml of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide +1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL, IL), heated for 60 min at 90°C, and 2 µl injected the following day and up to 8 d later into an HP5890 Series II GC and 5972 MS, fitted with an HP-5MS (crosslinked 5% PH ME Siloxane) 30 m × 0.25 mm × 0.25 µm film thickness capillary column (Hewlett-Packard, Avondale, PA, USA). The operating conditions were similar to that reported in Gebre *et al.* (1998). High concentrations of complex phenolic compounds (e.g. isorugosins) delayed derivatization, necessitating the repeated injections until maximal peak response was obtained. Peaks were quantified by area integration and concentration was determined based on the quantity of the internal standard and the response factors of the specific carbohydrates relative to the internal standard.

Experimental design and data analysis

The experimental design comprises two high-CO_2 plots with elevated [CO_2] and two control plots with ambient [CO_2]. The control plots were equipped with all of the air circulating equipment used in the high CO_2 plots, except for a CO_2-enrichment source. The control plots were located upwind from the high CO_2 plots to reduce the chances of CO_2 enrichment in the control plots. Significant stem respiration responses to treatment were determined with a repeated measures ANOVA model (STATISTIX for Windows; Analytical Software, Tallahassee, FL, USA). Individual plot averages (i.e. the average of six trees in each plot during a single averaged measurement time interval) were the experimental units ($n = 2$). Owing to limitations of the statistical software used in handling large data sets and in order to evaluate statistical differences at different times of the year, analyses were performed on segments of data covering time periods of 5–9 d. Only measured respiration rates were used in the analyses (i.e. no predicted data used). Data from 15 time-periods were statistically analysed (eight during the growing season and seven during the dormant season). Carbohydrate data presented are treatment means (± SE) of five trees per FACE plot with two replicate plots per treatment. Statistically significant ($P \leq 0.05$) treatment differences in carbohydrates were determined by one-sided Student's *t*-tests, with the plot considered as the experimental unit.

Results

Stem respiration

Stem respiration rates of individual trees varied considerably. For example, in mid-August 2000, individual tree rates ranched from 33 to 66 µmol CO_2 m^{-3} s^{-1} in control plots and from 40 to 94 µmol CO_2 m^{-3} s^{-1} in the CO_2-enriched plots. Plot-to-plot variation was higher for the control trees than for the CO_2-enriched trees, and within-plot variation was higher in the CO_2-enriched trees (Table 2). Rates in the CO_2-enriched plot 2 were consistently higher than in plot 1 and rates in the control plot 4 were consistently higher than in plot 5 throughout the study.

Table 2 Stem respiration rates by plots of sweetgum (*Liquidambar styraciflua*) trees during an 8-d period in mid-August 2000

Treatment	Plot	Respiration (µmol CO_2 m^{-3} s^{-1})
CO_2-enriched	1	65.5 ± 7.6
	2	68.8 ± 9.5
Control	4	54.7 ± 3.8
	5	38.4 ± 2.5

Values are means ± SE ($n = 6$).

Daily average and seasonal patterns of stem respiration and temperature are shown in Fig. 1. Respiration rates were consistently higher under CO_2 enrichment than at ambient [CO_2] throughout both the growing season and the dormant season. Averaged over an entire year, stem respiration was increased 33% by CO_2 enrichment because of a 23% increase in growth respiration and a 48% increase in maintenance respiration (Table 3). Differences in total respiration rates were statistically significant ($P \leq 0.01$) for each of the 15 periods tested throughout the growing season and the dormant season (Table 4). The highest rates in both treatments occurred during mid-June to mid-July and the lowest rates were recorded in early January, but they were very low throughout the winter season. Annual total R_g was 13.9 kg CO_2 m^{-3} yr^{-1} in CO_2-enriched trees and 11.4 kg CO_2 m^{-3} yr^{-1} in control trees (Fig. 2). Annual R_m was 11.7 CO_2 m^{-3} yr^{-1} in CO_2-enriched trees and 7.9 kg CO_2 m^{-3} yr^{-1} in control trees. We calculate from these totals that 40% of the increased stem respiration in the CO_2-enriched trees resulted from differences in growth rates and 60% from differences in maintenance costs.

The BAI for the study period averaged 23% higher in the CO_2-enriched trees than control trees, but treatment differences were greater during the latter part of the 2000 growing season than during the early part of this growing season (Fig. 1). By September 15, 2000, BAI had declined to near zero in both treatments and reached zero by mid-October It was during this time that respiration rates began to decline disproportionately to changes in stem temperature. Basal area growth was detectable with dendrometer bands by May 11, 2001 (in Fig. 1), and it was at this time that respiration rates began to increase disproportionately to changes in stem temperature.

Diurnal changes in cambial stem temperatures lagged nearly 2 h behind changes in air temperatures and stem respiration

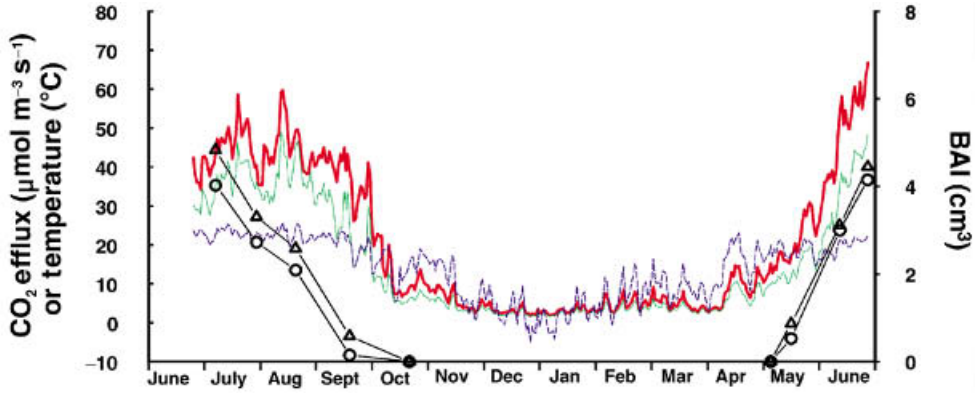

Fig. 1 Daily mean stem respiration rates in CO_2-enriched sweetgum (*Liquidambar styraciflua*) trees (red line), control trees (green line), and stem temperatures (blue line) between June 2000 and June 2001. The means (n = 2) were calculated from measurements taken at 112 min intervals on each of 6 trees in 2 plots of each treatment or in the case of power failure or equipment problems, rates were calculated using Q_{10} values and base rates. Basal area increments (BAI) of CO_2-enriched trees (triangles) and control trees (circlles) are also shown. BAI values are treatment means of the six 'respiration' trees in each plot. The BAI standard errors are not plotted but ranged from 7 to 14% of the means in the CO_2-enriched trees, and from 13 to 28% of the means in the control trees (n = 2).

Table 3 Mean, maximum, and minimum stem respiration rates of sweetgum (*Liquidambar styraciflua*) trees between June 21 2000 and June 21 2001 (n = 2)

	Respiration (μmol CO_2 m^{-3} s^{-1})								
	Total			Growth			Maintenance		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
High CO_2	18.5	80.0	1.2	10.1	62.9	0	8.4	27.6	1.2
Control	13.9	61.1	1.0	8.2	47.1	0	5.7	16.1	1.0

Treatment differences in mean R_t are assumed to be statistically significant because treatment differences in all blocks of data that were used in calculating the means were statistically significant ($P \leq 0.01$). Maintenance respiration was measured during the dormant season (November–March) and extrapolated through the entire year with dormant season Q_{10} and R_{10} values (Table 5) and growing season stem temperatures. Growth respiration was calculated by subtracting the calculated maintenance respiration from measured total respiration.

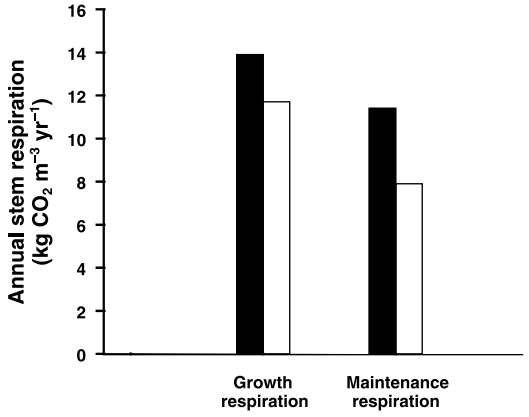

Fig. 2 Annual total stem growth respiration (R_g) and annual total stem maintenance (R_m) of sweetgum (*Liquidambar styraciflua*) trees. Annual total R_m = dormant season R_m plus growing season R_m. Dormant season R_m was calculated by summing measured dormant season daily average respiration rates. Growing season R_m was calculated from Q_{10} values and base rates at 10°C (R_{10}) determined during the dormant season measurements. Annual total R_g = annual total respiration (sums of measured daily average respiration rates minus predicted R_m). Closed columns, high CO_2; open columns, control.

rates lagged nearly 2 h behind changes in stem temperatures throughout the year (data not shown). A typical diurnal pattern for stem temperature and respiration is shown for early June 2001 (Fig. 3), with respiration rates steadily increasing from late morning until nearly midnight and then declining until midmorning of the next day.

Baseline respiration rates at 10°C (R_{10}) and Q_{10} values differed between treatments and between dormant season and growing season (Fig. 4 and Table 5). the R_{10} rates during the growing season were four times higher than dormant season R_{10} rates in the CO_2-enriched trees, and three times higher in control trees. Average respiration rates at 10°C were increased 73% by CO_2 enrichment during the growing season and 35% during the dormant season (Table 5 and Fig. 4).

The CO_2 on–off experiment

The enriched–control (E : C) ratios of daily average R_t declined from 1.4 immediately prior to shut-off of the CO_2 to about 1.25 at the time CO_2 was turned back on (Fig. 5a).

Table 4 Stem respiration rates of control and high CO_2 sweetgum (*Liquidambar styraciflua*) trees during various periods between June 21 2000 to June 21 2001

Dates	Control trees (μmol CO_2 m^{-3} s^{-1})	High-CO_2 trees (μmol CO_2 m^{-3} s^{-1})
Growing season		
2000		
June 21–27	29.5 ± 0.3	38.7 ± 0.5
July 15–20	44.0 ± 0.7	54.8 ± 0.9
July 21–27	38.9 ± 0.5	46.7 ± 0.7
July 28–August 1	33.5 ± 0.4	38.9 ± 0.7
August 8–14	43.2 ± 0.6	54.7 ± 1.2
August 15–20	42.9 ± 0.6	47.3 ± 0.8
August 20–26	36.4 ± 0.4	39.7 ± 0.6
2001		
April 2–9	6.1 ± 0.2	8.4 ± 0.3
Dormant season		
2000		
November 29–December 3	2.5 ± 0.07	3.4 ± 0.08
December 3–7	2.0 ± 0.04	2.6 ± 0.04
December 8–11	2.0 ± 0.05	2.9 ± 0.05
December 12–15	2.2 ± 0.06	2.8 ± 0.08
2001		
March 14–21	3.1 ± 0.06	3.9 ± 0.08
March 22–27	2.9 ± 0.07	3.5 ± 0.09
March 27–April 2	3.1 ± 0.05	3.6 ± 0.06

Values are plot means ± SE (n = 2). Differences in the means are all statistically significant ($P \leq 0.01$).

By day 3 after the CO_2 was turned back on R_t in CO_2-enriched trees had begun to increase, and by the day 6, rates in CO_2-enriched trees were once again about 1.4 times the rates in control trees. Treatment differences were highest during the day and lowest at night (Fig. 5b). This pattern of higher treatment differences in stem respiration during the day than during the night was observed throughout the annual cycle, including the dormant season (data not shown).

The E : C ratios of sucrose concentrations in the cambial tissue of the stem declined from 1.28 at the time of CO_2 shut-off to 1.03 at the time CO_2 was turned back on (Fig. 5a). Treatment differences in sucrose concentrations were statistically significant before the CO_2 was turned off but not after 4 d without CO_2 fumigation. There were no treatment differences in other carbohydrates analysed, including glucose and fructose concentrations (Table 6).

Discussion

Our findings of increased stem respiration under CO_2 enrichment agrees with some of the findings of Carey *et al.* (1996) for *Pinus ponderosa* grown in open-top field chambers. Their study found that treatment differences in stem respiration were significant only at temperatures above 21°C, whereas our study found statistically significant treatment differences in stem respiration across the full range of temperatures measured. They concluded that the response to CO_2 enrichment was due to higher maintenance cost because they calculated no significant difference in construction costs between treatments. We found responses of both R_g and R_m to CO_2 enrichment. By contrast, Janous *et al.* (2000) reported slightly lower growing-season-rates of stem respiration under CO_2 enrichment than in ambient air in *Picea abies* growing in open-top chambers. Wullschleger *et al.* (1995) reported higher rates of growing season stem respiration under CO_2 enrichment than in ambient air in *Quercus alba* saplings growing in open-top chambers, but they found no effects of

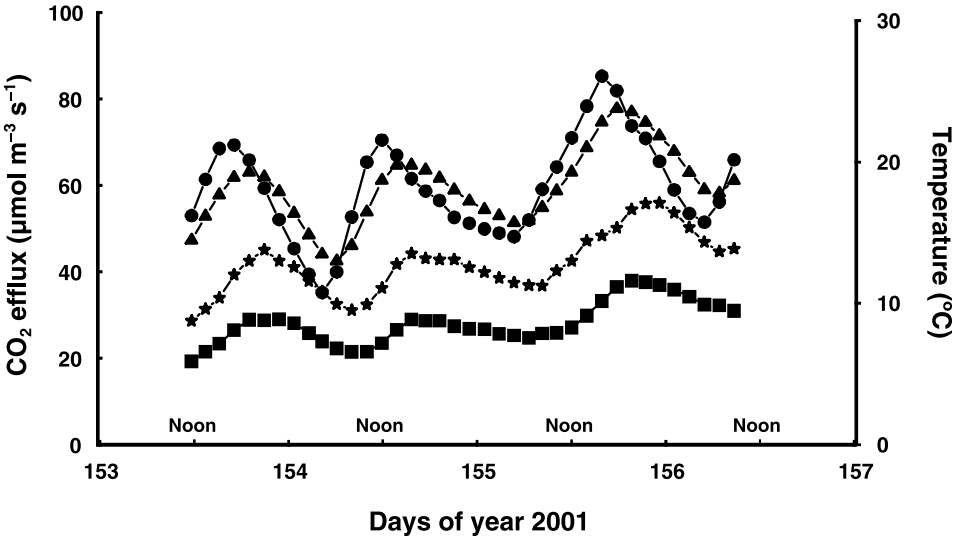

Fig. 3 Diurnal cycles of air and sweetgum (*Liquidambar styraciflua*) stem temperature and stem respiration by treatment (n = 2) are shown for a period between June 2 and June 5, 2001. Circles, air temperature; triangles, stem temperature; stars, respiration (high CO_2); squares, respiration (control).

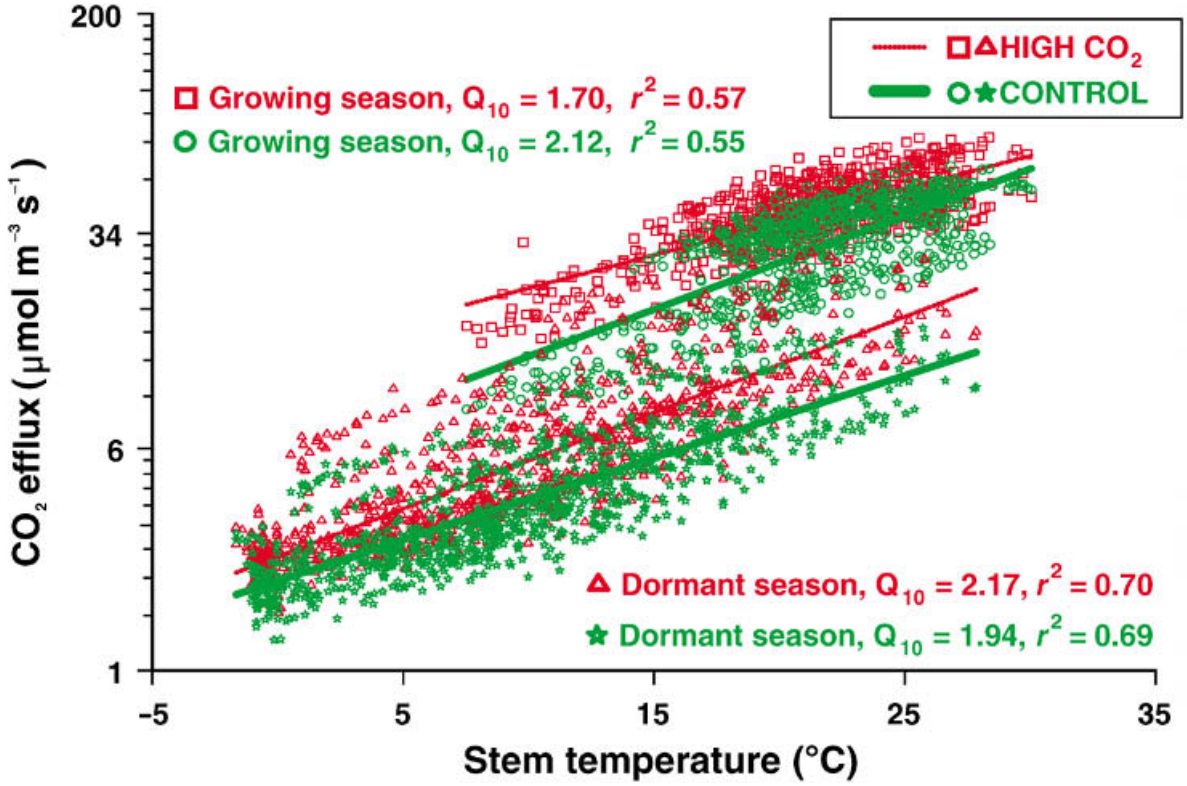

Fig. 4 Measured (at 112-min intervals) treatment average (n = 2) stem respiration plotted against stem temperature of sweetgum (*Liquidambar styraciflua*). Dormant season and growing season data are plotted separately. The Q_{10} values and R_{10} rates were calculated for each season and each treatment from the regression lines shown for each set of data.

Table 5 Growing season (April–October) and dormant season (November–March) Q_{10} values and respiration rates at 10°C (R10) by treatment

	Growing season		Dormant season	
Treatment	Q_{10}	R_{10} (μmol CO_2 m^{-3} s^{-1})	Q_{10}	R_{10} (μmol CO_2 m^{-3} s^{-1})
High CO_2	1.7	21.8	2.2	5.4
Control	2.1	12.6	1.9	4.0

Growing season Q_{10} values and R_{10} rates were calculated with regression analysis of stem temperatures and respiration rates measured from June 21 through October 2000; April–June 20, 2001 (See Fig. 3). Dormant season Q_{10} values and R_{10} rates were calculated with regression analysis of stem temperatures and respiration rates measured in November–March.

CO_2 enrichment on maintenance respiration. Wullschleger *et al.* (1995) concluded that there was no effect of CO_2 enrichment on either (growth or maintenance) respiratory process. The current study demonstrates higher respiration rates for both R_g and R_m in sweetgum grown under CO_2 enrichment. However, we suggest that both R_g and R_m rate responses may result from changes in growth rates and live to dead tissue ratios rather than from mechanistic alterations in the respiratory processes.

Annual total CO_2 efflux from stems at 1.3 m was 6.3 kg m^{-3} higher in CO_2-enriched trees than in control trees. Forty per cent of this difference can be accounted for by differences in R_g. The BAI based on changes in diameter growth was 18% higher in CO_2-enriched trees than in control trees during this study compared with our calculated 23% higher R_g stem respiration in the CO_2-enriched trees. During the late portion of the 2000 growing season (July–September), BAI and R_g in the high-CO_2 trees exceeded the control trees by 26% and 20%, respectively. During the early growing season of 2001 (May–June), treatment differences in BAI were only 10% vs 44% in R_g. Making growth comparisons based on wood volume increments with stem respiration rates across the growing season is cumbersome because of differences in rates of cell expansion vs cell wall thickening and increasing wood density as the season progresses (Sprugel & Benecke, 1991; Edwards & Hanson, 1996). This might explain some of the discrepancy between stem growth and stem respiration in the late summer of 2000 vs the early spring comparisons in 2001. However, we might have expected the opposite pattern because during the later portion of the growing season wood density generally increases without a proportionate increase in volume.

Given that R_g was calculated by subtracting maintenance respiration from total respiration and that R_g exceeded what would be expected based on BAI, we conclude one or more of the following: (1) the energy cost for growing new tissues was

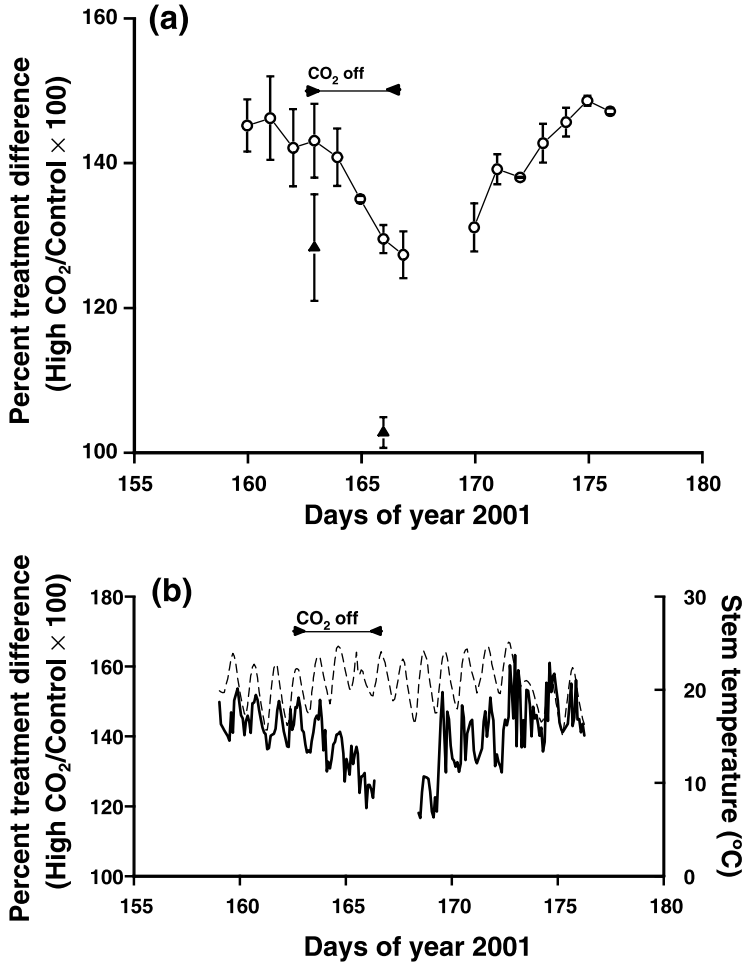

Fig. 5 (a) Ratios of stem respiration rates (rates in CO_2-enriched sweetgum, *Liquidambar styraciflua*, trees divided by stem respiration rates in control trees) are shown for a period of time just prior to stopping CO_2 fumigation, for 4 days when CO_2 was off and for a period of time beginning the day 3 after CO_2 was turned back on (open circles). Ratios of sucrose concentrations (concentrations in the high-CO_2 plots before and after the off–on period divided by concentrations in control plots just prior to turning off the CO_2) are also shown (filled triangles). Sucrose concentrations in the control did not change over the 4-d period. Sucrose concentration were determined for the cambium and adjacent tissues collected 1 d before CO_2 was turned off and 1 h before CO_2 was turned back on. Data symbols represent means ± 1 SE (n = 2). (b) The diurnal pattern of enriched–control (E : C) ratios in stem respiration (solid line) during the same period depicted in (a). Changes in stem temperatures (dashes line) measured at cambial depth are also shown.

Table 6 Concentrations of soluble carbohydrates in the stem tissue of sweetgum (*Liquidambar styraciflua*) trees from the two treatments before and after the CO_2 in the free-air CO_2 enrichment (FACE) plots was turned off for 4 d in early June 2001

		Carbohydrate concentrations (mg g^{-1} d. wt)					
Treatment	Time[1]	Fructose	Galactose	Glucose	Sucrose	Scylloinositol	Myoinositol
Control	CO_2 On	2.41 ± 0.27	0.44 ± 0.001	1.14 ± 0.02	7.48 ± 0.45[a]	0.62 ± 0.07	0.16 ± 0.01
High CO_2	CO_2 On	2.54 ± 0.10	0.52 ± 0.08	1.32 ± 0.14	9.60 ± 0.55[b]	0.63 ± 0.05	0.15 ± 0.01
High CO_2	CO_2 Off	2.75 ± 0.27	0.27 ± 0.02	1.15 ± 0.05	7.69 ± 0.16[a]	0.69 ± 0.09	0.14 ± 0.02

Values are means and standard errors (n = 2). Carbohydrate analysis was performed on 5 trees in each treatment plot. The values under the sucrose columns that are designated by different letters indicate statistical treatment differences ($P \geq 0.05$). There were no other statistical differences. [1]Time: CO_2 On, 1 d before CO_2 was turned off; CO_2 Off, 4 d after CO_2 was turned off.

higher in CO_2-enriched trees than in control trees; (2) some respiration was wasted when substrate concentrations exceed the levels required for growth and maintenance; (3) our growing season estimates of R_m in the CO_2-enriched trees may be too low.

We are unaware of any published data to support the idea that growing new tissues costs more energy in CO_2-enriched trees than in control trees. Carey *et al.* (1996) found no difference in construction costs in stems of young *P. ponderosa* trees grown under ambient air and CO_2 enrichment and Wullschleger *et al.* (1997) reported slightly lower construction costs of tissues of *Liriodendron tulipifera* and *Q. alba* trees grown under CO_2 enrichment than under ambient conditions. Wasteful respiration due to excess substrate is a possibility given the high photosynthesis rates in CO_2-enriched trees relative to the control trees (Gunderson *et al.*, 2002). According to Amthor (2000) some respiration may occur without benefit to the plant (i.e. heat and CO_2 are produced with no contribution to growth or maintenance). If a part of what we are calling R_g is actually wasted, and if the respiratory response is primarily a function of substrate availability and temperature,

we may be overestimating R_g in the CO_2-enriched trees. The possibility that we are underestimating R_m in the CO_2-enriched trees has merit because the growing season R_m rates were not measured, but were based on the dormant season Q_{10} values and the growing season temperatures. Amthor (1989) and Lavigne & Ryan (1997) reported that R_m increases with increases in relative growth rates, supporting our suggestion that we may be underestimating R_m during the growing season, especially in the CO_2-enriched trees. This would result in an overestimate of R_g by the subtraction technique used here.

Nevertheless, the second hypothesis that stem respiration increases under CO_2 enrichment because of increased stem growth rates is partly supported. We conclude that a portion (up to 40%) of the increased stem respiration under CO_2 enrichment resulted from increased stem growth. The cost for maintenance of living tissues in the CO_2-enriched sweetgum trees was 48% higher than in control trees. Gunderson *et al.* (2002) reported that photosynthesis during the year 2000 in this FACE study was 46% higher in CO_2-enriched trees than in control trees, indicating that an ample supply of carbon was available to support the increased R_m. Thus, our third hypothesis that maintenance respiration does not change in response to CO_2 enrichment is rejected. We speculate that the treatment response in R_m could be due in part to an increase in the proportion of living tissues in the stem wood of CO_2-enriched trees rather than to a direct effect on R_m rates. However, we have no data to support this possibility. The importance of the ratio of live to dead tissue in stems in respiration rates has been well documented (Kramer & Kozlowski, 1979; Ryan, 1990; Stockfors & Linder, 1998; Stockfors, 2000).

The lag in changes in stem temperature behind changes in air temperature and the lag in stem respiration in response to changes in stem temperature were expected (Ryan, 1990; Stockfors, 2000; Damesin *et al.*, 2002). The lag in changes in air temperature and stem temperature reflects the time required for stem mass to exchange heat with the air. Since we would expect a quick respiration rate response to a change in temperature, the lag in apparent stem respiration probably reflects the time required for respired CO_2 to diffuse from its origin to the atmosphere. However, we must keep in mind that the stem temperature was measured at only one depth (albeit at the depth of expected highest rates of respiratory activity) and both the rates of respiration and the temperature would be expected to change with depth.

The gradual reduction in stem respiration over 3–4 d after CO_2 enrichment was stopped and then the gradual increase after it was started again suggest that stem respiration is driven in part by photosynthates, specifically sucrose, transported from the leaves and that the respiratory response to available substrates occurs quickly. This suggestion is supported in this study by the decrease in sucrose concentrations in the stem tissues in a relatively short period during curtailment of CO_2 fumigation. Previous papers have reported quick responses of woody plant tissue respiration to substrate supply. For example, McLaughlin *et al.* (1977) demonstrated that $^{14}CO_2$ was respired from roots of a mature white oak tree within 18 h of the injection of [^{14}C]sucrose into the stem at 1.5 m above ground level. Edwards & McLaughlin (1978) reported that reducing sugar concentrations in the phloem of mature *L. tulipifera* trees tended to follow the same diurnal pattern as respiration rates, with highest sugar concentrations occurring in mid-afternoon and lowest concentrations from midnight to noon. Dewar *et al.* (1999) suggests that respiratory acclimation responses to temperature in leaves can be reconciled with substrate-based models in which respiration is limited by supply of photosynthates. Our study suggests that substrate availability plays a key role in woody tissue respiration and that respiration responds quickly (over the course of a few days) to changing atmospheric [CO_2], primarily in response to changes in photosynthate (sucrose) from the leaves. Only sucrose concentrations were increased by elevated [CO_2], whereas fructose and glucose concentrations did not change in response to treatments. The decline in sucrose coupled with a decline in R_t, but without a concomitant decline in glucose, suggests that monosaccharide turnover (i.e. monosaccharide flux rather than concentration) was increased under CO_2 enrichment. We conclude that the data support the hypothesis that elevated [CO_2]-induced increases in stem respiration are, in part, driven by increased substrate supply from leaves.

We observed that differences between the R_t of CO_2-enriched trees and control trees were greater during the day than at night throughout both the dormant season and the growing season. This can be explained for the dormant season by the higher Q_{10} values in CO_2-enriched trees than in control trees and by the fact that the Q_{10} lines converge at the lower temperatures. However, during the growing season, Q_{10} values were lower in CO_2-enriched trees than in control trees and the Q_{10} lines converge at the higher temperatures. Despite this, the treatment differences increase during the day when temperatures are higher than at night. This suggest that the R_m during the growing season has a higher Q_{10} response in the CO_2-enriched trees than in the control trees, because the observed higher Q_{10} for R_t in control trees would have caused the opposite response (i.e. treatment differences would have been less during the day than at night). Unfortunately, we cannot measure R_m directly during the growing season, but this observation provides additional support for the suggestion that we may be underestimating R_m in the CO_2-enriched trees.

Using only the measurements of stem respiration rates reported in this study to scale up to the whole-tree level would likely result in an underestimate of whole-tree woody tissue respiration, because the younger portions of the stem and the branches have much higher rates of respiration than the older portions of the stem. For example, a limited number of comparative measurements in this study indicated that the upper portion of the stem, near the top of the canopy, respired at rates about four times those in the lower portion of the stem (data not shown) and small branches (approx. 1.5 cm diameter)

respired at rates about six times those in the lower portion of the stem. Damesin *et al.* (2002) reported maximum rates in small branches (approx. 0.5 cm diameter) that were nearly 20 times higher than maximum rates at a height of 1.5 m on the stem of 30-yr-old *Fagus sylvatica* trees in France. Using only stem respiration rates from the stems at a height of 1.3 m and assuming the rates in upper stems and in branches to be equivalent to the rates in the lower stem portion, we calculated that stem respiration would account for about 5% of gross primary production (GPP) in this forest. This calculation assumes a stem wood volume of about 0.08 m^3 per tree based on average heights and diameters of these sweetgum trees. We plan to obtain additional measurements in the upper portions of the stems and of the branches in order to more accurately assess woody tissue respiration at the stand level. We hypothesize that when this is accomplished, our estimate of above-ground woody tissue respiration may exceed 10% of GPP. This assumes that our preliminary data showing a fourfold higher stem respiration rate in the upper stem than at a height of 1.3 m is substantiated and that there is a linear increase in rates with decreasing diameter from the base to the upper stem. Additional measurements of carbon losses from a full size-range of stems and branches, as well as from roots and leaves over a wide range of environmental conditions, are needed if the allocation of the additional carbon that is assimilated under CO_2 enrichment is to be accurately assessed at stand level.

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References

Amthor JS. 1989. *Respiration and crop productivity*. New York, NY, USA: Springer-Verlag.

Amthor JS. 2000. The McCree-de Wit-Penning de Vries-Thornley Respiration Paradigms: 30 Years Later. *Annals of Botany* **86**: 1–20.

Carey EV, Evan H, Delucia, Timothy Ball J. 1996. Stem respiration and construction respiration in *Pinus ponderosa* grown in different concentrations of atmospheric CO_2. *Tree Physiology* **16**: 125–130.

Damesin CE, Ceschia N, Le Goff J-M, Ottorini, Dufrene E. 2002. Stem and branch respiration of beech: from tree measurements to estimations at the stand level. *New Phytologist* **153**: 159–172.

Dewar RC, Medlyn BE, Mcmurtrie RE. 1999. Acclimation of the respiration/photosynthesis ratio to temperature: insights from a model. *Global Change Biology* **5**: 615–622.

Edwards NT, Hanson PJ. 1996. Stem respiration in a closed-canopy upland oak forest. *Tree Physiology* **16**: 433–439.

Edwards NT, McLaughlin SB. 1978. Temperature-independent diel variations of respiration rates in *Quercus alba* and *Liriodendron tulipifera*. *Oikos* **31**: 200–206.

Edwards NT Shugart HH Jr, McLaughlin SB, Harris WF, Reichle DE. 1981. Carbon metabolism in terrestrial ecosystems. In: Reichle DE, ed. *Dynamic properties of forest ecosystems*. London, UK: Cambridge University Press, 499–536.

Gebre GM, Tschaplinski TJ, Tuskan GA, Todd DE. 1998. Clonal and seasonal differences in leaf osmotic potentials and organic solutes of five hybrid poplar cl *I*s grown under field conditions. *Tree Physiology* **18**: 645–652.

Gunderson CA, Sholtis JD, Wullschlelger SD, Tissue DT, Hanson PJ, Norby RJ. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a Sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. *Plant, Cell & Environment* **25**: 379–393.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* **3**: 463–309.

Janous DR, Pokorny J, Brossaud, Marek MV. 2000. Long-term effects of elevated CO_2 on woody tissue respiration of Norway spruce studied in open-top chambers. *Biologia Plantarum* **43**: 41–46.

Kramer PJ, Kozlowski TT. 1979. Physiology of woody plants. Academic Press, New York, 811 p.

Lavigne MB, Ryan MG. 1997. Growth and maintenance respiration rates of aspen, black spruce and jack pine stems at northern and southern BOREAS sites. *Tree Physiology* **17**: 543–551.

Lavigne MB, Ryan MG, Anderson DE, Baldocchi DD, Crill PM, Fitzjarrald DR, Goulden ML, Gower ST, Massheder JM, McCaughey JH, Rayment M, Strieg RG. 1997. Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at 6 coniferous boreal sites. *Journal of Geophysical Research* **102** (d24): 28977–29985.

McCree KJ. 1970. An equation for the rate of respiration of white clover plants grown under controlled conditions. In Setlik I (Ed.) *Prediction and measurement of photosynthetic productivity*. PUDOC, Wageningen, Netherlands, pp. 221–229.

McLaughlin SB, Edwards NT, Beauchamp JJ. 1977. Spatial and temporal patterns in transport and respiratory allocation of [^{14}]sucrose by white oak (*Quercus alba*) roots. *Canadian Journal of Botany* **55**: 2971–2980.

Norby RJ, Todd DE, Fults J, Johnson DW. 2001. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist* **150**: 477–487.

Penning de Vries FWT. 1975. The cost of maintenance processes in plant cells. *Annals of Botany* **39**: 77–92.

Penning de Vries FWT, Brunsting AHM, van Laar HH. 1974. Products, requirements and efficiency of biosynthesis: a quantitative approach. *Journal of Theoretical Biology* **45**: 339–377.

Ryan MG. 1990. Growth and maintenance respiration in stems of *Pinus contorta* and *Picea engelmannii*. *Canadian Journal of Forest Research* **20**: 48–57.

Sprugel DG, Benecke U. 1991. Measureing woody-tissue respiration and photosynthesis. In Lassoie JP, Hinckley TM (Eds) *Techniques and Approaches in Forest Tree Ecophysiology*. CRC Press, Boca raton, FL, USA, pp. 329–355.

Stockfors J. 2000. Temperature variations and distribution of living cells within tree stems: implications for stem respiration modeling and scale-up. *Tree Physiology* **20**: 1057–1062.

Stockfors J, Linder S. 1998. Effect of nitrogen on the seasonal course of growth and maintenance respiration in stems of Norway spruce trees. *Tree Physiology* **18**: 155–166.

Wullschleger SD, Norby RJ. 2001. Sap velocity and canopy transpiration in a sweetgum stand exposed to free-air CO_2 enrichment (FACE). *New Phytologist* **150**: 489–498.

Wullschleger SD, Norby RJ, Hanson PJ. 1995. Growth and maintenance respiration in stems of *Quercus alba* after 4 years of CO_2 enrichment. *Physiologia Plantarum* **93**: 47–54.

Wullschleger SD, Norby RJ, Love JC, Runck C. 1997. Energetic costs of tissue construction in yellow-poplar and white Oak trees exposed to long-term CO_2 enrichment. *Annals of Botany* **80**: 289–297.